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Bioactivity, Chemistry and Processing

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Bioactivity, Chemistry and Processing

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Preface

Olives, olive oil, and the effect of their functional compounds as bioactives on human health have been discussed during the past several years all over the world. This interest is likely to continue for the years to come. People will learn more about the importance of olive oil and its role in reducing oxidative stress, a serious health risk factor.

The book provides thorough information about olives and olive oil, concerning mainly composition, analysis, fruit processing, quality, and use of by-products, and will help the reader to understand the importance of this commodity for the agricultural economy and the relevance of its bioactives to human health.

This book consists of 35 chapters contributed by distinguished authors and industry leaders in the field covering major areas of: production, postharvest handling, physicochemical characteristics, chemistry, processing, nutritional and phenolic composition, significance to human health, lipidomics, fingerprinting and DNA quality authentication, packaging and marketing, waste treatment, and utilization.

The book is probably the first one in the market providing much information for farmers, traders, olive oil mill operators, packaging unit owners, consumers, scientists, health professionals, and students. Readers will be familiarized with the significance of new trends of olive oil for health and economical aspects. It will also serve as a valuable reference text for agricultural scientists, nutritionists, dieticians, physicians, and anybody else related to health. Recent dimensions in scientific knowledge have revealed the importance of bioactive compounds of olives and olive oil for health. There is a great interest in the bioactive constituents of olives and olive oil and their functional properties. These are demonstrated clearly in the book.

The present book provides comprehensive coverage dealing with functional and nutraceutical properties of olive products based on their unique composition. The carefully selected topics of special importance will help the reader find answers to different questions quickly and simply. It is divided into several sections focusing on important issues that concern the scientific community as well as the olive oil industry. The issues are analyzed under separate topics. These topics offer an up-to-date view of not only the present situation of olives and olive oil but also the evolution concerning their functional value.

We trust that this book would meet the requirement for a good text in the field. The editors acknowledge many individuals for their help in conceptualizing and developing the book. Special thanks go to the Librarians of the European Library in Luxembourg, and especially to Mrs Hayat Benaissa, for the material provided. Special thanks are also extended to Nikos Sakellaropoulos for his intensive effort in the preparation of the book, as well as to George Firtinidis for his significant help in editing its bibliography and indexing, and finally to Mrs Eleftheria Karamesinis-Kiritsakis for her great help in proofreading most of the chapters. Our sincere thanks and appreciation to all authors for their outstanding contributions. Last but not least, we are grateful to our family members for their support and understanding.

Apostolos Kiritsakis
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1 Olive tree history and evolution

Giorgos Kostelenos and Apostolos Kiritsakis

1.1 Introduction

The olive tree, *Olea*, derives its name from the Greek word *elea* and is one of the oldest known cultivated trees in the world. It seems possible that when man first cut wild olive tree branches to kindle a fire or to use them as a weapon, he noted its potential uses as well. It is possible that when the cut branches were left partly covered on the ground, they sprouted and after a long time grew into wild olive trees. The "taming" of the wild olive and the emergence of the cultivated olive tree represent the triumph of a developing civilization (Kiritsakis, 1998). The olive tree has been cultivated for about 6000 years in the Mediterranean basin. Unquestionably, the cultivation of the olive tree began before the written word was invented.

Archaeological studies indicate that the original centers of olive cultivation were in Syria, Israel, Lebanon, Cyprus, and Crete. Paintings found in the Minoan palace of Knossos on Crete early in the 20th century show people consuming olives and using olive oil for cooking and as fuel in lamps. Huge clay containers (amphoras), used for the storage of olive oil in ancient times, exist even today in Knossos and Phestos. Many archaeologists believe that the wealth of the Cretan Minoan Kingdom (3500–1000 BC) was due to the successful trade in olive oil. Olive tree cultivation was spread from Crete to the rest of Greece. Around 600 BC, the olive tree was brought to Italy and to other Mediterranean countries from Greece or from North Africa. The olive tree was probably introduced to Spain by Greeks, Romans, and Arabs. Rome expanded olive cultivation to the entire Roman Empire under occupation. The olive tree was widely cultivated in southern Europe, and this is where the name *Olea europaea* comes from.

After the discovery of the North American continent, the olive tree was brought there by the Spanish settlers. Olive trees were first planted in California around 1800 AD, when seeds or cuttings were brought to San Diego by the Franciscan padres. In the earlier days, the cultivar from the San Diego Mission was the leading one in California. Despite the fact that Americans have also developed an olive oil–based cuisine, the local supply is still inadequate. In the 1930s and 1940s, many Californian olive groves were grafted to produce table olives, rather than oil olives. Thus, today California supplies only a small percentage of the olive oil consumed in the United States. Arizona is another state with commercial acreage planted with olive trees.

In modern times, the olive tree has been spread all over the world and has been successfully cultivated in many regions previously not known to have olive trees. Olive trees are now being grown commercially in about 30 countries located mainly between latitudes of 30° and 45°. However, the Mediterranean basin, which was its ancient home, has the largest number of olive trees and is still the main source of olive oil in the world.

1.2 The olive culture in the Mediterranean region

According to legend, the olive tree was a gift from the gods to the ancient Greeks. Historian Herodotus described Athens in the fifth century BC as a vast center of olive culture. Philosopher Aristotle elevated

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olive cultivation to a science. The olive tree was a spiritual treasure for the Greeks. Olive branches dipped in purified water were used in funeral ceremonies. A special wreath, made of olive branches wrapped with wool, was carried by singing boys during harvest festivals. The crowning wreaths (*kotinos*) for the winners of the ancient Olympic games, which were held at the Greek city Olympia, were made of a wild olive tree branch. Victorious athletes also received olive oil in a cup.

The olive tree was a symbol for the Romans just as it was for the Greeks and other nations. Romans and Greeks developed all aspects of olive cultivation, production, and processing. It was the Romans who invented the screw press (hydraulic press) for olive fruit processing, and that procedure remained mostly unchanged for about 2000 years. Rome realized the significance of the olive tree in Africa and boosted its cultivation there. Romans considered those who used animal fat instead of olive oil in their diet to be barbarians. Olives were considered as one of the most useful and delectable fruits, like figs and grapes. Unquestionably, olives and olive oil were the most valuable products in ancient times.

Olive oil, besides being a fatty nutrient, always had other various uses. It had a special place in the Orthodox Church ceremonies, and it was used for the consecration of the Orthodox priests and of the kings of Greece. In biblical history, the olive tree played its role. Noah, after the waters of the flood receded, sent a dove out from the Ark. The dove came back carrying an olive branch in its mouth, and the olive tree was recognized as the symbol of peace thereafter.

The olive tree also played an important role in the decoration of pottery and of murals on the walls of houses. The olive tree has great historical importance because of the antiquity of its culture and the extent of its influence upon the development of Western civilization. It is not a coincidence that Romans, Greeks, Syrians, Egyptians, Israelites, Arabs, Babylonians, and many other people in ancient history were considered to be “children of the olive land” and were nourished by the fruit of the olive tree.

Many olive cultivars have been developed over the centuries. Four of the cultivars most commonly used for oil production are ‘Koroneiki’ in Greece, ‘Frantoio’ in Italy, ‘Arbequina’ in Spain, and ‘Mission’ in the United States. Olive cultivars differ in the size and color of their fruit, in oil content, as well as in quality. Some cultivars produce oil superior to that of most others. Thus, olives vary from cultivar to cultivar, as do apples or other fruits.

Unlike other fruit-producing trees, however, olive trees live to be centuries old and sometimes thousands of years old. The aged trunks in the natural environment of an olive grove are eye-catching; they make people appreciate Nature’s work of art and lead to the trees’ conservation and their characterization as areas of “particular natural beauty” for some (Simantirakis & Lykoudi, 2001), or as “Monumental Olive Trees of the World” for others (Association of Cretan Olive Municipalities [ACOM], 2002) (Figure 1.1).

There are several monumental trees in many countries (Italy, Spain, Portugal, Cyprus, Greece, etc.). In Greece, for example, there are the olive trees of the goddess Athena, of Plato, and of Hippocrates. There is also the olive tree of Kalamata (Psyllakis *et al.*, 2003) with an 8-meter perimeter; it is more than 800 years old and is the only tree that survived the big fire (1821–1824) in Peloponnese. On the island of Crete, among the 20 ancient olive trees that are older than 1000 years, the famous “olive tree of Vouves” is probably one of the oldest olive trees in the world, still producing some fruit, with an age estimated between 2000 and 3000 years old. This natural monument attracts many visitors every year.

Figure 1.1 Monumental olive trees. Left: Mother tree of Kalamata olives. Center: Tragic physiognomy – trapped spirit of the wood. *Source*: Courtesy of Simantirakis. Right: Ancient olive tree of the editor’s area. *Source*: Courtesy of Mountakis.

1.3 Evolution of the olive tree from a botanical point of view

The olive tree (*Olea europaea* L.) is the most distinctive tree of the Mediterranean flora, and it can be found in all of the surrounding Mediterranean Sea countries. Native olive trees can also be found beyond the Mediterranean countries, such as in Portugal to the west and in Jordan, Iraq, Iran, and up to Turkmenistan to the east.

During the evolutionary course of the olive tree, more than 1500 olive cultivars were created by man, making its expansion possible not only throughout the Mediterranean basin and the Middle East, but also even further to the Americas, Oceania, Central and Southern Africa, East Asia, and Southeast Asia.

Worldwide, the cultivation of the olive tree occupies more than 9,800,000 hectares and it is the sixth most important crop for production of edible oils. Only 15% of the olive groves throughout the world are irrigated. Furthermore, about 20% of all the olive groves are considered as "marginal" because they occupy areas of low productivity, that is, areas that are not suited for other, more demanding fruit trees. Another 50% of the olive groves can be characterized as "traditional," and only 30% of the groves are "modern" – with young trees of an intensive form and satisfactory cultivation, which give 50% of the total olive products (Lombardo, 2007).

Regarding the cultivated land areas, Spain comes first in the world with approximately 2,572,500 hectares, followed by Tunisia with 1,780,000 hectares, Italy with 1,212,000, Greece with 900,000, Turkey with 778,000, Morocco with 735,000, and others. In recent decades, olive tree cultivation has spread to many countries. The successful mechanization of its cultivation, mostly in the form of hyper-intense linear olive groves, has found new grounds, potentials, and capability for a rapid expansion of olive cultivation in countries possessing large tracts of land but a limited or costly workforce.

1.3.1 Botanical classification

The olive tree *Olea europaea* L. belongs to the *Oleaceae* family. There are other known genera of decorative plants that belong to the same family group, such as: *Phillyrea*, *Osmanthus*, *Jasminum*, *Ligustrum* (privet), *Fraxinus* (ash), *Forsythia* (golden bell), and others.

According to a more recent classification, the olive tree (*Olea europaea* L.) is placed in the subfamily *Oleideae* and the genus *Olea*, which includes three subgenera: *Olea*, *Tetrapilus*, and *Paniculatae*. Subgenus *Olea* is divided into two sections: *Olea* and *Ligustroides*. The *Olea* genus includes 33 species totally. Nine of them belong to *Olea* subgenus (one in the *Olea* section, and eight in the *Ligustroides* section), one to the *Paniculatae* subgenus, and 23 to the *Tetrapilus* subgenus (Besnard *et al.*, 2009).

The *O. europaea* species has six subspecies:

1. *O. europaea* subspecies *cuspidata*, deriving from southern and eastern Africa and having spread from the Middle East to China
2. *O. europaea* subsp. *laperrinei*, deriving from central-southern Sahara and the eastern Sahel
3. *O. europaea* subsp. *maroccana*, coming from southwest Morocco, west of Mount Atlas
4. *O. europaea* subsp. *cerasiformis*, coming from the Canary and Madeira Islands, and Porto Santo
5. *O. europaea* subsp. *guanchica*, deriving from the Canary Islands
6. *O. europaea* subsp. *europaea*.

The *O. europaea* subsp. *europaea* subgenus is subdivided into two cultivars: *O. europaea* subsp. *europaea* var. *sylvestris*, which is the wild olive tree, and *O. europaea* subsp. *europaea* var. *europaea*, which is the cultivated one.

In order to make the distinction between the wild and the cultivated olive trees, we could define the wild olive tree, from a botanical point of view, as the subspecies or cultivar of olive tree that would never bear not even one descendant by self-fertilization that could produce large fruit or fruit of high oil content. Based on this definition, two kinds of olive trees can be regarded as wild.

1. The "genuine wild olive trees," in a botanical sense of the term, located in isolated areas away from the presence of man and other cultivated olive trees.

2. The "forest olive trees," that is, natural seedlings that can be found near other cultivated olive trees. These seedlings, which are usually found on mountains at altitudes from 700 to 1000 m, derive directly or indirectly from seeds of domesticated olive trees or from seeds that have directly or indirectly received the pollen of cultivated olive trees. In a real sense, these kinds of olive trees are nothing but tamed olive trees in a natural form, and under no circumstance should they be considered as "genuine wild olive trees."

The small leaves and dense leaf order, two characteristics often found on the "forest olive trees," should not be related to the features of the "genuine wild olive trees" because they are standard characteristics of every young olive tree and gradually fade when they start bearing. Apart from young seedlings, olive trees that derive from tissue culture may also have characteristics of temporary youthfulness.

The longer the olive tree is cultivated in an area, the harder it is to find genuine wild olive trees in that area, due to the ever-increasing presence and scattering of pollen from cultivated olive trees. On the contrary, the further back we go timewise, the higher the chances are of meeting them, mainly in the form of grafted subjects on perennial olive trees. This happened because, back then, in the majority of cases, the number of cultivated olive trees was much smaller and, therefore, so was the spreading of pollen from tamed olive trees in comparison with the number of wild olive trees and their pollen.

1.3.2 Origin and revolution of the olive tree

There have been several views presented by many researchers about the origin of the olive tree, such as the eastern Mediterranean Basin, Anatolia (southern Asia Minor), Syria, and central Mesopotamia. According to still other theories, there have been other places regarded as being the birthplace of the olive tree; these are further east of Asia Minor as far as the west shores of the Caspian Sea, south Caucasus, Sudan, Ethiopia, and others (Breton *et al.*, 2012).

Many of these claims are based on the existence of a large number of native wild olive trees in Anatolia (Pelletier), in Asia Minor (De Candolle), and in Syria, as well as on the existence of the species *Olea cuspidata* in Iran, which some assume has contributed toward the evolution of the cultivated olive tree. Other theories also claim that the cultivated olive tree probably derives, via the wild olive tree, from *Olea chrysophylla* Laxx (Blázquez, 1996). Using modern molecular methods, it has been proven that the tamed olive tree is a descendant of the wild olive tree (Breton *et al.*, 2012).

One of the most prevalent theories suggests that the cultivated olive tree was tamed and evolved approximately 6000–7000 years ago, between 4800 and 4300 BC (Zohary *et al.*, 2012), in the eastern Mediterranean Basin. It then moved on and spread initially in the Aegean (Cyclades, Crete, and mainland Greece), later on in the central and western Mediterranean, and from there it spread to the Americas, Oceania, southern Africa, and other places. However, for many researchers, the fact that genetic differences between domesticated and wild olive trees were found by using molecular markers in the western Mediterranean Basin is not sufficient evidence regarding the origin of the olive tree from the eastern part of the Mediterranean (Breton *et al.*, 2012). Thus, the places of origin and taming of the olive tree, in general, remain unknown, and many scientific groups in several countries are involved with the research in this field.

Many Mediterranean populations, however, played an important role in spreading the cultivation of the olive tree, such as the Hittites in Syria and Anatolia, the Jews, the Phoenicians, the Egyptians, the Hellenes, the Romans, and others. Signs excavated in Ebla, a district of northern Syria near Aleppo, dating back to the third millennium BC, prove extensive production of olive oil there (Blázquez, 1996). Ebla at that time ranked third in olive tree cultivation, and according to the existing records there were three olive groves in that area, two of them with 500 olive trees each and one with 1000 trees (Rodríguez, 1996). From the second half of the second millennium BC, more data are available regarding the olive oil of Syria. During the Late Bronze Age, in the Ugarit area of Syria (today called Ras Shamra), the production of olive oil was about 5000 tons. Exports of olive oil from that area were made to Cyprus, Asia Minor, and Egypt (Hadjisavvas, 2008). Despite the fact that there are no explicit reports in the Egyptian records, Egypt was the major destination of the olive oil produced on the Syrian-Palestinian coast (Hadjisavvas, 2008).

Evidence proves the spreading of olive tree cultivation in the Near East (Levant) since the Chalcolithic era (3700–3500 BC) (Kelder, 2009); however, the date of first cultivation of the olive tree in southern Mesopotamia seems to be unknown. In Egypt, olive oil is mentioned for the first time during Dynasty XVIII

(1570–1345 BC). Later, during the reign of Ramses II (1197–1165 BC), olive tree cultivation was practiced in Egypt; and, according to an inscription found in the temple of the god Ra in Heliopolis, the olive trees around the city produced the best-quality olive oil, used for the lighting of the palace. These olive trees, which were cultivated in the Nile Valley, are believed to have derived or originated from Syria (Blázquez, 1996), with whom the Egyptians traded.

During the era of Pharaohs Tuthmosis III and Akhenaten, and maybe even later during the era of Ramses II, there was important documented diplomacy and commerce between Egypt and Mycenae. During that period, there were at least three diplomatic delegations between Mycenaeans and Egyptians (Kelder, 2009) where olives and olive oil from Argolis were offered to the pharaohs.

On a sculptured stone discovered in the temple of the god Aten in Amarna, the new capital founded by Akhenaten the Reformer, the pharaoh is presented holding an olive tree branch with olives (Hadjisavvas, 2008). Additionally, a mural was discovered in Amarna depicting an olive tree and an olive leaf wreath (Kelder, 2009). Judging by the size and the shape of the leaves of the tree in the mural, it seems that the depicted olive tree is tamed and not derived from a seed. This fact confirms the existence of cultivated olive trees during that time.

The existence of diplomatic relations between the Mycenaeans and the Egyptians along with the appearance of the olive tree in Egypt not only show the pharaohs' interest in the olive tree and its oil, but also provide evidence of the direct or indirect involvement of the Mycenaeans in introducing cultivation of the olive tree on the banks of Nile. Therefore, it seems that even if the Mycenaeans did not directly offer young olive trees to the Egyptians, they at least helped them with their attempts to cultivate the trees (Kelder, 2009).

The presence of the olive tree in northern Africa dates back to the 12th millennium BC (Camps-Fabrer, 1996), but according to Pliny its cultivation was unknown in that part of the world until the sixth century BC. The same thing is confirmed by Diodorus of Sicily about the late fifth century BC, saying that at that time oil was imported in northern Africa from the Greek city of Akragantas and that, by the end of the fourth century BC, northern Africa was full of olive groves (Blázquez, 1996).

The Roman contribution to the expansion of olive tree cultivation to northern Africa was very determining. They made olive tree spreading easier by applying two very important arboricultural techniques: grafting, which made the wild olive trees productive, and transplantation. There is evidence of the presence of the olive tree in the Negev Desert dating back to 4200 BC, and clear indications about olive oil production on Mount Carmel from the sixth millennium BC. Olive tree cultivation in the area of today's Israel is believed to have started much later (around 3500 BC), but it was notably widespread in 1000 BC (Eitam, 1996). This fact is confirmed by archaeological findings at Tel Miqne Ekron, where one of the biggest olive oil processing units was discovered. According to Eitam (1996), there are specific reports about exports of important quantities of olive oil from Canaan to Egypt during the 15th century BC.

Archaeological findings of the olive tree have been found in all Mediterranean countries, with the oldest of them being in the eastern part and dating from the Late Paleolithic era to historical times. More specifically, the most ancient fragments of wild olive kernels, estimated to be approximately 19,000 years old, have been found on Ohalo II, a location near the modern city of Tiberias (Kislev *et al.*, 1992; Sarpaki, 2003).

In the broader Greek area, Aegean and mainland, the existence of the olive tree is confirmed to date back to 50,000 years ago. Some of the oldest Paleolithic findings of olive tree wood of the eastern Mediterranean have been found in Cave Kleisoura, eastern Peloponnese (Carrión *et al.*, 2010). However, the most characteristic findings are the fossilized olive leaves, 35,000 to 50,000 years old, which were discovered in volcanic ash on the Greek islands of Nisyros and Santorini (Thyra) (Figure 1.2).

Cyprus, Crete, and the islands of the Aegean Sea were the first areas in Greece where the olive tree was cultivated. Nevertheless, its intensive cultivation in these areas seems to have begun only toward the end of the Late Bronze Age (1600–1100 BC). At that time, copper and olive oil were possibly the two most important commodities (Hadjisavvas, 2008). Consequently, olives and olive oil were an important source of wealth and power during the Minoan and Mycenaean times. The economies of both civilizations largely depended on the production and trade of olive oil. It would not be an exaggeration if we characterized olive oil as the petroleum (fuel) of that time (Zerefos, 2013). Large depositories of olive oil have been discovered in Knossos, Pylos, and other parts of Crete and Peloponnese, respectively. Greeks at that time considered the quality of olive oil to be of great importance and were the first ones to identify and dissociate the wild from the tamed olive trees and their corresponding oils. The olive oils in the Mycenaean palaces were produced not only from domesticated trees but mostly from wild ones in a ratio of 2:7 (Kelder, 2009).

Figure 1.2 Fossilized olive leaf.

1.3.3 Domestication of the olive tree

In order to define the process of domestication and the origin of a cultivated olive tree, it would be useful first to designate some possible stages of this process. Bearing in mind the stages of domestication of other fruit trees by man, such as the fig, date, palm, and so on, we may suppose that the stages of domestication of the olive tree could be similar.

It is possible that the utilization of the wild and tamed olive tree began in the eastern Mediterranean very early, with its wood being used as fuel and construction material, and its leaves as animal feed. Additionally, the utilization of olive trees might have been generalized around 6000 BC with the development of olive oil production techniques from wild olive trees for medicinal and cosmetic use or as lamp fuel. Nevertheless, at that time the fruit could not be consumed in its natural form (Hadjisavvas, 2008).

It is possible that the reason for the first stage of human domestication of fruitful trees is that people had as a principal criterion the direct consumption of the ripe fruit straight from the trees – that is, consumption without any processing. If this is the case, the same criterion was true for the olive trees as well. Selection of olive trees based on the oil content of their fruit probably came later. Even much later, people probably thought of looking for ways of processing bitter olive fruit and producing olive oil from "tamed" olive trees.

1.4 A different approach

The theory of olive tree domestication, based on the fact that the starting point was the ability of some trees to produce edible fruit that needed no processing, can be supported not only by archaeological findings but also from the fact that a pre-Minoan multi-trunk olive tree was discovered on the island of Naxos, Greece. This tree is possibly the oldest olive tree in the world (Figure 1.3). It consists of several trunks covering a much larger trunk whose diameter is over 10 meters (Kostelenos, 2011, 2015). The age of this tree, judging by its diameter (ACOM, 2002), is estimated to be 4500–5000 years, and it belongs to the cultivar Throumbolia Aegaiou (Kostelenos, 2011, 2015), along with many other olive trees of different ages existing in the same location.

'Throumbolia Aegaiou' is a Greek olive tree cultivar found even nowadays in all of central and southern insular Greece, Attica, Euboea, Lesbos, Chios, Samos, the Dodecanese, Cythera, and Crete. It is the only known cultivar that under normal field conditions produces fruit that can be consumed in its natural form straight from the tree without any processing (Anagnostopoulos, 1930). The fruit of this particular cultivar is collected and marketed even today in Greece and is known to consumers under the name of Thrumbes.

We don't know if the domestication of the olive tree happened in only 10 different locations (Breton *et al.*, 2012), or if it happened in many places and not only in the Near East (Newton *et al.*, 2014) or only in the Near East and eastern Mediterranean during the Chalcolithic era around 4000 BC (Zohary & Spiegel-Roy, 1975),

Figure 1.3 The pre-Minoan multi-trunk olive tree.

or even between 3300 and 3100 BC (Kislev, 1995). What we know, however, is that the existence of the Naxos olive tree coincides with the starting point of global olive growing. This fact proves the continuous cultivation of domesticated olive tree cultivars in Greece since pre-Minoan times up to this day, and it supports the hypothesis of the domestication and origin of the cultivated olive tree in the eastern Mediterranean.

Furthermore, the Naxos olive tree, along with all other trees of the same cultivar but different ages existing in the same area, strengthens the base of the theory of Anagnostopoulos (1951), which is that the cultivated-domesticated olive tree derives from Crete or that the olive tree was domesticated in the broader Aegean area. Patac *et al.* (1954) also agrees with this theory, whereas Camps-Fabrer (1953) seems to be reserved about it. Anagnostopoulos (1951) talked about another ancient olive tree that existed on Iera Odos Street in Athens; it was known as "Plato's olive tree" and was in its half part 'Throumbolia Aegaiou', which is the same cultivar as that of the Naxos olive tree. It should be noted that Attica is the western boundary of this cultivar in the Greek area (Kostelenos, 2011, 2015).

Apart from the paleobotanical and archaeological evidence, the existence of a large number of perennial domesticated olive trees in the Aegean, Crete, and mainland Greece supports the opinion that the center of domestication of the olive tree is the Aegean and that the Hellenes (Greeks) were involved in its evolution. Additionally, ancient Greek literature in the form of myths managed to salvage and has available information on the geographical expansion of the olive tree 4000–6000 years ago, and also on the area of its domestication. Subsequently, two well-known myths will be explored. The first deals with wild olive trees, whereas the second is about tamed ones:

1. Pindaros in *Olympia* Γ' (*Olympian 3*) mentions that Hercules, when he returned to Greece from the shady banks of Istria (Danube), brought a wild olive tree with him and planted it in Olympia. This indicates two things: first, the initial contact of the Greeks with olive trees was with wild ones and not tamed ones; and, second, in earlier periods when the climate was warmer, such as the Minoan warm period (Grootes *et al.*, 1993), the cultivation of wild olive trees extended as far north as the banks of the Danube River (i.e., farther north than it is today). This first contact with the wild olive trees, as well as their presence so far north and away from the warm Aegean Sea, was something impressive for the ancient Greeks, remaining etched in their "collective memory" and expressed through the myth of Hercules. Branches from this very first wild olive tree, planted in Olympia, were used to make the olive wreaths given to winners of the Olympic games. During the Roman times, there was a temple with a statue in honor of "Hercules olivarius" on the island of Delos (Blázquez, 1996).
2. A second myth regarding the origin of olive trees is that of the dispute between the god Poseidon and goddess Athena, about which of the two should give their name to the newly founded city of Kekrops (Athens). The myth says that the council of gods gave the victory to Athena because she offered the first

Figure 1.4 Modern Throumbolia Aegaiou kernel.

> "domesticated-cultivated olive tree" as a gift to the city. According to tradition, this tree was planted on the Acropolis, at Erechtheion Temple, and the city was named after Athena. In another interpretation of the myth, the council of gods – that is, the entirety of natural laws (ecology, climate, and natural and economic conditions) – gave the victory to Athena, who symbolizes human intelligence (Anagnostopoulos, 1951). Possibly with this myth, two other facts are testified: first, that domestication of the olive tree happened in Greece; and, second, that it was carried out by the Greeks.

Although the myth of the dispute between Poseidon and Athena could be considered as an unsubstantiated exaggeration in order to document Greece as the place of origin of the domesticated olive tree, we should not overlook the fact that other Greek myths that were considered exaggerations up to 150–200 years ago, such as Homer's reports on Troy and Mycenae, were proved real when these cities came to light after archaeological excavations. In nowhere else in the Mediterranean, or any other place where the olive tree has been cultivated for the past 4500–5000 years, can one find kernels (Figure 1.4) that have the same macroscopic characteristics as the kernels found in the seventh-century BC excavations in Andros (Megaloudi, 2006) (Figure 1.5) and the Minoan III excavations in Crete, as presented by the archaeologist Chatzi-Vallianou (2003) (Figure 1.6).

We must note at this point that Minoans were the first people in the Mediterranean who could clearly tell apart the difference between the wild and the domesticated olive trees and their respective oils 3500 to

Figure 1.5 Olive kernel found in Andros.

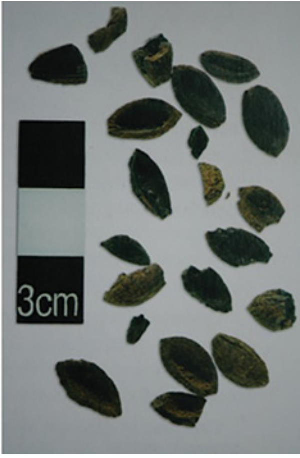

Figure 1.6 Protopalatial kernels (left) and Late Minoan III ones (right) in Crete.

4000 years ago (Vasilakis, 2003). To sum up, after carefully observing and then comparing the carbonized olive kernels found in the vicinity of Teleilat Ghassul, one may easily notice a heterogeneity in both shape and size. After doing the same with the olive kernels from Minoan Crete, however, a homogeneity in shape and size will be detected (Chatzi-Vallianou, 2003). This fact clearly suggests that the Teleilat Ghassul kernels belonged to heterogeneous plant material, possibly wild olive trees, whereas those from Minoan Crete belonged to a homogeneous plant, meaning cultivated olive trees.

It is possible that olive oils could be found throughout the eastern side of the Mediterranean, but they were produced from wild olive trees. Only in Minoan Crete, in the islands of the Aegean Sea, and in the Mycenaean mainland does it appear that domesticated olive trees produced edible fruit (Vasilakis, 2003) and, furthermore, tamed "sweet" olive oils not produced in other parts of the Mediterranean. This is possibly the most important reason why the Minoan and Mycenaean olives, edible olive oils, and cosmetic olive oils were in great demand in Egypt and elsewhere.

With the gradual expansion and increase in the number of domesticated olive trees, first in the eastern Mediterranean, then throughout the Mediterranean, and eventually in the rest of the world, the olive oils from wild olive trees were progressively replaced with oils from domesticated olive tree cultivars. Nowadays, however, some people are interested again in olive oils from wild olive fruit because of their high composition in phenolic compounds, and these are sold at much higher prices. The hypothesis of the olive tree's domestication in Greece is supported by the large number of cultivars mentioned in the ancient Greek literature, as well as the great variability among them. Tavanti (1819) reports five Jewish, three Egyptian, and 15 ancient Greek names of olive cultivars based on reports of the ancient Greek, Latin, and Jewish literature. Besides this, Lychnos (1948) refers to 16 ancient Greek olive tree cultivars (Table 1.1).

We must take into consideration the fact that in Greece as well as in other olive-growing countries, only less than half of the existing cultivars were recorded up to 100 years ago. As a result, the number of olive tree cultivars mentioned in the ancient Greek literature must have been only a small fraction of those that really existed, and proportionally the domesticated olive tree cultivars must have numbered at least 30.

Greece and the broader Aegean area have always been rich in olive tree cultivars and constitute a large "reservoir" of olive cultivars that has supplied many other parts of the Mediterranean in the past. Ramon Blanco (1927) reports that 'Arbequina', a Spanish olive tree cultivar, was introduced to Spain from Greece along with other olive cultivars by Duke Medinaceli, a Spaniard, around 500 years ago. Moreover, Rados Antonio Michieli Vitturi (Michieli Vitturi, 1788), while referring to the introduction and cultivation of the olive tree in Dalmatia, mentions that among the olive cultivars found in that area, several had been introduced from the "islands of the Archipelagos," that is, the islands of the Aegean Sea.

The broader Greek area and the Aegean Sea have not stopped producing up to this day new "original" olive cultivars. A characteristic example is the white fruit 'Asprolia Alexandroupolis' (*aspro* in Greek means

Table 1.1 The ancient Greek olive tree cultivars.

	Cultivar	Comments
1	'Kallistefanos'	The wild olive tree cultivar that existed in ancient Olympia and was used for making the wreaths for the Olympic Games
2	'Kotinos' or 'Fylia'	The wild olive tree that was also called Elaios, Agrifos, Agrippos, Agrielaios, Rahos Streptos, or Eiresioni
3	'Favlia' or 'Favlios'	A cultivar whose fruit turned white during ripeness
4	'Ehinos'	A cultivar whose leaves had a large curved thorn on the top
5	'Stemfylitis'	A cultivar whose olives were smashed and kept in brine
6	'Moria'	The first tamed olive tree cultivar found within the inner sanctum of the Acropolis temple
7	'Dryepis' or 'Ryssi'	A cultivar whose olives ripened on the trees or resembled acorns
8	'Rafanis'	A cultivar whose fruit looked like radish
9	'Nitris'	A cultivar whose olives were processed with salt
10	'Kolymvas', 'Niktris', or 'Vomvia'	Its olives were preserved fully in brine or olive oil
11	'Almas' or 'Hypoparthenos'	A cultivar whose fruit was prepared salted or preserved in brine
12	'Orhas' or 'Orhemon'	Olives in the shape of testicles
13	'Gergerimos' or 'Ishas'	The olives of this cultivar were left to drop from the trees.
14	'Goggylis'	A large fruited cultivar, similar to Almada, whose olives resembled dates
15	'Trampellos'	–
16	'Ishas'	Its olives resembled kidneys.

"white"), a cultivar from Thrace, Greece (Kostelenos, 2003), which is the only known white fruit cultivar in the world capable of producing large and potentially marketable olives (Figure 1.7). It has changed from what could be considered as a small fruited "wild" tree to a domesticated large fruited tree with olives that can no longer be considered wild, due to their size.

1.5 Conclusion

Eastern mainland Greece, and above all the Aegean Islands and Crete, could be considered at least as the primordial place of domestication and evolution of the olive tree and the development of olive growing as well. From this region, the cultivated olive tree spread throughout the Mediterranean and from there on to

Figure 1.7 Olive from the Asprolia Alexandroupolis cultivar.

the rest of the world. This is probably the reason why the olive tree is regarded as the tree of the Greeks (Bartolini & Petrucelli, 2002).

References

Anagnostopoulos, P. (1930) Cause of converting to natural tins the kind of "Thrubes" of the ripe oils. Athens Academy Records, 290–294 (in Greek).

Anagnostopoulos, P. (1951) The origin of the olive tree. Athens Academy Records, 224–237 (in Greek).

Association of Cretan Olive Municipalities (ACOM) (2002) The olive tree and olive oil in Crete: proceedings of the international symposium, Sitia, Crete, May 23–25, edited by N. Michelakis. ACOM, Rethimnon, Greece (in Greek).

Bartolini, G., & Petrucelli, R. (2002) Classification, origin, diffusion and history of the olive. FAO, Rome.

Besnard, G., Baradat, P., Breton, C., Khadari, B., & Bervillé, A. (2001) Olive domestication from structure of oleasters and cultivars using nuclear RAPDs and mitochondrial RFLPs. Genetics Selection Evolution 2001, 119.

Blanco, R. (1927) Estudio biometrico de la olive Arbequina. Gráficos Academia Mariana, Lérida, Spain.

Blázquez, M.J. (1996) Origine e diffusion della coltivazione. In: Enciclopedia Mondiale dell'Olivo (Italian language edition). COI, Barcelona, 19–21.

Breton, M.C., Warnock, P., & Bernillé, J.A. (2012) Origin and history of the olive. In: Olive germplasm, ed. Muzzalupo Innocento. TECH, Rijeka, Croatia, 3–22.

Camps-Fabrer, H. (1953) L'Olivier et l'huile dans l'Afrique romaine. Gouvernement General de l'Algerie, Algiers.

Camps-Fabrer, H. (1996) La coltura dell'olivo nell'Africa del Nord. In: Enciclopedia Mondiale dell'Olivo (Italian language edition). COI, Barcelona, 30–33.

Carrión, Y., Ntinou, M., & Batal, E. (2010) *Olea europaea* L. in the North Mediterranean basin during pleniglacial and the Early-Middle Holocene. Quarternary Science Reviews 20, 952–968.

Chatzi-Vallianou, D. (2003) Olive cultivation and olive oil production in Ancient Crete. In: The olive tree and olive oil in Crete: proceedings of the international symposium, Sitia, Crete, May 23–25, edited by N. Michelakis. Association of Cretan Olive Municipalities (ACOM), Rethimnon, Greece, 86–104 (in Greek).

Eitam, D. (1996) La coltivazione dell'olivo nell'antico Israel. In: Enciclopedia Mondiale dell'Olivo, Italian language ed. COI, Barcelona, 36–41.

Grootes, P.M., Stuiver, M., White, J.W.C., Johnsen, S., & Jouzel, J. (1993) Comparison of oxygen isotope from the GISP2 and GRIP Greenland ice core. Nature 366, 552–554.

Hadjisavvas, S. (2008) The olive tree and olive oil in the ancient Greek world. PIOP, Athens (in Greek).

Kelder, M.J. (2009) Royal gift exchange between Mycenae and Egypt: olives as "greeting gifts" in the Late Bronze Age Eastern Mediterranean. American Journal of Archaeology 113, no. 3, 339–352.

Kiritsakis, A. (1998) Olive oil: from the tree to the table, 2nd ed. Food and Nutrition Press, Trumbull, CT, 1–18.

Kislev, E.M. (1995) Wild olive stones at submerged Chalcolithic Kfar Samir, Haifa, Israel. Journal of the Israel Prehistoric Society 26, 134–145.

Kislev, E.M., Nabel, D., & Carmi, I. (1992) Epipalaeolithic (19.000 BP) cereal and fruit diet at Ohalo II, Sea of Galilee, Israel. Review of Palaeobotany and Palynology 73, 161–166.

Kostelenos, G. (2003) Native olive tree varieties of northern Greece. In: Olive oil production. Euripos Publishing, Athens, 22–29 (in Greek).

Kostelenos, G. (2011) Elements of olive cultivation: history, description and geographical allocation of the olive tree varieties in Greece. n.p., Galatas Trizinias, Greece (in Greek).

Lombardo, N. (2007) Aspetti generali dell'olivicoltura. In: Olea Trattato di olivicoltura. Edagricole, Bologna, 3–11.

Lychnos, N. (1948) The olive tree and its cultivation, vol. 1. Ministry of Agriculture, Athens (in Greek).

Megaloudi, F. (2006) Plants and diet in Greece from Neolithic to Classic periods: the archaeobotanical remains. BAR International Series 1516. Archaeopress, Oxford.

Michieli Vitturi, R.A. (1788) Sull'introduzione degli ulivi nei territorj mediterranei della Dalmazia e sulla loro coltivazione. In: Memorie della pubblica societa economica di Spalato. Stamperia Coleti, Vinegia.

Newton, C., Lorre, C., Sauvage, C., Ivorra, S., & Terral, JF. 2014. On the origins and spread of *Olea europaea* L. (olive) domestication: evidence for shape variation of olive stones at Ugarit, Late Bronze age, Syria: a window on the Mediterranean Basin and on the westward diffusion of olive varieties. Vegetation History and Archaeobotany 23, 567–575.

Patac, L., Cadahia, P., & Del Campo, E. (1954) Tratado de olivicoltura. Sindacato nacional del olivo, Madrid.
Psyllakis, N., Psyllaki, M. & Kastanas, I. (2003) The olive oil: the culture of olive tree. Edit Karmanor, Heraclion, Greece, 61 (in Greek).
Rodríguez, R.J. (1996) Economia oleicola dell'antichitá. In: Enciclopedia Mondiale dell'Olivo (Italian language ed.). COI, Barcelona, 47–49.
Sarpaki, A. (2003) Tracing the presence of the olive tree in prehistoric Crete. In: The olive tree and olive oil in Crete: proceedings of the international symposium, Sitia, Crete, May 23–25, edited by N. Michelakis. Association of Cretan Olive Municipalities (ACOM), Rethimnon, Greece, 44–52 (in Greek).
Simantirakis, V., & Lykoudi, M. (2001) The olive "Kalistefanos." Edit Epfesus Publishing, Greece, 9.
Tavanti, G. (1819) Trattato teorico – pratico completo sull'olivo, vol. 1 Stamperia Piatti, Florence (in Italian).
Vasilakis, A. (2003) Olive tree cultivation and olive oil production in Minoan Crete: Testimony of the Monuments. In: The olive tree and olive oil in Crete: proceedings of the international symposium, Sitia, Crete, May 23–25, edited by N. Michelakis. Association of Cretan Olive Municipalities (ACOM), Rethimnon, Greece, 53–65 (in Greek).
Zerefos, C. (2013) The Mediterranean is the most vulnerable area on the planet. Real Newspaper, Realplanet, 7, August 25 (in Greek).
Zohary, D., Hopf, M., & Weiss, E. 2012. Domestication of plants in the old world, 4th ed. Oxford University Press, New York.
Zohary, D., & Spiegel-Roy, P. (1975) Beginnings of fruit growing in the old world. Science 187, 319–327.

2 Botanical characteristics of olive trees: cultivation and growth conditions – defense mechanisms to various stressors and effects on olive growth and functional compounds

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2.1 Introduction

In the Mediterranean basin, olive trees (*Olea europaea* L.) have been a typical example of cultivation. They spread on sloping arid areas and rain-fed conditions, where other fruit trees could not grow (Loumou & Giourga, 2003). This type of cultivation protects land from erosion, and adds income to small farmers offering work on a seasonal basis. Nowadays, there has been a continuously increasing interest in olive cultivation. Growing demand for olive products, olive oil and olives, is ascribed to awareness of consumers for products of high nutritional value and health benefits (Visioli *et al.*, 2002; Kiritsakis, 2007). In the Mediterranean Basin, olive remains a very significant species environmentally and socioeconomically (Conde *et al.*, 2008). During recent decades, big progress has been made in olive cultivation in three main European Union (EU) olive-producing countries, Spain, Italy, and Greece.

Nowadays, olive cultivation is spread worldwide, introducing conventional integrated systems with irrigation and fertilization regimes. Since the 1980s, new orchards have been created using high-density (HD) planting systems, ranging between 250 and 400 trees/ha, instead of the traditional system ranging between 100 and 300 trees/ha (Connor *et al.*, 2014). During the last two decades, a more intensive planting system, the super-high-density (SHD) system consisting of 1500 to 2200 trees/ha, has been developed mainly in Australia, Argentina, California, Chile, Morocco, Portugal, Spain, and Tunisia (Tous *et al.*, 2010). Additionally, new machinery designed for pruning and harvest of olive trees has been introduced to balance the high yields at a short harvest time with the high manual harvest costs, and to maintain a high-quality product (Connor *et al.*, 2014). In HD systems, mobile trunk shakers with umbrella catch frames have been used for harvesting individual trees, and side-by-side trunk shakers are used for harvesting vigorous trees, whereas small straddle harvesters of grape type have been used in SHD systems (Connor *et al.*, 2014). However, in older olive orchards, there are issues related to harvesting, such as tree dimensions and pruning requirements, cultivar vigor, and fruiting habits, which are still being studied (Tombesi *et al.*, 2014).

Among other cultivars, 'Arbequina', 'Arbosana', and 'Koroneiki' are most suitable for SHD systems (Godini *et al.*, 2011). These three cultivars exhibit good attributes, such as semi-dwarf habit and early bearing of tree, as well as successful control of mechanical and manual pruning. They also exhibit fruit resistance to impact bruising, and give oil of high quality (Godini *et al.*, 2011). In Argentina, the cultivars 'Arbequina', 'Arauco', 'Barnea', Coratina', 'Frantoio', 'Hojiblanca', 'Manzanilla de Sevilla' and 'Picual' have been used in HD systems (Gómez del Campo *et al.*, 2010).

However, more studies are needed for HD and especially SHD planting systems. Microenvironmental conditions (humidity, aeration, and solar radiation), cultivar sensitivity to diseases, and the growth of tree

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branches and canopy in connection with harvesting machines are some areas to be examined in the long term (Castro-Garcia *et al.*, 2012; Connor *et al.*, 2014). Breeding olive genotypes that need lower levels of fruit removal force (FRF) per fruit weight could be another approach for effective mechanical harvesting (León *et al.*, 2006).

Fruit bruising during mechanical harvesting is a major limiting factor, especially in harvesting of table olives. Bruising may create fruit darkening, even within one hour, due to cell rupture and oxidation of phenolic compounds (Segovia-Bravo *et al.*, 2011). Olives are usually harvested at an immature stage that requires high FRF for detachment from the tree. Therefore, immature fruit are more susceptible to bruising and darkening. Immediately after harvest and during transport to factories, treatments like immersion of olives in antioxidant solutions (Segovia-Bravo *et al.*, 2011), or in glycerol and then in nitrogen atmosphere, reduced olive darkening (Sánchez *et al.*, 2013). An additional approach to ameliorate the bruising problem is selection and use of cultivars resistant to bruising. Among three cultivars studied by Jiménez-Jiménez *et al.* (2013), 'Manzanilla' was more susceptible to bruising than 'Hojiblanca' and 'Gordal Sevillana'. Ferguson and Castro-García (2014) have recently achieved adapting 'Manzanillo' table olive trees for mechanical pruning and harvesting at cost and efficiency competitive to those for manual harvesting.

Nevertheless, besides any trend and advantages of new dense olive cultivation and mechanical harvesting, traditional olive farming remains of great importance in sloping areas with water shortage and non-fertile soil. Therefore, techniques facilitating traditional cultivation have to be developed in parallel with those of HD and SHD orchards.

2.1.1 Classification – taxonomic hierarchy

The olive tree belongs to the family Oleaceae, which has 30 genera and 180 species. The species *Olea europaea* originates from the eastern Mediterranean, where it has been cultivated continuously and has been expanded to become the predominant one within olive groves worldwide. Many scientists consider that *Olea europaea* is not a true species, but one group of species with a genotype of 2x = 46 chromosomes, derived from hybridism and mutation of genotypes from tropical and subtropical Afro-Asian species, such as *Olea chrysophilla* and *Olea excelsa*. Along the historical lifespan of the olive tree, the above genotypes were crossed under various climatic conditions to constitute nowadays the most cultivated species, *Olea europaea*. This emblematic crop of the Mediterranean Basin has conserved a very wide germplasm, estimated in many groups, and more than 2600 cultivars (Therios, 2009). Recent studies evidenced the genotypic variation and diversity within plants of the same *Olea* species (Angiolillo *et al.*, 1999; Baldoni *et al.*, 2002).

In the Mediterranean region, the main olive subspecies are the native *Olea europaea Oleaster* (wild olive) and the cultivated *Olea europaea Sativa* (Breton *et al.*, 2006). Over recent decades, due to the economic and nutritional importance of olive crops, particular attention was paid to research of DNA markers and their application, and important results in genome analysis were reported. The available data from analysis of genetic variability in complex of *Olea europaea* are related to application of molecular markers to develop efficient tools for traceability of olive oil origin. Recently the ongoing genomic research made efforts to identify sequences, in particular those expressed during fruit development and in pollen allergens. The sequencing of the chloroplast genome opened the olive genomic era, providing new information on olive nucleotide sequence (Bracci *et al.*, 2011). Classical taxonomy of the olive plant by rank, scientific name, and common name is listed here (USDA NRCS, 2015):

Kingdom: Plantae – plants
Subkingdom: Tracheobionta – vascular plants
Superdivision: Spermatophyta – seed plants
Division: Magnoliophyta – flowering plants
Class: Magnoliopsida – dicotyledons
Subclass: Asteridae
Order: Scrophulariales
Family: Oleaceae – olive family
Genus: *Olea* L. – olive
Species: *Olea europaea* L. – olive
Subspecies: *Olea europaea* L. ssp. *europaea* – European olive

2.2 Botanical characteristics

Olive is a densely grown evergreen tree with silver-green leaves, thin branches, and small, white, fragrant flowers, producing a lot of pollen. It is a long-lived tree reaching an age of more than 1000 years, and an average height of 15–20 m. Under pruning agricultural practices, height is limited to 4–5 m (Martin, 1994).

2.2.1 Anatomy – morphology

The main anatomic parts of the olive tree are the root system, trunk, main branches, leaves, buds, inflorescences and flowers, and fruit. These parts perform important physiological and growth functions of the olive tree and are described in detail in this section.

2.2.1.1 Root system

During their first 3–4 years, olive trees (derived either from seedlings or from asexual propagation) develop a vertical root system, which is gradually replaced by a side-root system, limited at a soil depth of 1 m. It lacks a dominant taproot system and the thick roots grow in the top 20 cm, while the main proportion of roots grow at a soil depth of 60–70 cm (Therios, 2009). Rootstocks originating from seedlings (not rooted cuttings) with vigorous scions develop a deep and branched root system. In shallow heavy soils, with claypan and hardpan layer, the roots grow close to the soil surface. In light-textured and/or sandy soils, the root system is extensive, trying to reach into moist soil. In dry climates, the side growth of the roots can extend up to 12 m away from the trunk and 6 m in depth, covering an area seven to eight times greater than the leaf area (Fernández *et al.*, 1990). Roots are derived from carbon fixation of the spheroblasts existing in the ovoid hypertrophic tissue, lying between the trunk and the root crown (xylopode) of the olive tree. This form of root system is more efficient in water absorption than a deep-root system (Therios, 2005). The root system of olive trees is commonly in symbiosis with endotrophic mychorizzae, especially in poor soils (Mancuso & Rinaldelli, 1996).

Storage of carbon and nitrogen nutrients: under normal conditions, carbon in the form of starch and soluble carbohydrates, and nitrogen in the form of amino acids and proteins, are stored in the root system to induce root growth, bud flowering, and shoot growth. Root growth is negatively affected by severe pruning and certain soil conditions, such as accumulation of (a) fungi, bacteria, and nematodes; (b) excess soil water; and (c) excess chlorine ($>$0.5% Cl) and sodium ($>$0.2%) salts (Therios, 2009).

2.2.1.2 Above-the-ground parts (trunk, main branches, leaves, buds, inflorescences and flowers, and fruit)

Trunk: The olive trunk is cylindrical, with yellowish to dark wood, and an uneven and partially swollen surface.

Main branches: In traditional olive groves, the main branches start at a height of 1.2 m, while in modern, dense ones they start at a height of 20–40 cm from the bottom of the trunk. The main branches give secondary and tertiary branches (shoots), bearing leaves, flowers, and fruit. According to Martin (1994) and Therios (2009), shoots are classified into four categories:

Vegetative shoots bearing only buds to produce new shoots and leaves;
Flowering shoots bearing buds to produce flowers and fruit;
Mixed shoots bearing vegetative and flowering buds; and
Vigorous water sprouts growing vertically, which should be removed.

Leaves: The leaves are silver-green, feather-shaped, covered by a layer of wax and cutin (cuticle). Their stomata are almost lying in the lower surface, nestled in trichomes; they protect leaves from water loss and UV radiation, and increase by threefold the efficient leaf surface (Fernández *et al.*, 2012). During the

Figure 2.1 Growth phases of an olive tree flower from blooming until petal drop.

transition period from juvenile to adult leaves, chemical, anatomical, and compositional changes occur, affecting the optical properties of leaves (García *et al.*, 2000).

Buds: They are classified as either vegetative or flowering, producing shoots and leaves or flowers and fruit, respectively. Vegetative buds are small and conical, while flowering ones are bigger and spherical. Latent buds grow in cases of severe pruning or frost damage.

Flowers and inflorescences: Olive inflorescence is a panicle, which originates from flowering buds in the axil of each leaf and contains 15–30 flowers. The number of flowers per inflorescence varies with cultivar, winter chilling, soil moisture during its development, and the N content of olive tree leaves. Small, creamy-white olive flowers are protected by thick leaves. Olive flowers are distinguished by two types, the hermaphrodite (perfect) having both male and female organs, and the staminate (imperfect) having only stamens and a rudimentary pistil. Imperfect flowers do not set fruit. Figure 2.1 shows the growth phases of an olive tree flower until petal drop.

Fruit: Olive fruit is a spherical or elliptic drupe of small, medium, or large size, and it can be separated into three distinct anatomical parts. (a) The epicarp (skin) accounts for 1–3% of drupe weight. (b) The mesocarp (pulp or flesh) is the edible portion and accounts for 70–80% of the whole fruit. Water content of the mesocarp is 70–75%, and olive oil ranges between 14 and 15% in green table olives, and up to 30% in black mature olives. (c) The endocarp (stone or pit) represents 10–27% of the olive by weight and contains the seed (Farinelli *et al.*, 2002). Each seed consists of the seed coat and the endosperm, containing an embryo, two cotyledons, a radicle, and a plumule. The size and shape of olive fruit vary with cultivar, fruit load, soil fertility, soil water, and farming practices. Cultivars with large fruit size are used for table olive processing, while those with small fruit size for olive oil processing. Ripe fruit is mainly purplish black, and in some cultivars greenish or coppery brown.

2.2.2 Flowering, pollination, and fruit set

2.2.2.1 Flowering

The annual cycle of olive flower phenology includes bud formation during the previous summer, dormancy during the cold period, bud burst in late winter, and flower structure development starting with bud burst and ending with flowering in spring (Porlingis & Dogras, 1969). Within 14 days after full bloom, most olive flowers drop, and optimum yield can be achieved when only 1 or 2% of these flowers remain to develop fruit. Temperature and photoperiod (light) exert the strongest effects on the olive flowering process. Olive flowering induction requires a period of low temperatures (winter chilling 0 to −7 °C) and a photoperiod of sunlight, affecting the photosynthesis rate (Palliotti & Bongi, 1996). Other factors affecting the flowering process are the cultivar; duration of juvenility; growth regulator concentrations in leaves, nodes, and fruit; oleuropein levels; fruit thinning; date of harvesting; and nutrient and water stress (Ülger *et al.*, 2004; Malik & Bradford, 2006).

2.2.2.2 Pollination

Pollen grains are formed in the stamens of olive flowers. The shape and form of the olive flower structure create air flow patterns that release and direct pollen to the stigma surface. Olive flowers are mainly wind pollinated. Fertilization capacity increases with the presence of well-developed embryo sacs in the ovules, and the ovule longevity. Only one developed ovule is enough for seed formation. Out of the four ovules contained in an embryo sac, only one is fertilized and the other three degenerate and shrink. Embryo sac degeneration and ovule viability are affected by the cultivar, water stress, heat stress, and the nitrogen concentration of olive leaves (<1.3%) (Martins *et al.*, 2006). Pollination in olives takes place during the full blooming stage (5–6 days). The selection of the most appropriate pollinators is very important, as the flowering period, the amount of pollen, and its germination capacity vary between cultivars and range from low levels to 40–50%. They are also affected by various factors such as temperature, relative humidity, wind, and rain. Extreme climatic conditions (very low or high temperatures, dry and hot winds, and rain during the period of blooming) reduce the pollen germination and the pollen tube growth (Galán *et al.*, 2001). The imperfect or staminate flowers play a vital role for olive tree pollination, by enhancing pollen donation to fertilize other flowers, and by attracting the pollinators to offer enough pollen to olive flowers (Cuevas & Polito, 2004). According to Polito (2011), the *effective pollination period* (EPP) includes three temperature-dependent factors:

Stigma receptivity: ability of stigma to support pollen germination
Pollen tube growth rate: time required for pollen tubes to grow through style to ovule
Ovule viability: time that ovule is capable of being fertilized.

According to Farinelli *et al.* (2006) as well as Guerin and Sedgley (2007), there is a compatibility relationship between some main cultivars and their appropriate pollinators:

Main cultivar	*Compatible pollinator(s)*
‘Arbequina’	‘Carolea’ and ‘Kalamon’
‘Barnea’	‘Kalamon’ and ‘Mission’
‘Frantoio’	‘Coratina’, ‘Kalamata’, and ‘Mission’
‘Kalamon’	‘Arbequina, ‘Barnea’, ‘Ascolana semitenera’, ‘Carolea’, ‘Frantoio’, ‘Giarraffa’, ‘Koroneiki’, ‘Leccino’, ‘Maurino’, and ‘Manzanillo’
‘Koroneiki’	‘Mission’ and ‘Hojiblanca’
‘Mission’	‘Koroneiki’ and ‘Arbequina’
‘Manzanillo’	‘Gordal’, ‘Leccino’, ‘Maurino’, ‘Nocellara Etnea’, ‘Picholine’, and ‘Santa Caterina’

2.2.2.3 Fruit set

Fertilization triggers the fruit set. After fertilization, petals and stamens are abscised and the ovary increases up to 50-fold by cell division, expansion, and/or differentiation to produce olive fruit. The most favorable

temperature for higher fruit set is 25 °C (Rapoport *et al.*, 2004; Polito, 2011). According to Cuevas *et al.* (1995), inadequate fertilization and fruit drop are caused by (a) incompatible pollinating cultivars, (b) lack of pollinator trees, (c) pest and disease problems, (d) strong winds, (e) water and nutrient stress, and (f) unfavorable climatic conditions. The determining factors for regular fruit set are (a) inflorescence load and distribution, (b) size and number of florets per inflorescence, (c) percentage of hermaphrodite flowers, (d) ovarian size, and (e) physiological condition of ovules and their longevity (Lavee *et al.*, 1999).

2.3 Cultivation and growth conditions

2.3.1 Climatic conditions

Olive is a perennial subtropical tree cultivated mostly in the temperate and subtropical zones between 30 and 45° north and south latitudes, under various climatic and soil conditions. Olive trees need mild winters (4 °C minimum) and warm and dry summers (40 °C maximum) with a mean annual temperature of 15–20 °C. Spring frosts are not determining factors in olive culture, due to late blooming of olives (Palliotti & Bongi, 1996). Frost before harvesting, however, can cause shrinkage of olive fruit and even necrosis of the whole tree. Risk of frost damage can be reduced by certain farming practices, such as (a) reduction of irrigation and N fertilization, (b) late pruning, and (c) use of cold-hardy cultivars. Calcium and copper-containing chemicals sprayed on olive trees increase their cold tolerance. Cold acclimation of olive trees is related to the cryoprotective protein osmotin (D'Angeli & Altamura, 2007). Some of the most frost-resistant cultivars are 'Cornicabra', 'Arbequina' and 'Picual', 'Mission', 'Leccino', 'Carolea', 'Chemlali', 'Moraiolo', and 'Picholine' (Barranco *et al.*, 2005). Although olive trees can tolerate wind, areas affected by strong winds should be avoided for olive cultivation. Cold, moist, and hot winds during spring can cause blossom drop and growth reduction, while high temperatures and hot winds during summer can instigate fruit drop (Denney *et al.*, 1985). In the Mediterranean countries, altitudes greater than 800 m are not appropriate for olive cultivation, due to frost risk and shorter vegetative periods. In tropical regions, olive is not cultivated due to the lack of low temperatures (0–7 °C) required for flowering and bud differentiation. Some olive cultivars could produce fruit even under tropical conditions, if they are cultivated at adequate altitude to meet needs for low temperatures, under supplemental pollination. Hail and high relative humidity are responsible for disease problems, fruit damage, and olive oil quality defects, such as reduction of functional compounds of olive oil (Ayerza & Sibbett, 2001; Ayerza & Coates, 2004).

2.3.2 Soil conditions

Olive trees are low demanding and can grow well even in poor, dry, calcareous, and stony soils. They are very tolerant to water stress, especially when their root system is dense. Clay soils with high moisture content, which immobilize K and P, are not suitable for olives. The best fitting soils for annual bearing of olive trees are deep, sandy-loam soils adequately supported with N, P, K, and water (Therios, 2009). Also, olive trees grow and produce better in soils with sodium chloride (NaCl) content less than 1g/l, and medium acid and alkaline pH. Alkaline soils with pH higher than 8.5 are not suitable for olive growth. Soils with high boron content reduce toxicity problems of olive trees (Chatzissavvidis, 2002).

2.3.3 Factors affecting olive growth and composition

Under regular climatic conditions, the olive fruit growth and ripening process takes about 5 months. The olive fruit, on average, contains water (50%), protein (1.6%), oil (22%), carbohydrate (19.1%), cellulose (5.8%), inorganic substances (1.5%), and phenolic compounds (1–3%). Furthermore, some other important compounds are present in olive fruit, such as pectin, organic acids, and pigments (Kiritsakis, 1998). Olive trees produce two main products, namely table olives and olive oil, both main components of the traditional "Mediterranean diet." Worldwide consumption of olives and olive oil has increased significantly due to the rising awareness of functional foods with beneficial effects on optimal nutrition and health (Trichopoulou,

2003; Knoops *et al.*, 2004). Olive and olive oil composition and properties may alter because of genetic (cultivar), environmental, and horticultural factors. The most acknowledged factors are cultivar, nutrition (fertilization), irrigation, fruit ripening stage (maturity index), and olive oil processing techniques. Other farming practices such as pruning and weed, pest, and disease management have a direct effect on growth and productivity of olive trees, and indirectly on the content of phenolic compounds and thus on olive oil quality (Covas *et al.*, 2006; Covas, 2008).

2.3.3.1 Genetic factors

Olive cultivar, ripening stage, and harvest time have significant influences on the total amount of phenols and bitterness intensity of virgin olive oil (VOO) (Kevin *et al.*, 2003). Many studies have demonstrated that several characteristics of the olive tree and olive fruit are genetically controlled. Variations within primary olive cultivars' traits, such as oil content, cold hardiness, fruit size, polyphenol content, and pollination compatibility, are shown in Table 2.1 (Vossen, 2007).

2.3.3.2 Nutrition (fertilization)

Several studies have been conducted and published showing the influence of macronutrients on the growth of the olive tree, olive fruit, and olive oil. Macronutrients nitrogen (N), potassium (K), and other minerals taken up, as well as their accumulation in olive tree, are influenced by various factors, such as water availability, fertilizer composition, application methods, timing, soil physical and chemical properties, tree nutritional status, and environmental conditions. Therefore, the evaluation of the nutritional status of the tree is more important than the application dose rates of nutrients (Marschner, 1995; Bar-Yosef, 1999). Recent studies have been dealing with the interaction between plant nutrition and oil quality, based on factors that influence levels of N and K in the olive tree, fruit, and leaves. Olive oil quality is a complex combination of parameters that influence sensory evaluation, nutritional values, health benefits, and oil stability. Fernández-Escobar *et al.* (2009) reported that in a long-term N trial, high N fertilization decreased the polyphenol content in olive oil, and thus decreased the oil stability. Recently, Erel *et al.* (2013) studied the interaction between the main nutrients (N and K) of olive trees, and olive oil quality parameters. This study demonstrated that the N level of olive trees has a major effect on main olive oil quality components, including polyphenol content, free fatty acids (FFAs), as well as oleic and polyunsaturated fatty acids (PUFAs). The increased N level of leaves linearly decreases olive oil phenolic content, indicating a protein–phenol competition in leaves. Increased fruit N level increases olive oil PUFAs and linolenic (ω3) and linoleic (ω6) acids, but decreases oleic acid. The influence of olive tree nitrogen (N) status on olive oil components is shown in Figure 2.2 (Erel *et al.*, 2013).

High fruit load reduces nitrogen concentration of the fruit and leaf. The nitrogen content of the fruit is affected by nitrogen concentration in the irrigation solution, potassium (K) availability, and fruit load. An increased K level affects olive oil quality indirectly, as it increases nitrogen accumulation. Balanced nitrogen fertilization in olive trees cultivated for oil ensures its good quality (Erel *et al.*, 2013).

2.3.3.3 Alternate bearing in olive tree

Alternate bearing is a widespread pomology phenomenon among crop plants, and is defined as the tendency of certain fruit trees to produce a high-yield crop one year ("on-year"), followed by a low-yield or even no-crop year ("off-year") (Lavee, 2007). The olive tree is particularly known for its tendency to bear fruits in an uneven manner (alternate bearing, biennial bearing, uneven bearing, or periodicity) (Ben-Gal *et al.*, 2011). Alternate bearing is a built-in character of olive trees, controlled by a continuous and complex interaction between vegetative and reproductive development of olive buds. Severe economic problems are usually added to olive cultivation due to biannual alternate bearing in fruit production of olives. The difference in crop load between the fully productive year (on-year) and the following one with the reduced production (off-year) may range from 5 to 30 t/ha, and this phenomenon is mainly attributed to genetic factors (Lavee,

Table 2.1 Variations of the world's primary olive cultivars in traits, for comparison.

Cultivar	Oil (%)	Cold hardiness	Fruit size	Polyphenol content	Pollenizer cultivars[1]
'Aglandau'	23–27	Hardy	Medium	Medium	Self-compatible
'Arbequina'	22–27	Hardy	Small	Low	Self-compatible
'Ascolano'	15–22	Hardy	Large	Medium	'Manzanillo' and 'Mission'
'Barnea'	16–26	–	Medium	Medium	Self, 'Manzanillo', and 'Picholine '
'Barouni'[2]	13–18	Hardy	Large	Medium	'Manzanillo', 'Ascolano', and 'Mission'
'Bosana'	18–28	–	Medium	High	'Tondo de Cagliari' and 'Pizzé Carroga'
'Bouteillan'	20–25	Hardy	Medium	Medium	'Aglandau' and 'Melanger Verdale'
'Chemlali'	26–28	–	Very small	High	Self-compatible
'Coratina'	23–27	Hardy	Medium	Very high	Self, 'Cellina di Nardo', and 'Ogliarola'
'Cornicabra'	23–27	Hardy	Medium	Very high	Self-compatible
'Empeltre'	18–25	Sensitive	Medium	Medium	Self-compatible
'Frantoio'	23–26	Sensitive	Medium	Medium-high	'Pendolino', 'Moraiolo', and 'Leccino'
'Farga'	23–27	Hardy	Medium	Medium	–
'Hojiblanca'	18–26	Hardy	Large	Medium	Self-compatible
'Kalamon'	15–25	Moderate	Large	Medium	–
'Koroneiki'	24–28	Sensitive	Very small	Very high	'Mastoides'
'Leccino'	22–27	Hardy	Medium	Medium	'Frantoio', 'Pendolino', and 'Moraiolo '
'Manzanillo'[3]	15–26	Sensitive	Large	High	'Sevillano' and 'Ascolano'
'Maurino'	20–25	Hardy	Medium	High	'Lazzero' and 'Grappolo'
'Mission'[3]	19–24	Hardy	Medium	High	'Sevillano' and 'Ascolano'
'Moraiolo'	18–28	Sensitive	Small	Very high	'Pendolino' and 'Maurino'
'Pendolino'	20–25	Hardy	Medium	Medium	'Moraiolo', 'Frantoio', and 'Leccino'
'Picholine'	22–25	Moderate	Medium	High	Self and 'Aglandau'
'Picual'	24–27	Hardy	Medium	Very high	Self and 'Picudo'
'Picudo'	22–24	Hardy	Large	Low	–
'Sevillano'[2]	12–17	Hardy	Very large	Low	'Manzanillo', 'Mission', and 'Ascolano'
'Taggiasca'	22–27	Sensitive	Medium	Low	Self-compatible

*Oils with high polyphenol content have longer shelf life and are generally more bitter and pungent.
[1] Most olive cultivars are somewhat self-incompatible. They will usually set a better crop with cross-pollination, especially under adverse weather conditions. 'Leccino', 'Pendolino', 'Moraiolo', and 'Maurino' are self-sterile and require a pollen source from another cultivar.
[2] 'Barouni' and 'Sevillano' are not compatible cross-pollenizers for each other.
[3] 'Manzanillo' and 'Mission' are not compatible cross-pollenizers for each other.
Source: Vossen (2007).

2007). An off year is characterized by vigorous vegetative growth bearing buds. Under favorable conditions, a portion of buds will be induced, differentiated, and developed into floral buds, being the potential for fruit production the following year. This is the reason that pruning during the on year favors the production during the following off year. The harvest yield variation depends on factors such as genetic, physiological, and environmental conditions (Ben-Gal *et al.*, 2011). The main causal factor of alternate bearing is inhibition of olive flower bud induction by the seed of growing fruit, due to competition for nutrients.

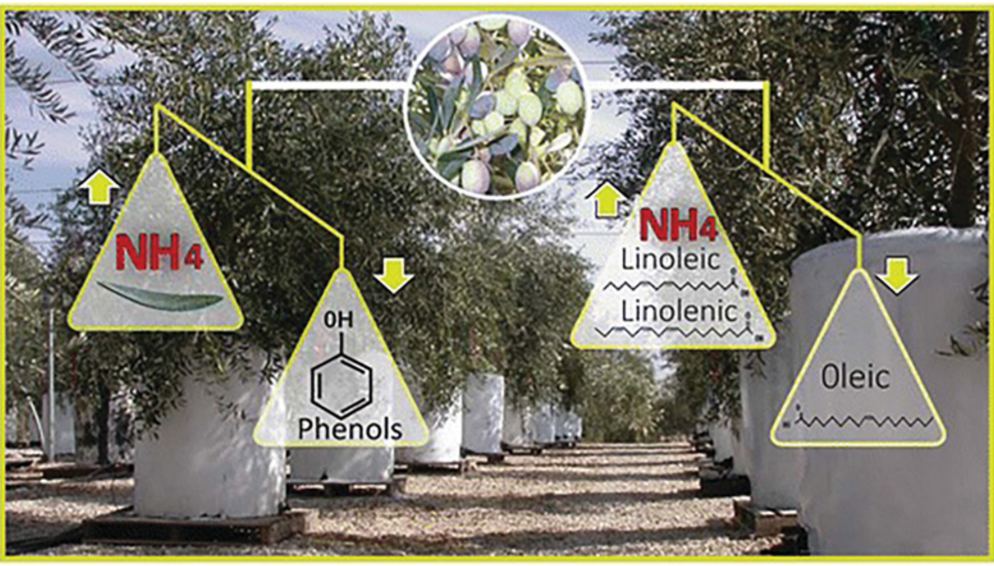

Figure 2.2 The influence of olive tree nitrogen (N) status on olive oil components. *Source*: Erel *et al.* (2013). Reproduced with permission of American Chemical Society.

Nutrient deficiency, especially of N during the period of flower bud differentiation, affects vegetative development, size of fruit, and flowering capacity in the following spring (Fernández-Escobar *et al.*, 2004). The life cycle of the olive tree undergoes transitions from a juvenile to adult stage of vegetative phase, and afterwards enters the reproductive phase, which is under tight control of a complex genetic network (Huijser & Schmid, 2011).

The discovery of control mechanisms is a crucial step to understand the molecular basis of alternate bearing tendencies. It has been found that microRNA (miRNA) gene expression regulators are involved in several physiological processes. A comprehensive study on olive miRNA related to alternate bearing has shown that regulation of miRNA under different developmental phases can control nutrition, hormone, and flowering processes, having a noteworthy impact on olive tree alternate bearing (Yanik *et al.*, 2013). Three main factors have been suggested as influencing olive alternate bearing, namely, flowering-site limitation, endogenous plant growth hormones control, and carbohydrate storage control (Goldschmidt, 2005). Endogenous control includes the involvement of growth regulators or protein content and composition, differences in polyamines concentrations, and time patterns of their variations (Pritsa & Voyatzis, 2005). Concerning phenolic compounds, no major qualitative differences were found between various tissues (new and old leaves, and fruit tissues), but distinct differences between tissues with respect to quantifiable phenols were established (Lavee, 2007; Ryan *et al.*, 2003). However, no competition for metabolites between fruit and vegetative growth was determined (Fernández-Escobar, 2004). The olive tree preserves carbohydrate reserves for its survival, under fluctuations of Mediterranean climate (Bustan *et al.*, 2011). Climate, however, has a crucial effect on increasing or decreasing alternating tendency each year. A wide range of climatic changes during the annual growth, could affect an activation of olive metabolic pathways, resulting in intensified alternate bearing. In fact, there is a continuous and complex interaction between environmental factors and both vegetative and floral buds, as has been suggested by Lavee (2007).

Various horticultural means have been applied to reduce the intensity of olive alternate bearing. Agronomical practices such as pruning, thinning, girdling, irrigation schedule, and other cultural and nutritional measures can reduce and even eliminate alternate bearing in regions with favorable and stable climatic conditions. However, under unstable and extreme environmental conditions, these horticultural strategies have been proven ineffective to control the alternate bearing phenomenon of olives (Lavee, 2007; Therios, 2009).

2.4 Defense mechanisms against various stresses

2.4.1 Development of defense mechanisms against drought

Olive trees, among the oldest species domesticated in the Mediterranean Basin, are well adapted to semi-arid regions; indeed, the basin seems to be the largest area in the world suitable for olive cultivation (Tanasijevic *et al.*, 2014). Mediterranean areas could be characterized by irregular frequency of raining during winters, soil moisture fluctuation, and dry summers with high solar radiation (Sofo *et al.*, 2008). Irrigation is an essential factor increasing the productivity of the olive tree and olive oil quality characteristics. Under irrigation and/or rain-fed conditions, variations in chemical composition and sensory qualities of VOO have been reported. Water stress has a negative effect on tree productivity and oil quality (Gomez-Rico *et al.*, 2007). It has also been observed that high irrigation (no drought stress) levels decrease phenolic content and oxidative stability of VOO (Artajo *et al.*, 2006; Berenguer *et al.*, 2006), as shown in Table 2.2.

Dag *et al.* (2014a), however, showed that deficit irrigation rather lowered not just FFAs, but also the total phenolic content (TP) and the ratio of monounsaturated fatty acids (MUFAs) to PUFAs, whereas no effect on peroxide value (PV) was observed (Table 2.2). Melgar *et al.* (2008) suggested that an irrigation system with a low water dose is not worthwhile under environments with relatively high mean annual precipitation. The above contradictory results indicate the need for detailed conditions under which the experiments are carried out. Solar radiation and warm or hot weather are closely connected with tree water needs. However, the olive tree is much more resistant to drought, irradiation, salinity, and heat than most other common crops.

There is a series of studies on the development of various physiological, morphological, and biochemical mechanisms of olive trees when exposed to various stresses and in particular to drought. Water deficit initially promotes a high water gradient between olive roots and leaves, while leaves could remain active for photosynthesis and transpiration. Under these unfavorable conditions, products of photosynthesis become available for root growth but not for canopy growth. An extending root system could better cope with water deficit and uptake of nutrients for the tree (Moreno *et al.*, 1996). Indicatively, inorganic cations in drought-stressed leaves remain at similar levels to those of irrigated ones except for calcium (Ca), which showed higher levels in stressed leaves (Sofo *et al.*, 2008). Calcium increase could be attributed to its alleviating effect on stressed tissue (Blumwald, 2000). Recently, in a study under rain-fed conditions and five irrigation levels, it was found that water deficit did not influence leaf nitrogen, magnesium, sodium, and zinc concentrations, but decreased phosphorous, potassium, boron, and chloride, and tended to decrease calcium, manganese, and iron, whereas the nutrient concentrations were cultivar dependent (Zipori *et al.*, 2015).

When drought intensity or/and its duration increases, a reduction in leaf cell turgor and an increase in cell wall stiffness occur by lignin synthesis, and by deposition to cell wall that might result in photosynthesis prevention and transpiration decrease (Sofo *et al.*, 2008) or inhibition of photosynthesis associated with photosystem II (Angelopoulos *et al.*, 1996). Under drought, Sofo *et al.* (2008) observed increases in lipoxygenase activity (LOX) and in malonaldehyde (MDA) concentration, which indicate membrane lipid oxidation, which is related to photosynthetic apparatus lesion. In the 'Koroneiki' cultivar, water stress resulted in increased LOX and MDA concentration, indicating the oxidation of the lipid membrane. Chlorophyll loss and increases in carotenoids were promoted by increased water deficit. After rewatering, the recovering of trees subjected to moderate stress was related to an increased enzymatic antioxidant system (Doupis *et al.*, 2013) (Table 2.2). Oleuropein, hydroxytyrosol, total phenolic concentration, and antioxidant capacity increased in leaves in response to water stress, with the oleuropein increases being the major change (Petridis *et al.*, 2012).

Stress is usually accompanied with increased levels of reactive oxygen species (ROS), radicals or nonradicals such as $^1O_2$, H_2O_2, HOCl, and $HO^\bullet$; and reactive nitrogen species (RNS), which are toxic molecules that are capable of causing oxidative damage to proteins, DNA, and lipids (Apel & Hirt, 2004; Møller *et al.*, 2007). Cellular antioxidants, including enzymes and various compounds, are key to protecting the cells against damage due to ROS. Accumulation of ROS during stress greatly depends on balance between their production and scavenging (Mittler *et al.*, 2004). However, generated ROS due to stress, such as drought or salinity, could also be channeled by the plant, in order to activate defense mechanisms and act as signals (Miller *et al.*, 2010). Indeed, olive trees under water deficit conditions increased activities of antioxidant enzymes, ascorbate peroxidase (APX) and catalase (CAT) in leaves, as well as superoxide dismutase (SOD) and peroxidase (POD) in leaves and root, whereas they decreased the activity of polyphenol oxidase (PPO)

Table 2.2 Major indicative changes in olive oil quality or compounds and enzyme activities in olive fruit and olive leaves related to various stresses in olive trees.

Type of stress	Responding part of the tree and cultivar	Stressed/non-stressed	References
Drought	Fruit: 'Arbequina'	H: Hydroxytyrosol, tyrosol, and other simple phenols	Artajo *et al.* (2006)
Drought	Fruit: 'Arbequina I-18'	H: TP, oxidative stability, C18:1/C18:2	Berenguer *et al.* (2006)
Drought (rain-fed)	Fruit: 'Cornicabra'	H: TP, B (K_{225}), MUFA/PUFA; L: Oil (%); NS: FFA, PV, chlorophyll, carotenoids	Gómez-Rico *et al.* (2007)
Drought	Fruit: 'Koroneiki'	H: C18:2, 3,4-DHPEA-EDA, 3,4-DHPEA-EA, TToc, TSt, TVol; L: Weight, oil (%), C18:1; NS: FFA, PV	Stefanoudaki *et al.* (2009a)
Drought	Leaves: 'Koroneiki', 'Kalamon', 'Megaritiki', 'Gaidourelia'	H: TP, oleuropein, hydroxytyrosol	Petridis *et al.* (2012)
Drought	Leaves: 'Koroneiki'	H: SOD, APX, GPX, carotenoids; L: CAT, chlorophyll	Doupis *et al.* (2013)
Drought	Fruit: 'Arbequina'	L: Water (%); NS: Oil (%)	Gómez-del-Campo *et al.* (2014)
Drought	Fruit: 'Koroneiki'	L: FFA, TP, MUFA/PUFA in oil; NS: PV in oil	Dag *et al.* (2014a)
Salinity	Fruit: 'Barnea'	H: TP, α-Toc; NS: FFA, PV, FA profile	Wiesman et al.*et al.* (2004)
Salinity	Fruit: 'Koroneiki'	H: TP, 3,4-DHPEA-EDA; L: C18:1/C18:2,	Stefanoudaki *et al.* (2009b)
Hot weather (through early ripening)	Fruit: 'Barnea'	H: FFA; NS: TP (oleuropein aglycon derivatives), MUFA/PUFA	Dag *et al.* (2014b)
Hot weather (through early ripening)	Fruit: 'Coratina'	H: FFA, lignans; L: MUFA/PUFA; NS: TP	Dag *et al.* (2014b)
Hot weather (through early ripening)	Fruit: 'Picual'	H: FFA, lignans; NS: TP, MUFA/PUFA	Dag *et al.* (2014b)
R-W after drought + irradiation	Leaves and roots: 'Coratina'	H: PPO; L: SOD, CAT, APX, POD (faster under shade)	Sofo *et al.* (2004)
Salinity + solar radiation	Leaves: 'Cipressino'	H: Mannitol, zeaxanthin, flavonoids (mannitol and flavonoids under sunlight irrespectively of root salinity)	Remorini *et al.* (2009)
Wastewater	Fruit: 'Maiatica'	L: TP; NS: FFA, K_{232}, K_{270}	Palese *et al.* (2006)

Abbreviations: H, higher; L, lower; NS, nonsignificant change/not consistent change during ripening or years; R-W, rewatering; FA, fatty acids; FFA, free fatty acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; MUFA/PUFA, monounsaturated/polyunsaturated; PV, peroxide value; B, bitterness absorbance at (K_{225}), K_{232}, and K_{270}, ultraviolet absorbancies at the corresponding wavelengths (indicating degree of oxidation); TP, total phenolic concentration; TToc, total tocopherol content, α-Toc, α-tocopherol; TSt, total sterol content; TVol, total volatiles; 3,4-DHPEA-EDA, oleuropein-aglycone di-aldehyde; 3,4-DHPEA-EA, oleuropein-aglycone mono-aldehyde; SOD, superoxide dismutase; APX, ascorbate peroxidase; GPX, guaiacol peroxidase; CAT, catalase; POD, peroxidase; PPO, polyphenol oxidase.

in leaves and root, in order to protect oxidation of phenols (Sofo *et al.*, 2004) (Table 2.2). Therefore, ROS could also be viewed as a prerequisite to induce acclimation mechanisms (Foyer & Noctor, 2005; Suzuki *et al.*, 2014). Plant hormones also play a protective signaling role in response to drought and other stresses. Cross-talking observations among polyamines and hormones, along with changes in ROS and alteration of ion homeostasis, have been reported (Anwar *et al.*, 2015).

In olives, accumulated proline along with malate and carbohydrates are main constituents contributing to osmotic adjustment, one of the most important mechanisms that is also developed in response to water stress (Sofo *et al.*, 2004; 2008). The olive tree is capable of synthesizing both the polyol mannitol and oligosaccharides of the raffinose family, as end products of photosynthesis. Mannitol, being a major carbohydrate in leaves and fruit, is synthesized in mature leaves, used for energy supply or as a carbon source (Flora & Madore, 1993), and used for osmotic adjustment (Sofo *et al.*, 2008). An increase in transport activity of mannitol was indeed observed under drought and salinity stress (Conde *et al.*, 2011). No negative effects on photosynthesis have been observed in plants even at high mannitol concentrations (Bolouri-Moghaddam *et al.*, 2010), while it was suggested that mannitol concentration in olive fruit might indicate the potential for oil biosynthesis (Wodner *et al.*, 1988).

2.4.2 Defense mechanisms against combined stresses (drought, salinity, radiation, and heat)

It is noteworthy that combined effects of two or more stresses, such as drought and heat or heat and radiation, are usually additive, indicating an independence of each defense mechanism, at least to some extent (Miller *et al.*, 2010). Different but simultaneous responses to stresses might be regulated by a coordination of signals and combination of pathways (Suzuki *et al.*, 2014). Young olive trees subjected to root salinity and solar radiation, in a stress combination, showed higher mannitol and flavonoids, as well as sodium, chloride, potassium, and magnesium concentrations, in leaves under sunlight (temperature >33 °C) than in semishaded ones (Remorini *et al.*, 2009) (Table 2.2). After rewatering trees, Sofo *et al.* (2004) observed decreases in SOD, CAT, APX, and POD activities that were faster in plants under semishaded conditions, indicating the additive stress of radiation. The resulting increased PPO activity after rewatering can be ascribed to its role in the removal of damaged proteins and regulation of the redox state of phenols (Table 2.2).

2.5 Factors affecting olive growth and functional compounds

2.5.1 Effects of heat, salinity, and irrigation systems on olive growth

According to studies on the prediction of temperature and net radiation increase in the Mediterranean area, this climate change in the near future will have a serious negative effect on olive cultivation (Chartzoulakis, 2005; Tanasijevic *et al.*, 2014). In such a case, the olive cultivation would face the challenge of choice between the high-water-demanding intensive systems and the low rain-fed traditional system (Tanasijevic *et al.*, 2014). Irrigation treatment with wastewater can affect the olive oil composition and decreases the total phenolic content (TP), but does not affect the FFA content and the specific ultraviolet absorbance K_{232} and K_{270} (Palese *et al.*, 2006) (Table 2.2). However, many quality criteria should be evaluated prior to the use of low-quality water (Palese *et al.*, 2009).

Experimental results of some study cases dealing with irrigation applying wastewater have been very promising. Olive groves of 'Koroneiki' in Crete, Greece, irrigated with secondary and tertiary treated wastewater for three growing seasons, exhibited higher *Esherichia coli*, magnesium, calcium, and boron soil concentrations. Nevertheless, the researchers achieved a safe irrigation similar to that with tap water with no negative effects on tree and fruit growth, and no contamination on leaves and fruit (Petousi *et al.*, 2015). Recently, efforts have been made toward the application of olive mill wastewater (OMW) to olive orchards, and results appear promising for its use during winter but not during summer (Steinmetz *et al.*, 2015). Additionally, the technology of thermal imaging can be used for determining representative trees from which information can be provided, for orchard irrigation toward water economy. The salinity

tolerance of olives is based on the retention of sodium and chlorine ions in the olive root (Marschner, 1995). When salinity is at low or moderate levels, the root system regulates the concentration in xylem sap and prevents toxicity in the aerial parts, whereas an ionic balance is achieved by potassium increases in new leaves and decreases in root and old leaves (Slama, 1986). In contrast, the tolerance to salinity was attributed to accumulation of salt in the leaf vacuoles, as suggested by Loreto and Bongi (1987). In all cases of salt stress, salinity decreases both photosynthesis and growth of the tree (Loreto *et al.*, 2003), while extended growth reduction depends on salt concentration and duration of exposure (Chartzoulakis, 2005).

Screening of olive cultivars for salinity tolerance seems to be an alternative to irrigation with saline water. Marin *et al.* (1995) found that ‘Arbequina’, ‘Nevadillo’, ‘Lechin de Sevilla’, ‘Jabanula’, ‘Escarabajuelo’, ‘Canivano’, and ‘Picua’ are the most tolerant cultivars found among 26 tested, but the study was carried out on young plants grown in pots. Irrigation with saline water did not change olive oil quality parameters, such as free acidity, peroxide value, and fatty acids profile, but increased the content of polyphenols and vitamin E (Wiesman *et al.*, 2004) (Table 2.2).

2.5.2 Effects of various stresses on fruit weight, anatomy, and composition

The effects of irrigation deficit on olive fruit have attracted much attention. The increased cuticle thickness has been observed in ‘Kalamata’ (Patumi *et al.*, 2002) and ‘Arbequina’ olives (Gómez-del-Campo *et al.*, 2014). The same authors reported that in mesocarp cells, the oil accumulation decreased along with the cell size but not the cell number, while these effects on mesocarp cells were recovered after irrigation, before harvest. The same mesocarp recovery has also been observed in ‘Picual’ (Moriana *et al.*, 2003).

In traditional non-irrigated (rain-fed) ‘Koroneiki’ groves, water deficit decreased fruit weight, oil content, and oleic acid, but increased linoleic and linolenic acids, total sterols, total tocopherols, and total volatiles. In one out of two years’ study, water deficit affected the phenolic and sterol profile, while total phenolic content was increased in comparison with the content of irrigated trees (Stefanoudaki *et al.*, 2009a) (Table 2.2). Another study on ‘Koroneiki’ olives, however, cultivated in SHD orchards and irrigated by five different water levels, showed that water stress did not affect the total phenolic content and PV of the oil (Dag *et al.*, 2014a). This last finding is promising for SHD orchards, when it is well known that water stress can limit the tree vigor. Major relative changes in oil quality or compounds and enzyme activities in leaves and fruit related to various stresses in olive trees are shown in Table 2.2.

In general, it is known that saline water decreases fruit weight and oil content (Weisman *et al.*, 2004; Stefanoudaki *et al.*, 2009b), whereas it increases total phenolic content (Stefanoudaki *et al.*, 2009b) (Table 2.2) and sodium and chlorine ions in the fruit (Chartzoulakis *et al.*, 2004). Potassium, added to alleviate the stress of saline water, promoted coloring and ripening of olives (Chartzoulakis *et al.*, 2004).

It has been found that ‘Konservolia’ olives, harvested at a dark green state and exposed to 20 °C in air for 7 days, showed an increase in oleuropein content by 2.53-fold, reaching a level of 14 mg g^{-1} dw, whereas under these conditions most other determined phenolic compounds decreased, along with loss of color and firmness (Tsantili *et al.*, 2012). This unexpected result was further confirmed by HPLC-ESI-MS (high-performance liquid chromatography–electrospray–mass spectrometry). In green fruit, the increase in oleuropein content could possibly be attributed to a defense mechanism after detachment from the tree. Kubo *et al.* (1985) suggested that oleuropein is responsible for the release of phytoalexins, and acts against pathogens as being a protein cross-linker (Konno *et al.*, 1999) that decreases the nutritive value of dietary protein. Oleuropein degradation has been achieved successfully long ago. However, the whole biosynthetic pathway is not yet fully elucidated, despite the fact that a proposed mechanism for biosynthesis from 7-ketologanin (Damtoft *et al.*, 1993) has been accepted (Gutierrez-Rosales *et al.*, 2010).

2.5.3 Fruit growth, maturation, and ripening physiology

Among common crops, olives exhibit the longest duration of fruit growth on the tree, including the fully ripe stage. The entire fruit development can be extended to seven months after full bloom (Bodoira *et al.*, 2014). Although photosynthesis occurs at the green fruit stage and contributes to fruit carbon economy (Sánchez, 1995), the long-lasting fruit development might reflect, at least partially, the slow supply of metabolites

and nutrients from tree to fruit. The priority of the olive tree is to survive under unfavorable conditions (Tanasijevic *et al.*, 2014), and this is probably related to olive alternate bearing. After fruit set, vegetative and reproductive growth occur simultaneously and a competition for assimilates between them might occur. This could be a reason for the long duration of fruit development. Cherbiy-Hoffmann *et al.* (2013) suggested that fruit growth has priority in photosynthesis products over vegetative growth, at low and medium photosynthetic active radiation (PAR).

During different developmental stages, considerable alterations occur in fruit composition. The different stages are discriminated either by the days elapsed from the full bloom (DEFB) or by a ripening color index. This index can be best determined by peel and flesh color, the so-called maturity index (MI) (Hermoso *et al.*, 1991). MI classifies randomly harvested olives into eight categories. The eight-point scale starts from fruit with dark green color in peel (MI = 0) and ends up with black color in peel and purple in flesh (MI = 7). In cases where changes in color do not have a good relationship with oil accumulation (in olives for oil production) or firmness decrease (in olives for table use), an additional index would be useful to determine the harvest timing, meaning a detachment index (Camposeo *et al.*, 2013).

Fruit growth shows a double sigmoidal curve divided into three main distinct phases. During the first and third phases, the fruit size increases rapidly, while during the second phase it increases slowly (Bodoira *et al.*, 2014; Gómez-del-Campo *et al.*, 2014). A typical example is given for 'Arauco', which exhibits a whole growth period of 215 DEFB and a slow phase between 111 DEFB and 142 DEFB (Bodoira *et al.*, 2014). Oil accumulation starts very early (at about 30 DEFB) (Bodoira *et al.*, 2014; Gómez-del-Campo *et al.*, 2014) and continues at a very slow rate at approximately 80 DEFB. Then, oil synthesis occurs at a high rate for approximately 60 days, until green maturation. In particular, the green maturation occurs when fruit has reached its final size.

Based on fruit ripening, there has been an argument concerning the classification of olives in climacteric (Ranalli *et al.*, 1998) or non-climacteric fruits (Kader, 2002). Olives exhibit responses to ethylene that are not common in fruit. In green harvested olives, ethylene treatment does not promote the ripening process, but prevents softening and reddening (Shulman *et al.*, 1974; Tsantili & Pontikis, 2004). Endogenous ethylene is not detectable in olives before green maturation. Afterward, it ranges between a few nmol kg^{-1} h^{-1} and approximately 100 nmol kg^{-1} h^{-1} at 20 °C, depending on the cultivar and ripening stage (Tsantili *et al.*, 2012; Tsantili, 2013).

Recently, proteins were identified and related to metabolic changes during olive development (Bianco *et al.*, 2013). The comparison between proteomic and transcriptomic data strengthened the non-climacteric behavior of olives (Alagna *et al.*, 2012). Fruit ripening has been described as a controlled oxidative process where H_2O_2 and ROS accumulation is balanced with antioxidant systems, as suggested in the case of the climacteric fruit of tomato (Jiménez *et al.*, 2002). In olives, a transcription response to oxidative stress is induced, but the protein levels of enzymes responsible for ROS detoxification are controlled at posttranscriptional levels (Bianco *et al.*, 2013).

2.5.4 Changes in fruit composition and functional compounds during fruit development

Mature olive pulp consists of 65–70% water and 30–35% oil (Therios, 2009). Among the major fatty acids, oleic acid increases in parallel with oil accumulation and varies between 55 and 83% (m/m methyl esters), while linoleic acid ranges between 3.5 and 21%, and palmitic acid between 7.5 and 20% (Boskou, 1996; Kiritsakis, 1998).

Olive oil has a unique composition with beneficial effects on human health (Conde *et al.*, 2008). Apart from the fatty acids content, other constituents or microconstituents are also important for human health. They include tocopherols, polar phenolic compounds, phytosterols, triterpenic hydrocarbon squalene, terpenic acids, and carotenes (Kalogeropoulos & Kaliora, 2015). Phenolic compounds in olive pulp range from 1 to 3% (w/w), and they include phenolic acids (such as caffeic acid and its sugar ester verbascoside), alcohols (tyrosol, which is a hydrolysis product of ligstroside and hydroxytyrosol that results from oleuropein hydrolysis), flavonoids (rutin, luteolin, luteolin-7-glucoside, luteolin-4-glucoside, quercetin, cyaniding-3-glucoside, and cyaniding-3-rutinoside), and lignans and secoiridoids (mainly oleuropein, ligstroside, demethyloleuropein, elenolic acid, and elenolic acid linked to tyrosol or 3,4-DHPEA-EDA) (Charoenprasert

& Mitchell, 2012;. Tsantili, 2013). Other important microconstituents are the maslinic and ursolic acids located in the cuticular lipid layer of the fruit, and oleocanthal or p-HPEA-EDA that may be present in VOO (Stefanoudaki *et al.*, 2009b; Kalogeropoulos & Kaliora, 2015).

During fruit development, the most prevalent change is the decrease in oleuropein (Jemai *et al.*, 2009). Oleuropein is the most important secoiridoid glycoside, produced through glycosidase enzymes of the fruit (Ryan & Robards, 1998; Morelló *et al.*, 2005). In the literature, oleuropein varies from 15 mg g^{-1} (dw) in young ‘Arbequina’ olives (Morelló *et al.*, 2005) to 2.5 mg g^{-1} (dw) in ripe ‘Kalamon’ olives at MI 6 (Tsantili, 2013). In ripe fruit, oleuropein is replaced by demethyloleuropein and hydroxytyrosol (Esti *et al.*, 1998; Obied *et al.*, 2008). However, hydroxytyrosol levels do not exhibit consistent changes during ripening, ranging between 300 mg kg^{-1} (Esti *et al.*, 1998) and approximately 70,000 mg kg^{-1} (Vinha *et al.*, 2005). A decreasing trend in total phenolic compounds (Morelló *et al.*, 2005) in 3,4-DHPEA-EDA and tyrosol, but also an increase in demethyloleuropein, was also observed, which corresponds to the decrease in oleuropein (Alagna *et al.*, 2012). These authors studied 12 cultivars and classified them into two groups; one included cultivars with low phenolic content and another with high content. These differences might be related to modulation of phenolic biosynthesis through enrichment of olives with phenolic compounds, a case that could be investigated in breeding programs.

2.6 Conclusion

Olive trees genetically have a long life that may reach several centuries. This fact indicates that they have the potential to tolerate various types of stress. The effort to identify genes and signaling components, such as transcription factors and protein kinases involved in plant stress adaptation, would be substantial to face the new trends of intensive olive cultivation with a high water demand. It is generally accepted that stress induces synthesis of phenolic compounds that have antioxidant activity. In the case of olive trees under various stresses, the increased antioxidant enzymatic systems and antioxidant compounds, like phenolic and others, activate a common defense mechanism throughout all tissues, which responds effectively to stress situations. Therefore, it has been thought that enrichment of leaves and fruit with these compounds might result in olive tree responses to stress situations, in order to protect its own tissues. However, a plethora of scientific studies confirmed that these olive compounds exhibit bioactive and functional properties, resulting in human health promotion and disease risk reduction. The advantageous properties of these functional compounds, along with the agreeable taste of olive products, justify the increased consumer preference in olive oil and table olives.

Recently, alternative dietary enrichment of fruit or other responding tissues with various “controlled” stresses has been attempted. However, each plant tissue has a certain potential to cope with a stressful situation at a particular time, and therefore an excessive stress could not always be overcome. In case of olive products, retention of bioactive compounds that already exist in the raw product is of interest. Cultivation practices, harvesting time, transport, storage, and industry processing are critical elements for high-quality olive products with high functional value.

References

Alagna, E., Mariotti, R., Panara, E., Caporali, S., Urbani, S., Veneziani, G., Esposto, S., Taricchi, A., Rosati, A., Rao, R., Perrotta, G., Servilli, M., & Baldoni, L. (2012) Olive phenolic compounds: metabolic and transcriptional profiling during fruit development. BMC Plant Biology 12, 162. Available from http://www.biomedcentral.com/1471-2229/12/162.

Angelopoulos, K., Dichio, B., & Xiloyannis, C. (1996) Inhibition of photosynthesis in olive tress (*Olea europea* L.) during water stress and rewatering. Journal of Experimental Botany 47, 1093-1100.

Angiolillo, A., Mencuccini, M., & Baldoni, L. (1999) Olive genetic diversity assessed using amplified fragment length polymorphisms. Theoretical and Applied Genetics 98, 411–421.

Anwar, R., Mattoo, A.K., & Handa, A.K. (2015) Polyamine interactions with plant hormones: crosstalk at several levels. In: Polyamines: universal molecular nexus for growth, survival, and specialized metabolism, ed. T. Kusano & H. Suzuki. Springer, Tokyo, 267–302.

Apel K., & Hirt H. (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology 55, 373–399.

Artajo, L.S., Romero, M.P., Tovar, M.J., & Motilva, M.J. (2006) Effect of irrigation applied to olive trees (*Olea europaea* L.) on phenolic compound transfer during olive oil extraction. European Journal of Lipid Science and Technology 108, 19–27.

Ayerza, R., & Coates, W. (2004) Supplemental pollination increasing olive (*Olea europaea*) yields in hot, arid environments. Exploratory Agriculture 40, 480–491.

Ayerza, R., & Sibbett, G.S. (2001) Thermal adaptability of olive (*Olea europaea* L.) to the Arid Chaco of Argentina. Agriculture, Ecosystems and Environment 84, 277–285.

Baldoni, L., Guerriero, C., Sossey-Aloui, K., Abbott, A.G., Angiolillo, A., & Lumaret, R. (2002) Phylogenetic relationships among Olea species based on nucleotide variation at a non-coding chloroplast DNA region. Plant Biology 4, 346–351.

Barranco, D., Ruiz, N., & Gómez-del-Campo, M. (2005) Frost tolerance of eight olive cultivars. HortScience 40, 558–560.

Bar-Yosef, B. (1999) Advances in fertigation. Advances in Agronomy 65, 1– 77.

Ben-Gal, A., Dag, A., Basheer, L., Yermiyahu, U., Zipori, I., & Kerem, Z. (2011) The influence of bearing cycles on olive oil quality response to irrigation. Journal of Agricultural and Food Chemistry 59, 11667–11675.

Berenguer, M.J., Vossen, P.M., Grattan, R.S., Connell, J.H., & Polito, V.S. (2006) Tree irrigation levels for optimum chemical and sensory properties of olive oil. HortScience 41, 427–432.

Bianco, L., Alagna, F., Baldoni, L., Finnie, C., Svensson, B., & Perrotta, G. (2013) Proteome regulation during *Olea europea* fruit development. PLoS ONE 8(1), e53563.

Blumwald, E. (2000) Sodium transport and salt tolerance. Current Opinion in Cell Biology 12, 431–433.

Bodoira, R., Torres, M., Pierantozzi, P., Taticchi, A., Servilli, M., & Maestri, D. (2014) Oil biogenesis and antioxidant compounds from 'Arauco' olive (*Olea europea* L.) cultivar during fruit development and ripening. European Journal of Lipid Science and Technology 117, 377–388.

Bolouri-Moghaddam, M., Le Roy, K., Xiang, L., Rolland, F., & Van den Ende, W. (2010) Sugar signaling and antioxidant network connections in plant cells. FEBS Journal 277, 2022–2037.

Boskou, D. (1996) History and characteristics of the olive tree. In Olive Oil Chemistry and Technology, ed. D. Boskou. American Oil Chemists' Society Press, Champaign, IL, 13–19.

Bracci, T., Busconi, M., Fogher, C., & Sebastiani, L. (2011) Molecular studies in olive (*Olea europaea* L.): overview on DNA markers applications and recent advances in genome analysis. Plant Cell Reports 30, 449–462.

Breton, C., Medail, F., Pinatel, C., & Berville, A. (2006) From olive tree to Oleaster: origin and domestication of *Olea europaea* L. in the Mediterranean basin. Cahiers Agriculture 15, 329–336.

Bustan, A., Avni, A., Lavee, S., Zipori, I., Yeselson, Y., Schaffer, A.A., Riov, J., & Dag, A. (2011) Role of carbohydrate reserves in yield production of intensively cultivated oil olive (*Olea europaea* L.) trees. Tree Physiology 31, 519–530.

Camposeo, S., Vivaldi, G.A., & Gattullo, C.E. (2013) Ripening indices and harvesting times of different olive cultivars for continuous harvest. Scientia Horticulturae 151, 1–10.

Castro-García, S., Blanco Roldán, G.L., Jiménez-Jiménez, F., Gil-Ribes, J.A., Ferguson, L., Glozer, K., Krueger, W.H., Fichtner, E.J., Burns, J.K., Miles, J.A., & Rosa, U.A. (2012) Preparing Spain and California table olive industries for mechanical harvesting. Acta Horticulturae 965, 29–39.

Charoenprasert, S., & Mitchell, A. (2012) Factors influencing phenolic compounds in table olives (*Olea europea* L.). Journal of Agricultural and Food Chemistry 60, 7081–7095.

Chartzoulakis, K.S. (2005) Salinity and olive: growth, salt tolerance, photosynthesis and yield. Agricultural Water Management 78, 108–121.

Chartzoulakis, K., Psarras, G., Vemmos, S., & Loupassaki, M (2004) Effects of salinity and potassium supplement on photosynthesis, water relations and Na, Cl, K and carbohydrate concentration of two olive cultivars. Agricultural Research 27, 75–84.

Chatzissavvidis, C. (2002) Study of boron toxicity in olive plants. PhD thesis, School of Agriculture, Aristotle University, Thessaloniki, Greece.

Cherbiy-Hoffmann, S.U., Hall, A.J., Searles, P.S., & Rousseaux, M.C. (2013) Fruit yield, and vegetative growth responses to photosynthetically active radiation during oil synthesis in olive trees. Scientia Horticulturae 150, 110116.

Conde, C., Delrot, S., & Geros, H. (2008) Physiological, biochemical and molecular changes occurring during olive development and ripening. Journal of Plant Physiology 165, 1545–1562.

Conde, A., Silva, P., Agasse, A., Conde, C., & Gerós, H. (2011) Mannitol transport and mannitol dehydrogenase activities are coordinated in *Olea europaea* under salt and osmotic stresses. Plant and Cell Physiology 52, 1766–1775.

Connor, D.J., Gómez-delCampo, M., Rousseaux, M.C., & Searles, P.S. (2014) Structure and productivity of hedgerow olive orchards: a review. Scientia Horticulturae 169, 71–93.
Covas, M.I. (2008) Bioactive effects of olive oil phenolic compounds in humans: Reduction of heart disease factors and oxidative damage. Inflammopharmacology 16, 216–218.
Covas, M.I., Nyyssonen, K., & Poulsen, H.E. (2006) The effect of polyphenols in olive oil on heart disease risk factors. Annals of International Medicine 145, 333–431.
Cuevas, J., & Polito, V.S. (2004) The role of staminate flowers in the breeding system of *Olea europaea* (Oleaceae): an andromonoecious, wind-pollinated taxon. Annals of Botany 93, 547–553.
Cuevas, J., Rallo, L., & Rapoport, H.F. (1995) Relationships among reproductive processes and fruitlets abscission in Arbequina olive. Advances in Horticultural Science 9, 92–96.
D'Angeli, S., & Altamura, M.M. (2007) Osmotin induces cold protection in olive trees by affecting programmed cell death and cytoskeleton organization. Planta 225, 1147–1163.
Dag, A., Harlev, G., Lavee, S., Zipori, L., & Kerem, Z. (2014a) Optimizing olive harvest time under hot climatic conditions of Jordan Valley, Israel. European Journal of Lipid Science and Technology 116, 169–176.
Dag, A., Naor, A., Ben-Gal, A., Harlev, G., Zipori, I., Schneider, D., Birger, R., Peres, M., Gal. Y., & Kerem, Z. (2014b) The effect of water stress on super high density 'Koroneiki' olive oil quality. Journal of the Science of Food and Agriculture 95, 2016–2020.
Damtoft, S., Franzyk, H., & Jensen, S.R. (1993) Biosynthesis of secoiridoid glucosides in Oleaceae. Phytochemistry 34, 1291–1299.
Denney, J.O., McEachern, G.R., & Griffiths, J.F. (1985) Modeling the thermal adaptability of the olive (*Olea europaea* L.) in Texas. Agricultural and Forest Meteorology 35, 309–327.
Doupis, G., Bertaki, M., Psarras, G., Kasapakis, I., & Chartzoulakis, K. (2013) Water relations, physiological behaviour and antioxidant defence mechanism of olive plants subjected to different irrigation regimes. Scientia Horticulturae 153, 150–156.
Erel, R., Kerem, Z., Ben-Gal, A., Dag, A., Schwartz, A., Zipori, I., Basheer, L., & Yermiyahu, U. (2013) Olive (*Olea europaea* L.) tree nitrogen status is a key factor for olive oil quality. Journal of Agricultural and Food Chemistry 61, 11261–11272.
Esti, M., Cinquanta, L., & La Notte, E. (1998) Phenolic compounds in different olive varieties. Journal of Agricultural and Food Chemistry 46, 32−35.
Farinelli, D., Boco, M., & Tombesi, A. (2002) Intensity and growth period of the fruit components of olive varieties. Acta Horticulturae 568, 607–610.
Farinelli, D., Boco, M., & Tombesi, A. (2006) Results of four years of observations on self-sterility behaviour of several olive cultivars and significance of cross-pollination. In: Biotechnology and quality of olive tree products around the Mediterranean Basin, ed. T. Caruso, A. Motisi, & A. Sebastiani. Olivebioteq, Second International Seminar 1, 275–282.
Ferguson, L., & Castro-García, S. (2014) Transformation of an ancient crop: preparing California 'Manzanillo' table olives for mechanical harvesting. HortTechnology 24, 274–280.
Fernández-Escobar, R., García-Novelo, J.M., Molina-Soria, C., & Parra, M.A. (2012) An approach to nitrogen balance in olive orchards. Scientia Horticulturae 135, 219–226.
Fernández-Escobar, R., Marin, L., Sánchez-Zamora, M., García-Novelo, J., Molina-Soria, C., & Parra, M. (2009) Long-term effects of N fertilization on cropping and growth of olive trees and on N accumulation in soil profile. European Journal of Agronomy 31, 223–232.
Fernández-Escobar, R., Moreno, R., & Sanchez-Zamora, M.A. (2004) Nitrogen dynamics in the olive bearing shoot. HortScience 39, 1406–1411.
Fernández, J.E., Moreno, F., & Martin-Aranda, J. (1990) Study of root dynamics of olive trees under drip irrigation and dry farming. Acta Horticulturae 286, 263–266.
Flora, L.L., & Madore, M.A. (1993) Stachyose and mannitol transport in olive (*Olea europea* L.). Planta 189, 484–490.
Foyer, C.H., & Noctor, G. (2005) Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. The Plant Cell 17, 1866–1875.
Galán, C., Garcia-Mozo, H., Cariñanos, P., Alcázar, P., & Dominguez-Vilches, E. (2001) The role of temperature in the onset of the *Olea europaea* L. pollen season in southwestern Spain. International Journal of Biometeorology 45, 8–12.
Garcia, J.L., Avidan, N., Troncoso, A., Sarmiento, R., & Lavee, S. (2000) Possible juvenile-related proteins in olive tree tissues. Scientia Horticulturae 85, 271–284.
Godini, A., Vivaldi, G.A., & Camposeo, S. (2011) Olive cultivars field-tested in super-high-density system in Southern Italy. California Agriculture 65, 39–40.
Goldschmidt, E. E. (2005) Regulatory aspects of alternate-bearing in fruit trees. Italus Hortus 12, 11–17.

Gómez-del-Campo, M., Moralles-Sillero, A., Vita Serman, F., Rousseaux, M.C., & Searles, P.S. (2010) Olive growing in the arid valleys of Northwest Argentina (Provinces of Catamarcaand La Rioja). Olivae 114, 23–45.

Gómez-del-Campo, M., Pérez-Expósito, M.A., Hammami, S.B.M., Centeno, A., & Rapoport, H.F. (2014) Effect of varied summer deficit irrigation on components of olive fruit growth and development. Agricultural Water Management 137, 84–91.

Gomez-Rico, A., Desamparados Salvador, M., Moriana, A., Perez, D., Olmedilla, N., Ribas, F., & Fregapane, G. (2007) Influence of different irrigation strategies in a traditional Cornicabra cv. olive orchard on virgin olive oil composition and quality. Food Chemistry 100, 568–578.

Guerin, J., & Sedgley, M. (2007) Cross pollination in olive cultivars. RIRDC Publication No 07/169. RIRDC, Barton, Australia.

Gutiérrez-Rozales, F., Romero, M.P., Casanovas, M., Motilva, M.J., & Minquez-Mosquera, M.I. (2010) Metabolites involved in oleuropein accumulation and degradation in fruits of *Olea europea* L.: Hojiblanca and Arbequina varieties. Journal of Agricultural and Food Chemistry 58, 12924–12933.

Hermoso, M., Uceda, M., Garcia, A., Morales, B., Frias, M.L., & Fernandez, A (1991) Elaboracion de aceite de calidad. Serie Apuntes, 5/92. Consejeria de Agricultura y Pesca, Seville.

Huijser, P., & Schmid, M. (2011) The control of developmental phase transitions in plants. Development 138, 4117–4129.

Jemai, H., Bouaziz, M., & Sayadi, S. (2009) Phenolic composition, sugar contents and antioxidant activity of Tunisian sweet olive cultivar with regard to fruit ripening. Journal of Agricultural and Food Chemistry 57, 2961–2968.

Jiménez, A., Greissen, G., Kular, B., Firmin, G. Robinson, S., Verhoeyen, M., & Mullineaux, P. (2002) Changes in oxidative processes and components of the antioxidant system during tomato fruit ripening. Planta 214, 751–758.

Jiménez-Jiménez, F., Castro-García, S., Blanco-Roldán, G.L., Ferguson, L., Rosa, U.A., & Gil-Ribes, J.A. (2013) Table olive cultivar susceptibility to impact bruising. Postharvest Biology and Technology 86, 100–106.

Kader, A.A. (2002) Postharvest biology and technology: An overview. In: Postharvest technology of horticultural crops, 3rd ed., ed. A.A. Kader. Publication 3311. University of California, Agricultural & Natural Resources, Oakland, CA, 39–47.

Kalogeropoulos, N., & Kaliora, A.C. (2015) Effect of fruit maturity on olive oil phenolic composition and antioxidant capacity. In: Olive and olive oil bioactive constituents, ed. D. Boskou. AOCS Press, Urbana, IL, 123–145.

Kevin, D., Rade, D., Trucelj, D., Mokrovãak, Î., Nederal, S., & Benãiç, D. (2003) The influence of variety and harvest time on the bitterness and phenolic compounds of olive oil. European Journal of Lipid Science and Technology 105, 536–541.

Kiritsakis, A. (1998) Olive oil, 2nd augmented and rev. ed. Food and Nutrition Press, Trumbull, CT.

Kiritsakis, A. (2007) Olive oil, table olives and olive paste. Akritidis Brothers, Thessaloniki, Greece. (in Greek).

Knoops, K.T., De Groot, L.C., & Kromhout, D. (2004) Mediterranean diet, lifestyle factors, and 10-year mortality in elderly European men and women. Journal of the American Medical Association 292, 1433–1439.

Konno, K., Hirayama, C., Yasui, H., & Nakamura, M. (1999) Enzymatic activation of oleuropein: a protein crosslinker used as a chemical defense in the privet tree. Proceedings of the National Academy of Sciences 96, 9159–9164.

Kubo, I., Matsumoto, A., & Takase, I. (1985) A multichemical defense mechanism of bitter olive *Olea europaea* (Oleaceae). Is oleuropein a phytoalexin precursor? Journal of Chemical Ecology 11, 251–263.

Lavee, S. (2007) Biennial bearing in olive (*Olea europaea*). Annales (Series Historia Naturali) 17, 101–112.

Lavee, S., Rallo, L., Rapoport, H.F., & Troncoso, A. (1999) The floral biology of the olive: II. The effect of inflorescence load and distribution per shoot on fruit set and load. Scientia Horticulturae 82, 181–192.

León, L., de L Rosa, R., Barranco, D., & Rallo, L. (2006) Selection for fruit removal force and related characteristics in olive breeding progenies. Australian Journal of Experimental Agriculture 45, 1643–1647.

Loreto, F., & Bongi, G. (1987) Control of photosynthesis under salt stress in the olive. In: International Conference on Agrometeorology, ed. F. Prodi, F. Rossi, & G. Cristoferi. Fondazione Cesena Agricoltura, Cesena.

Loreto, F., Centritto, M., & Chartzoulakis, K. (2003) Photosynthetic limitations in olive cultivars with different sensitivity to salt stress. Plant, Cell & Environment 26, 595–601.

Loumou, A., & Giourga, C. (2003) Olive groves: the life and identity of the Mediterranean. Agriculture and Human Values 20, 87–95.

Malik, N.S.A., & Bradford, J.M. (2006) Changes in oleuropein levels during differentiation and development of floral buds in 'Arbequina' olives. Scientia Horticulturae 110, 274–278.

Mancuso, S., & Rinaldelli, E. (1996) Response of young mycorrhizal and nonmycorrhizal plants of olive tree (*Olea europaea* L.) to saline conditions. II. Dynamics of electrical impedance parameters of shoots and leaves. Advances in Horticultural Science 10, 135–145.

Marin, L., Benlloch, M., & Fernandez-Escobar, R. (1995) Screening for olive cultivars for salt tolerance. Scientia Horticulturae 64, 113–116.

Marschner, H. (1995) Mineral nutrition of higher plants, 2nd ed. Academic Press, London.

Martin, G.C. (1994) Botany of the olive. In: Olive production manual, ed. L. Ferguson, G.S. Sibbett, & G.C. Martin. Publication 3353. University of California, Division of Agriculture and Natural Resources, Berkeley, CA, 19–21.

Martins, P.C., Cordeiro, A.M., & Rapoport, H.F. (2006) Flower quality in orchards of olive, *Olea europaea* L., cv. 'Morisca'. Advances in Horticultural Science 20, 262–266.

Melgar, J.C., Mohamed, Y., Navarro, C., Parra, M.A., Benlloch, M., & Fernández-Escobar, R. (2008) Long-term growth and yield responses of olive trees to different irrigation regimes. Agricultural Water Management 95, 968–972.

Miller, G., Suzuki, N., Ciftci-Yilmaz, S., & Mittler, R. (2010) Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant, Cell & Environment 33, 453–467.

Mittler R., Vanderauwera S., Gollery M., & Van Breusegem F. (2004) Reactive oxygen gene network of plants. Trends in Plant Science 9, 490–498.

Møller, I.M., Jensen, P.E., & Hansson, A. (2007) Oxidative modifications to cellular components in plants. Annual Review of Plant Biology 58, 459–481.

Morelló, J.R., Romero, M.P., Ramo, T., & Motilva, M.J. (2005) Evaluation of L-phenylalanine ammonia-lyase activity and phenolic profile in olive drupe (*Olea europea* L.) from fruit setting period to harvesting time. Plant Science 168, 65–72.

Moreno, F., Fernández, J.E., Clothier, B.E., & Green, S.R. (1996) Transpiration and root water uptake by olive trees. Plant and Soil 184, 85–96.

Moriana, A., Orgaz, F., Pastor, M., & Fereres, E. (2003) Yield responses of mature olive orchard to water deficits. Journal of the American Society for Horticultural Science 123, 425–431.

Obied, H.K., Prenzler, P.D., Servili, M., Taticchi, A., Esposto, S., & Robards, K. (2008) Biosynthesis and biotransformations of phenol-conjugated oleosidic secoiridoids from *Olea europaea* L. Natural Product Reports 25, 1167–1179.

Palese, A.M., Celano, G., Masi, S., Xiloyannis, C. (2006) Treated municipal wastewater for irrigation of olive trees: effects on yield and oil quality. In: Biotechnology and quality of olive tree products around the Mediterranean Basin, ed. T. Caruso, A. Motisi, & A. Sebastiani. Olivebioteq, Second International Seminar, 123–129.

Palese, A.M., Pasquale, V., Celano, G., Figliuolo, G., Masi, S., & Xiloyannis, C. (2009) Irrigation of olive groves in Southern Italy with treated municipal wastewater: effects on microbiological quality of soil and fruits. Agriculture, Ecosystems and Environment 129, 43–51.

Palliotti, A., & Bongi, G. (1996) Freezing injury in the olive leaf and effects of mefluidide treatment. Journal of Horticultural Science 71, 57–63.

Patumi, M., d'Andria, R., Marsilio, G., Fontanazza, G., Morelli, G., & Lanza, B. (2002) Olive and olive oil quality after intensive monocone olive growing (*Olea europaea* L. cv. Kalamata) in different irrigation regimes. Food Chemistry 77, 27–34.

Petousi, I., Fountoulakis, M.S., Saru, M.L., Nikolaidis, N., Fletcher, L., Stentiford, E.I., & Manios, T. (2015) Effects of reclaimed wastewater irrigation on olive (*Olea europea* L. cv 'Koroneiki') tress. Agricultural Water Management 160, 33–40.

Petridis, A., Therios, I., Samouris, G., Koundouras, S., & Giannakoula, A. (2012) Effect of water deficit on leaf composition, gas exchange, oxidative damage and antioxidant activity of four Greek olive (*Olea europea* L.) cultivars. Plant Physiology and Biochemistry 60, 1–11.

Polito, V. (2011, April 12) Pollination and fruit set. Presented at Sacramento Valley Olive Day, UC Davis, Department of Plant Sciences, UC Davis Olive Center, Davis, CA. Available from http://ceglenn.ucdavis.edu/files/90444.pdf

Porlingis, I.C., & Dogras, K. (1969) Time of flower differentiation of the olive as related to temperature and variety. Annals of the Agricultural and Forestry School, Aristotelian University of Thessaloniki 13, 321–341.

Pritsa, T.S., & Voyiatzis, D.G. (2005) Correlation of ovary and leaf spermidine and spermine content with the alternate bearing habit of olive. Journal of Plant Physiology 162, 2084–2091.

Ranalli, A., Tombesi, A., Ferrante, M.L., & De Mattia, G. (1998) Respiratory rate of olive drupes during their ripening cycle and quality of oil extracted. Journal of the Science of Food and Agriculture 77, 359–367.

Rapoport, H.F., Manrique, T., & Gucci, R. (2004) Cell division and expansion in the olive fruit. Acta Horticulturae 636, 461–465.

Remorini, D., Melgar, J.C., Guidi, L., Degl'Innocenti. E., Castelli, S., Traversi, M.L., Massai, R., & Tattini, M. (2009) Interaction effects of root-zone salinity and solar irradiance on the physiology and biochemistry of *Olea europea*. Environmental and Experimental Botany 65, 210–219.

Ryan, D., & Robards, K. (1998) Phenolic compounds in olives. Analyst 123, 31R–44R.
Ryan, D., Prenzler, P.D., Lavee, S., Antolovich, M., & Robards, K. (2003) Quantitative changes in phenolic content during physiological development of the olive (*Olea europea*) cultivar Hardy's Mammoth. International Journal of Food Chemistry 51, 2532–2538.
Sánchez, J. (1995) Olive oil biogenesis. Contribution of fruit photosynthesis. In Plant lipid metabolism, ed. J.-C. Kader & P. Mazliak. Kluwer Academic, Dordrecht, 564–566.
Sánchez, A.S., Romero, C., Ramírez, E., & Brenes, M. (2013) Storage of mechanically harvested Manzanilla olives under controlled atmospheres. Postharvest Biology and Technology 81, 60–65.
Segovia-Bravo, K.A., Jarén-Galán, M., Garcia-Garcia, P., & Garrido-Fernandez, A. (2011) Effect of bruising on respiration, superficial color, and phenolic changes in fresh Manzanilla olives (*Olea europea pomiformis*): development of treatments to mitigate browning. Journal of Agricultural and Food Chemistry 59, 5456–5464.
Shulman, Y., Erez, A., & Lavee, S. (1974) Delay in ripening of picked olives due to ethylene treatments. Scientia Horticulturae 2, 21–27.
Slama, F. (1986) Intervention des racines dans le sensibilite ou tolerance a NaCl des plantes cultivées. Agronomie 6, 651–658.
Sofo, A., Dichio, B., Xiloyannis, C., & Masia, A. (2004) Effects of different irradiance levels on some antioxidant enzymes and on malondialdehyde content during rewatering in olive tree. Plant Science 166, 293–302.
Sofo, A., Manfreda, S., Fiorentino, M., Dichio, B., & Xiloyannis, C. (2008) The olive tree: a paradigm for drought tolerance in Mediterranean climates. Hydrology and Earth System Sciences 12, 293–301.
Stefanoudaki, E., Williams, M., Chanzoulakis, K., & Harwood, J. (2009a) Effect of irrigation on quality attributes of olive oil. Journal of Agricultural and Food Chemistry 57, 7048–7055.
Stefanoudaki, E., Williams, M., Chanzoulakis, K., & Harwood, J. (2009b) Olive oil qualitative parameters after orchard irrigation with saline water. Journal of Agricultural and Food Chemistry 57, 1421–1425.
Steinmetz, Z., Kurtz, M.P., Dag, A., Zipori, I., & Schaumann, G.E. (2015) The seasonal influence of olive mill wastewater applications on an orchard soil under semi-arid conditions. Journal of Plant Nutrition and Soil Science 178, 641–648.
Suzuki, N., Rivero, R.M., Shulaev, V., Blumwald, E., & Mittler, R. (2014) Abiotic and biotic stress combinations. New Phytologist 203, 32–43.
Tanasijevic, L., Todorovic, M., Pereira, L.S., Pizzigalli, C., & Lionello, P. (2014) Impacts of climate change on olive crop evapotranspiration and irrigation requirements in the Mediterranean region. Agricultural Water Management 144, 54–68.
Therios, I. (2005) Olive production. Gartaganis Publications, Thessaloniki, Greece.
Therios, I.N. (2009) Olives. Crop Production Science in Horticulture series. CABI, London, 237.
Tombesi, S., Farinelli, D., Molfese, M., Cipolletti, M., & Visco, T. (2014) Pruning in young high density hedgerow olive orchards. Acta Horticulturae 1047, 385–390.
Tous, J., Romero, A., & Hermoso, J.F. (2010) New trends in olive orchard design for continuous mechanical harvesting. Advances in Horticultural Science 24, 43–52.
Trichopoulou, A., Costacou, T., Bamia, C., & Trichopoulos, D. (2003) Adherence to a Mediterranean diet and survival in a Greek population. New England Journal of Medicine 348, 2599–2608.
Tsantili, E. (2013) Quality attributes and their relations in fresh black ripe 'Kalamon' olives (*Olea europea* L.) for table use – phenolic compounds and total antioxidant capacity. International Journal of Food Science and Technology 49, 657–665.
Tsantili, E., Kafkaletou, M., Roussos, P.A., & Christopoulos, M.V. (2012) Phenolic compounds, maturation and quality in fresh green olives for table use during exposure at 20 °C after preharvest ReTain treatment. Scientia Hoertuculrurae 140, 26–32.
Tsantili, E., & Pontikis, C. (2004) Response to ethylene and its interactive effects with N6-benzyladenine (BA) in harvested green olives during ripening. Postharvest Biology and Technology 33, 153–162.
Ülger, S., Sonmez, S., Karkacier, M., Ertoy, N., Akdesir, O., & Aksu, M. (2004) Determination of endogenous hormones, sugars and mineral nutrition levels during the induction, initiation and differentiation stage and their effects on flower formation in olive. Plant Growth Regulation 42, 89–95.
USDA NRCS (2015) *Olea europaea* L. ssp. *Europaea*, European olive, PLANTS Profile. USDA NRCS, National Plant Data Team. Available from http://plants.usda.gov/java/reference?symbol=OLEUE&sort=Documentation
Vinha, A.F., Ferreres, F., Silva, B.M., Valentão, P., Gonçalves, A., Pereira, J.A., Oliveira, M.B., Seabra, R.M., & Andrade, P.B. (2005) Phenolic profiles of Portuguese olive fruits (*Olea europaea* L.): influences of cultivar and geographical origin. Food Chemistry 89, 561–568.
Visioli, F., Poli, A., & Galli, C. (2002) Antioxidant and other biological activities of phenols from olives and olive oil. Medical Research Reviews 22, 65–75.
Vossen, P.M. (2007) Organic olive production manual. Publication no. 3505. University of California, Division of Agriculture and Natural Resources, Berkeley, CA.

Wiesman, Z., David, Y., & Dom, N.B. (2004) Optimization of saline water level for sustainable Barnea olive and oil production in desert conditions. Scientia Horticulturae 100, 257–266.

Wodner, M., Lavee, S., & Epstein, E. (1988) Identification and seasonal changes of glucose, fructose and mannitol in relation to oil accumulation during fruit development in *Olea europea* (L.). Scientia Horticulturae 36, 47–54.

Yanik, H., Turktas, M., Dundar, E., Hernandez, P., Dorado, G., & Unver, T. (2013) Genome-wide identification of alternate bearing-associated microRNAs (miRNAs) in olive (*Olea europaea* L.). BMC Plant Biology 13, 10. doi: 10.1186/1471-2229-13-10.

Zipori, I., Yermiyahu, U., Erel, R., Presnov, E., Faingold, I., Ben-Gal, A., & Dag, A. (2015) The influence of irrigation level on olive tree nutritional status. Irrigation Science 33, 277–287.

3 Conventional and organic cultivation and their effect on the functional composition of olive oil

Nikolaos Volakakis, Emmanouil Kabourakis, and Carlo Leifert

3.1 Introduction

Global demand for organic foods has risen sharply over the last 20 years, resulting in a rapid expansion of organic olive oil production and consumption over the same time period. The main reasons for increasing consumer demand for organic products, including olive oil and table olives, have been related to (a) consumer concerns about negative environmental and human health impacts of agrochemicals (e.g., mineral fertilizers, synthetic chemical pesticides, and growth regulators and hormones) used in conventional production, (b) consumer perceptions of higher nutritional value of organically produced foods, and (c) ethical concerns of consumers (Anonymous, 1997, 2002; Benbrook, 2002; Yiridoe *et al.*, 2005; Oughton & Ritson, 2007; Vassiliou *et al.*, 2008). Organic food production in the European Union (EU) is based on organic standards set by the European Community (EC), and all organic producers are regularly audited and certified (EC, 2014). Organic crop production standards prohibit the use of synthetic chemical crop protection products and certain mineral fertilizers (all N, water-soluble P-fertilizers such as superphosphate, and KCl) to reduce environmental impacts (nitrate leaching, phosphate runoff, and pesticide contamination of groundwater) and the risk of pesticide residues being present in crop plants (Baker *et al.*, 2002). Instead, they prescribe regular use of organic fertilizer (e.g., manure and compost) inputs, the use of legume crops in the rotation or as cover crops (to increase soil N levels), and preventative and nonchemical crop protection methods (e.g., the use of crop rotation, more resistant and tolerant varieties, mechanical and flame weeding, and biological disease and pest control products). However, organic standards permit the use of certain plant or microbial extract and/or mineral (e.g., Cu and S based) crop protection products, and the use of mineral micronutrient fertilizers (e.g., Fe, Zn, and B) if deficiency can be clearly demonstrated by soil and plant analysis (Lampkin, 2002; Hansen, 2010; EC, 2014).

As a result, fertilization and crop protection protocols used in organic and conventional crop production differ significantly (Lampkin, 2002; Hansen, 2010). For example, organic olive production relies mainly on (a) mechanical weed control (supplemented on some farms with animal grazing); (b) mass trapping and *Bacillus thuringiensis* (Bt) products for the control of olive fly and olive moth pest, respectively; and (c) animal manures and legume cover crops for soil fertility building. In contrast, in more intensive conventional olive production, (a) herbicides (e.g., glyphosate) are increasingly used for weed control, especially in sloppy olive orchards; (b) synthetic chemical pesticides (e.g., organophosphorus pesticides) are routinely used for pest control; and (c) mineral NPK products are the main fertilizers used (Kabourakis, 1996, 1999; Volakakis, 2009).

In this chapter, we review the existing knowledge on differences in (a) productivity, (b) environmental impact, (c) pesticide residues, and (d) other nutritionally relevant quality parameters between organic and conventional olive oil production systems. Thus, productivity and environmental impact in this review are

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essential to allow potential tradeoffs between yield, environmental, and olive oil quality parameters to be identified.

3.2 Productivity

A recent meta-analysis showed that, different to annual crops such as cereals, field vegetables, and oilseed (where yields are lower in organic compared to conventional production systems), yields in perennial fruit crops such as olive may be similar or even slightly higher in organic compared to conventional production systems (Seufert *et al.*, 2014). It is important to note that only a very limited number of publications were available for comparing organic and conventional olive production systems for inclusion in this meta-analysis. However, a farm survey (carried out in the Messara Valley in Crete), which compared productivity and olive oil quality parameters in organic and conventional production systems, showed similar results as the meta-analysis (Volakakis, 2009). Numerically, both olive fruit and oil yields were slightly (approximately 10%) higher in organic systems, but the difference in yields between organic and conventional systems was not statistically significant (Table 3.1). Kabourakis (1996) and Vassiliou (2000) also reported no yield reduction in an agronomic and economic study. Furthermore, a more recent farm survey that examined the agronomic and environmental impact of olive production yields between organic and conventional systems found that the difference was not statistically significant (Gkisakis *et al.*, 2015, 2016). In contrast, other studies reported lower yields for organic compared to conventional production systems (Kiritsakis, 1998; Tzouvelekas *et al.*, 2001; Parra Lopez & Calatrava Requena, 2005).

Due to the higher labor demands, other input costs, as well as the organic certification cost, the financial viability of organic olive production systems relies on a price premium being achieved in the market (Kabourakis, 1996, 2000; Vassiliou, 2000; Volakakis, 2009).

3.3 Environmental impact

The environmental benefits of organic production are widely accepted by EU/national government subsidies. Environmental benefits include (a) reduced pollution of surface water and groundwater from leaching

Table 3.1 Effects of and interactions between (a) year (2006, 2007, or 2008), (b) production system (organic or conventional), and (c) location (plains or hills) on yields.

Main effect means		Olive fruit yield (t olives ha^{-1})	Olive oil yield (kg oil ha^{-1})
Year	2006	6.0 (1.0)	1184 (190)
	2007	4.8 (0.9)	983 (202)
	2008	5.9 (1.0)	1190 (181)
Production system	Organic	5.9 (0.8)	1182 (148)
	Conventional	5.2 (0.8)	1066 (160)
Location	Plains	7.3 (0.6)	1426 (135)
	Hills	3.7 (0.7)	803 (142)
ANOVA results (*p*-values)			
	Main effects		
	Year	0.4760	0.5858
	Production system	0.4943	0.5585
	Location	0.0016	0.0059
	3-way interactions		
	Year × Production system	0.4090	0.5583
	Year × Location	0.0080	0.0101
	Production system × Location	0.4721	0.3669
	Year × Production system × Location	0.3033	0.4758

Note: The values represent means (SE).

and/or runoff of mineral N and P fertilizers and chemosynthetic crop protection products (Porter *et al.*, 1993; Drinkwater *et al.*, 1998; Stolze *et al.*, 2000; Pesticide Action Network, 2002); (b) increased biodiversity in agricultural ecosystems with respect to birds, invertebrates, and non-crop plant populations (Eggers, 1984; Pysek & Leps, 1991; Stoate *et al.*, 2001; Berry *et al.*, 2004; Critchley *et al.*, 2004, 2006; Bengtsson *et al.*, 2005; Pysek *et al.*, 2005); and (c) reductions in energy use (and associated CO_2 carbon emissions) (Fluck, 1992; Helsel, 1992; Dubois *et al.*, 1999; Cormack, 2000; Pretty *et al.*, 2002). Although there are very few studies for the environmental benefits of organic olive production, it is assumed that it provides similar benefits. Less intensively managed organic olive orchards appeared to support higher diversity (Ruano *et al.* 2004; Solomou & Sfougaris 2011; Gkisakis *et al.*, 2015) and contribute to the landscape diversity (Stobbelaar *et al.*, 2000), although overall environmental benefits in organic olive production vary and depend on the intensity of management and the applied management practices (Gkisakis *et al.*, 2016).

3.4 Pesticide residues

A recent meta-analysis of published information on pesticide residues in organic and conventional crops (Smith-Spangler *et al.*, 2012; Barański *et al.*, 2014) showed that frequency of occurrence of detectable pesticide residues was more than four times higher in conventional than in organic crops, with residues present in approximately 50 and 10% of crop samples, respectively. Both studies concluded that consumption of organic crops is an efficient way to avoid food-based exposure to pesticide residues.

The problem of pesticide residues in conventional olive oil is well documented and was linked to the use of relatively persistent organophosphate (OP) pesticide products for olive fly control (Lentza-Rizos & Avramides, 1995). More recently, the increasing use of the organophosphorus herbicide glyphosate in conventional olive production has also become a concern, since glyphosate, together with two OP insecticides (malathion and diazinon), was recently reclassified by the International Agency for Research on Cancer (IARC) as “probably carcinogenic to humans” (Guyton *et al.*, 2015).

A study in Crete in 2001 and 2002 showed that OP residues can be detected in both organic and conventional olive oil, but that residue levels are significantly lower in organic compared to conventional olive oil (Tsatsakis *et al.*, 2003). The finding of OP residues in organic oil samples was attributed to cross-contamination of organic orchards from pesticide application in conventional orchards (Tsatsakis *et al.*, 2003). In response, additional quality assurance practices (e.g., the non-harvesting of trees bordering conventional orchards, testing for pesticide residues of oil from all organic fields, and rejection of contaminated oils for marketing into organic supply chains) were introduced in organic olive oil production in Greece based on a recommendation originally made by Kabourakis (1999). A more recent survey in the Messara Valley in Crete showed that organic olive oil samples tested positive for OP contamination at a very low frequency, suggesting that the additional quality assurance measures are working well. However, it should be pointed out that these quality assurance practices (especially the non-use of trees bordering conventional orchards and the testing for pesticide residues) result in significant additional costs for organic olive oil producers.

3.5 Oil composition and quality

Apart from minimizing the risk of agrochemical residues being present in crops, the agronomic protocols used in organic farming systems may also affect mineral uptake patterns and metabolic processes in crop plants. Recent studies have shown that switching from mineral to organic fertilizer use results in significant differences in gene and protein expression patterns, and as a result secondary metabolite profiles; for example, approximately 10% of proteins were found to be either up- or downregulated in response to contrasting fertilizer inputs in potato and wheat (Lehesranta *et al.*, 2007; van Dijk *et al.*, 2009, 2010, 2012; Rempelos *et al.*, 2013; Tétard-Jones *et al.*, 2013). In addition, switching from pesticide-based conventional to non-chemical, “organic” crop protection strategies was also shown to have a significant effect on gene and protein expression patterns (Lehesranta *et al.*, 2007; van Dijk *et al.*, 2009, 2010, 2012). It is therefore not surprising that two recent meta-analyses of published data on the nutritional composition of organic and conventional crops showed substantial differences in nutritionally relevant compounds between the two

Table 3.2 Effects of and interactions between (a) year (Y; 2007 or 2008), (b) production system (S; organic or conventional), and (c) location (L; plains or foothills) on olive fruit composition and olive oil quality characteristics.

							ANOVA results (*p*-values)						
	Year		Production system		Geographic location		Main effects			Interactions			
Parameter assessed	**2006–2007**	**2007–2008**	**Org.**	**Con.**	**Plains**	**Hills**	**Y**	**S**	**L**	**Y × S**	**Y × L**	**S × L**	**Y × S × L**
Olive fruit composition													
Water content (%)	48.0 (1.5)	53.8 (1.2)	49.1 (1.4)	52.8 (1.8)	49.2 (1.1)	52.7 (1.9)	0.0018	0.0186	0.0226	0.7021	0.9776	0.0290	0.3986
Oil content (% fresh weight)	25.3 (0.9)	20.5 (0.7)	23.9 (1.2)	21.9 (1.1)	23.8 (0.9)	22.0 (1.4)	0.0004	0.0336	*0.0628*	0.3487	0.5068	0.0298	0.3359
Oil content (% dry weight)	48.6 (0.7)	44.4 (1.0)	46.8 (1.3)	46.2 (1.0)	46.7 (1.0)	46.4 (1.3)	0.0213	0.7012	0.8264	0.5157	0.4330	0.4854	0.7529
Olive oil quality parameters													
Free acidity (%)	0.31 (0.02)	0.40 (0.00)	0.37 (0.02)	0.34 (0.02)	0.34 (0.02)	0.37 (0.02)	<0.0001	0.0056	0.0391	0.1610	0.0410	*0.0668*	0.5212
Peroxide value (meq O_2 kg oil^{-1})	5.32 (0.24)	3.06 (0.39)	4.27 (0.59)	4.11 (0.47)	3.68 (0.49)	4.71 (0.50)	0.0003	0.6808	0.0274	0.1678	0.4250	0.4945	0.3157
K_{270}	0.171 (0.008)	0.206 (0.012)	0.198 (0.013)	0.179 (0.011)	0.178 (0.008)	0.199 (0.015)	0.0245	0.1860	0.1431	0.8563	*0.0737*	0.2086	0.9014
K_{232}	1.729 (0.040)	1.753 (0.071)	1.795 (0.054)	1.686 (0.054)	1.668 (0.041)	1.814 (0.059)	0.6925	0.1066	0.0418	0.5693	0.0290	0.3045	0.8925
K_{262}	0.203 (0.010)	0.226 (0.016)	0.224 (0.014)	0.204 (0.013)	0.207 (0.012)	0.221 (0.016)	0.2744	0.3218	0.4786	0.4786	0.1533	0.3753	0.7059
K_{268}	0.180 (0.009)	0.196 (0.014)	0.198 (0.012)	0.178 (0.011)	0.178 (0.009)	0.197 (0.013)	0.3320	0.2322	0.2431	0.4441	0.1074	0.3771	0.5796
K_{274}	0.163 (0.007)	0.186 (0.015)	0.183 (0.013)	0.165 (0.011)	0.161 (0.009)	0.188 (0.014)	0.1374	0.2325	*0.0937*	0.4050	0.0862	0.2385	0.7550
ΔK	−0.0032 (0.0036)	−0.0103 (0.0012)	−0.0062 (0.0034)	−0.0072 (0.0025)	−0.0063 (0.0035)	−0.0072 (0.0024)	0.1755	0.8442	0.8541	0.8442	0.8245	0.5112	0.5761

Note: The values represent means (SE).

Table 3.3 Effect of, and interaction between (a) production system (S; organic or conventional) and (b) geographic location (L; plains or foothills) on fatty acid composition (%) of olive oil.

	Production system		Geographic location		ANOVA results (*p*-value)		
					Main effects		Interaction
Fatty acid	**Organic**	**Conventional**	**Plains**	**Hills**	**S**	**L**	**S × L**
SFA							
Myristic	0.0119	0.0117	0.0116	0.0119	0.8366	0.7324	0.6176
C14:0	(0.0005)	(0.0007)	(0.0005)	(0.0006)			
Palmitic	12.07	12.18	11.54	12.71	0.7149	0.0026	0.7338
C16:0	(0.33)	(0.26)	(0.12)	(0.26)			
Heptadecaonic	0.0392	0.0355	0.0372	0.0374	*0.0827*	0.9175	0.5718
C17:0	(0.0012)	(0.0014)	(0.0011)	(0.0017)			
Stearic	2.49	2.53	2.46	2.56	0.7679	0.4443	0.4489
C18:0	(0.11)	(0.07)	(0.08)	(0.10)			
Arachidic	0.437	0.436	0.434	0.438	0.9068	0.5885	0.1265
C20:0	(0.007)	(0.004)	(0.005)	(0.006)			
Behenic	0.146	0.144	0.146	0.144	0.2599	0.3386	0.0061
C22:0	(0.001)	(0.002)	(0.001)	(0.002)			
MUFA							
Palmitoleic	0.777	0.812	0.712	0.877	0.4940	0.0056	0.6257
C16:1 c9	(0.045)	(0.045)	(0.019)	(0.043)			
Heptadecanoic	0.068	0.065	0.067	0.066	0.3553	0.6779	0.4618
C17:1 c10	(0.002)	(0.002)	(0.002)	(0.002)			
Oleic	76.49	76.49	77.41	75.57	0.9993	*0.0503*	0.2265
C18:1 c9	(0.86)	(0.45)	(0.38)	(0.74)			
Eicosenoic	0.277	0.284	0.288	0.273	0.2383	0.0255	0.1394
C20:1 c11	(0.007)	(0.003)	(0.003)	(0.005)			
PUFA							
Linoleic	6.29	6.06	5.97	6.39	0.6322	0.3794	*0.0748*
C18:2 c9 c12	(0.43)	(0.26)	(0.28)	(0.41)			
α-linolenic	0.710	0.740	0.722	0.728	0.4086	0.8564	0.7712
C18:3 c5 c9 c12	(0.025)	(0.021)	(0.021)	(0.026)			
Eicosadienoic	0.139	0.142	0.144	0.137	0.3419	*0.0853*	0.9079
C20:2 c11 c14	(0.002)	(0.003)	(0.002)	(0.003)			

Note: The values represent means (SE); SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

categories (Brandt *et al.*, 2013; Barański *et al.*, 2014). Specifically, organic crops were shown to have higher antioxidant activity and higher concentrations of a range of antioxidants, (poly)phenolics, and/or vitamins and lower concentrations of the toxic metal cadmium. However, there are very few studies comparing the nutritional quality of organic and conventional olive oil, and from the data available there appear to be few differences in the nutritional quality of the two categories of olive oil (Gutierrez *et al.*, 1999; Parra Lopez & Calatrava Requena, 2005; Samman *et al.*, 2008). This was confirmed by a recent oil quality survey in the Messara Valley (Volakakis, 2009). While production year and location of olive orchards (extensively managed orchards in the foothills vs. more intensively managed orchards on the plains) had significant effects on standard oil quality parameters, the production system (organic vs. conventional) had a very limited effect (Table 3.2).

Since it is well known that organic management practices can significantly affect the fatty acid composition of meat and milk fat (Średnicka-Tober *et al.*, 2016), a similar study (Volakakis, 2009) also compared the fatty acid composition in olive oil from organic and conventional farms. However, no significant differences in fatty acid composition were observed (Table 3.3). Similar results were reported by Gutiérrez *et al.* (1999) and Samman *et al.* (2008).

It should be noted that both olive oil yield and quality are significantly reduced by high levels of olive fly fruit infestation (Neuenschwander & Michelakis, 1978; Samman *et al.*, 2008). This may explain the slightly but significantly higher levels of acidity in organic olive oil reported by Volakakis (2009) (Table 3.2). The prohibition against using OP pesticides for the control of olive fly results in higher olive fly infestation in organic production systems (Vassiliou, 2000). In organic production, mass-trapping systems are widely used for olive fly control (although some farmers also use pesticides permitted under EU-organic regulations, such as rotenone). Both mass trapping and crop protection products permitted under organic farming standards are thought to be less effective than pesticide-based crop protection, especially in areas with higher pest population pressure (Kabourakis, 1999, 2000; Parra Lopez & Calatrava-Requena, 2005; Volakakis *et al.*, 2012). However, according to our knowledge, there are no studies comparing the efficacy of mass trapping with pesticide-based olive fly control protocols.

3.6 Conclusion

The most important obstacle preventing further increases in demand for organic foods is the higher price of organic products. Prices for organic table olives, olive oil, and other processed olive products are currently between 50 and 100% higher than those of equivalent products from conventional production. This is mainly attributed to higher costs of inputs (e.g., manure, legume seed, and mass-trapping systems), quality assurance, and certification in organic production systems. However, there are still relatively few studies comparing the yields and cost structures in organic and conventional production systems.

The available evidence suggests that organic olive oil and table olive production systems result in (a) lower environmental impact (especially with respect to nitrate leaching, phosphorus runoff, and pesticide contamination) and (b) lower environmental and food-based exposure of consumers to potentially harmful OP and other pesticides. In contrast, there is currently no evidence for substantial differences in other olive quality parameters such as acidity (free fatty acid) and fatty acid composition.

It seems that there are no significant differences in the antioxidant composition, nutritional quality, and functional value between organic and conventional production. However, higher prices for organic olive oil and table olives are currently mainly justified by evidence for lower negative environmental and pesticide exposure related to human health impact.

References

Anonymous (1997) Organic and ethical foods: market intelligence. Mintel International Group Ltd., Rockville, MD.

Anonymous (2002) Organic farming, food quality and human health: a review of the evidence. The Soil Association, Bristol, UK.

Baker, B.P., Benbrook, C.M., Groth, E., & Benbrook, L.K. (2002) Pesticide residues in conventional, integrated pest management (IPM)-grown and organic foods: insights from three US data sets. Food Additives & Contaminants 19, 427–446.

Barański, M., Średnicka-Tober, D., Volakakis, N., Seal, C., Sanderson, R., Stewart, G.B., Benbrook, C., Biavati, B., Markellou, E., Giotis, H., Gromadzka-Ostrowska, J., Rembiałkowska, E., Skwarło-Sonta, K., Tahvonen, R., Janovská, D., Niggli, U., Nicot, P., & Leifert, C. (2014) Higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops: a systematic literature review and meta-analysis. British Journal of Nutrition 112, 794–811.

Benbrook, C.M. (2002) Organochlorine residues pose surprisingly high dietary risks. Journal of Epidemiology and Community Health 56, 822–823.

Bengtsson, J., Ahnstrom, J., & Weibull, A.C. (2005) The effects of organic agriculture on biodiversity and abundance: a meta-analysis. Journal of Applied Ecology 42, 261–269.

Berry, P., Ogilvy, S., & Gardner, S., ADAS. (2004) Integrated farming and biodiversity. English Nature Research Reports no. 634. English Nature, Worcester.

Brandt, K., Średnicka-Tober, D., Barański, M., Sanderson, R., Leifert, C., & Seal, C. (2013) Methods for comparing data across differently designed agronomic studies: examples of different meta-analysis methods used

to compare relative composition of plant foods grown using organic or conventional production methods, and a protocol for a systematic review. Journal of Agricultural and Food Chemistry 61, 7173–7180.

Cormack, W.F. (2000) Energy use in organic farming systems: final report of Defra project OF0182. Available from http://www.defra.gov.uk/research/project_data

Critchley, C.N.R., Allen, D.S., Fowbert, J.A., Mole, A.C., & Gundrey, A.L. (2004) Habitat establishment on arable land: assessment of an agri-environment scheme in England, UK. Biological Conservation 119, 429–442.

Critchley, C.N.R., Fowbert, J.A., & Sherwood, A.J. (2006) The effects of annual cultivation on plant community composition of uncropped arable field boundary strips. Agriculture Ecosystems and Environment 113, 196–205.

Drinkwater, L.E., Wagoner, P., & Sarrantonio, M. (1998) Legume-based cropping systems have reduced carbon and nitrogen losses. Nature 396, 262–264.

Dubois, D., Gunst, L., Fried, P.M., Stauffer, W., Spiess, E., Alfoldi, T., Fliessbach, A., Frei, R., & Niggli, U. (1999) DOC-trial, yields and energy use efficiency. Agrarforschung 6, 293–296.

Eggers, T. (1984) Changes in arable weed vegetation. Mitteilungen aus der Biologischen Bundesanstalt fur Land- und Forstwirtschaft Berlin-Dahlem 223, 62–65.

European Community (EC). (2014) Commission implementing Regulation (EU) No 836/2014 f 31 July 2014 amending Regulation (EC) No 889/2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. In: Official Journal of the European Communities. 2008R0889 - EN-01.01.2015-010.003- 1. EC, Brussels, Belgium.

Fluck, R.C. (1992) Energy Analysis of Agricultural Systems. In: Energy in World Agriculture, vol. 6, ed. R.C. Fluck. Elsevier Science Publishing, Amsterdam, 45–52.

Gkisakis, V.D., Kollaros, D., Bàrberi, P., Livieratos, I.C., & Kabourakis, E.M. (2015) Soil arthropod diversity in organic, integrated, and conventional olive orchards and different agroecological zones in Crete, Greece. Agroecology and Sustainable Food Systems 39, 276–294.

Gkisakis, V.D., Volakakis N., Kollaros, D., Bàrberi, P., & Kabourakis, E.M. (2016) Soil arthropod community in the olive agroecosystem, determined by environment & farming practices in different management systems and agroecological zones. Agriculture, Ecosystems & Environment 218, 178–189.

Gutierrez, F., Arnaud, T., & Albi, M.A. (1999) Influence of ecological cultivation on virgin olive oil quality. Journal of the American Oil Chemists' Society 76, 617–621.

Guyton, K.Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., Scoccianti, C., Mattock, H., & Straif, K. (2015) Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. Lancet Oncology 16, 490–491.

Hansen, A.L. (2010) Organic farming manual, a comprehensive guide to starting and running a certified organic farm, Storey Publishing, North Adams, MA.

Helsel, Z.R. (1992) Energy and alternatives for fertiliser and pesticide use. In: Energy in World Agriculture, vol. 6, ed. R.C. Fluck. Elsevier Science Publishing, Amsterdam, 177–210.

Kabourakis, E. (1996) Prototyping and dissemination of ecological olive production systems: a methodology for designing and dissemination of prototype ecological olive production systems (EOPS) in Crete. Thesis, Landbouw Universiteit, Wageningen.

Kabourakis, E. (1999) Code of practices for ecological olive production systems. Olivae 77, 46–55.

Kabourakis, E. (2000) Learning processes in designing and disseminating ecological olive production systems in Crete. In: Knowing and learning for change in agriculture: case studies from industrialised countries, ed. M. Cerf, D. Gibbon, B. Hubert, J. Jiggins, M. Paine, J. Proost, & N. Rolling. INRA Editions, Paris, 97–111.

Kiritsakis, A. (1998) Olive oil: from the tree to the table, 2nd ed. Food and Nutrition Press, Trumbull, CT.

Lampkin, N.H. (2002) Organic farming, 2nd ed. Old Pond Publishing, Sheffield, UK.

Lehesranta, S.J., Koistinen, K.M., Massat, N., Davies, H.V., Shepherd, L.V., McNicol, J.W., Cakmak, I., Cooper, J., Lück, L., Kärenlampi, S.O., & Leifert, C. (2007) Effects of agricultural production systems and their components on protein profiles of potato tubers. Proteomics 7, 597–604.

Lentza-Rizos, C., & Avramides, E.J. (1995). Pesticide residues in olive oil. Reviews in Environmental Contamination and Toxicology 141, 111–134.

Neuenschwander, P., & Michelakis, S. (1978) Infestation of *Dacus oleae* (Gmel.) (Diptera, Tephritidae) at harvest time and its influence on yield and quality of olive oil in Crete. Zeitschrift für Angewandte Entomologie 86, 420–433.

Oughton, E., & Ritson, C. (2007) Food consumers and organic agriculture. Handbook of Organic Food Quality and Safety. CRC Press, Boca Raton, FL, 74–94.

Parra Lopez, C., & Calatrava Requena, J. (2005) Factors related to the adoption of organic farming in Spanish olive orchards. Spanish Journal of Agricultural Research 3, 5–16.

Pesticide Action Network (2002) Pesticide impacts in the environment. Available from http://www.pan-europe.info/About%20pesticides/Impacts%20environment.shtm.

Porter, W.P., Green, S.M., Debbink, N.L., & Carlson, I. (1993) Groundwater pesticides: interactive effects of low concentrations of carbamates aldicarb and methomyl and the triazine metribuzin on thyroxine and somatrophin levels in white rats. Journal of Toxicology and Environmental Health 40, 15–34.

Pretty, J.N., Ball, A.S., Li, X.Y., & Ravindranath, N.H. (2002) The role of sustainable agriculture and renewable-resource management in reducing greenhouse-gas emissions and increasing sinks in China and India. Philosophical Transactions of the Royal Society of London Series A-Mathematical Physical and Engineering Sciences 360, 1741–1761.

Pysek, P., & Leps, J. (1991) Response of a weed community to nitrogen fertilization: a multivariate analysis. Journal of Vegetation Science 2, 237–244.

Pysek, P., Jarosik, V., Kropac, Z., Chytry, M., Wild, J., & Tichy, L. (2005) Effects of abiotic factors on species richness and cover in Central European weed communities. Agriculture Ecosystems and Environment 109, 1–8.

Rempelos, L., Cooper, J., Wilcockson, S., Eyre, M., Shotton, P., Volakakis, N., Orr, H.C., Leifert, C., Gatehouse, R.M.A., & Tétard-Jones, C. (2013) Quantitative proteomics to study the response of potato to contrasting fertilization regimes. Molecular Breeding 31, 363–378.

Ruano, F., Lozano, C., Garcia, P., Peña, A., Tinaut, A., Pascual, F., & Campos, M. (2004) Use of arthropods for the evaluation of the olive-orchard management regimes. Agricultural and Forest Entomology 6, 111–120.

Samman, S., Chow, J.W.Y., Foster, M.J., Ahmad, Z.I., Phuyal, J.L., & Petocz, P. (2008) Fatty acid composition of edible oils derived from certified organic and conventional agricultural methods. Food Chemistry 109, 670–674.

Seufert, V., Ramankutty, N., & Foley, J.A (2014) Comparing the yields of organic and conventional agriculture. Nature 485, 229–232.

Smith-Spangler, C., Brandeau, M.L., Hunter, G.E., Bavinger, J.C., Pearson, M., Eschbach, J.P., Sundaram, V., Liu, H., Schirmer, P., Stave, C., Olkin, I., & Bravata, D.M. (2012) Are organic foods safer or healt hier than conventional alternatives? A systematic review. Annals of Internal Medicine 157, 348–366.

Średnicka-Tober, D., Marcin Barański, M., Seal, C.J., Sanderson, R., Benbrook, C., Steinshamn, H., Gromadzka-Ostrowska, J., Rembiałkowska, E., Skwarło-Sońta, K., Eyre, M., Cozzi, G., Krogh Larsen, M., Jordon, T., Niggli, U., Sakowski, T., Calder, P.C., Burdge, G.C., Sotiraki, S., Stefanakis, A., Stergiadis, S., Yolcu, H, Chatzidimitriou, E., Gillian Butler, G., Gavin Stewart, G., & Leifert, C. (2016) Higher PUFA and omega-3 PUFA, CLA, α-tocopherol and iron, but lower iodine and selenium concentrations in organic milk: a systematic literature review and meta- and redundancy analyses. British Journal of Nutrition 115, 1043–1060.

Solomou, A., & Sfougaris, A. (2011). Comparing conventional and organic olive groves in central Greece: plant and bird diversity and abundance. Renewable Agriculture and Food Systems 26, 297–316.

Stoate, C., Boatman, N.D., Borralho, R.J., Carvalho, C.R., de Snoo, G.R., & Eden, P. (2001) Ecological impacts of arable intensification in Europe. Journal of Environmental Management 63, 337–365.

Stobbelaar, D.J., Kuiper, J., van Mansvelt, J.D., & Kabourakis, E. (2000) Landscape quality on organic farms in the Messara Valley, Crete: organic farms as components in the landscape. Agriculture, Ecosystems and Environment 77, 79–93.

Stolze, M., Piorr, A., Haring, A., & Dabbert, S. (2000) The environmental impacts of organic farming in Europe. University of Hohenheim Publications, Stuttgart.

Tétard-Jones, C., Shotton, P.N., Rempelos, L., Cooper, J., Eyre, M., Orr, H.C., Leifert, C., & Gatehouse, M.R.A. (2013) Quantitative proteomics to study the response of wheat to contrasting fertilisation regimes. Molecular Breeding 31, 379–393.

Tsatsakis, A.M., Tsakiris, I.N., Tzatzarakis, M.N., Agourakis, Z.B., Tutudaki, M., & Alegakis, A.K. (2003) Three-year study of fenthion and dimethoate pesticides in olive oil from organic and conventional cultivation. Food Additives and Contaminants 20, 553–559.

Tzouvelekas, V., Pantzios, C.J., & Fotopoulos, C. (2001) Technical efficiency of alternative farming systems: the case of Greek organic and conventional olive-growing farms. Food Policy 26, 549–569.

van Dijk, J.P., Cankar, K., Hendriksen, P.J.M., Beenen, H.G., Zhu M., Scheffer, S., Shepherd, L.V., Stewart, D., Davies, H.V., Leifert, C., Wilkockson, S.J., Gruden, K., & Kok, E.J. (2012). The identification and interpretation of differences in the transcriptomes of organically and conventionally grown potato tubers. Journal of Agricultural and Food Chemistry 60, 2090–2101.

van Dijk, J.P., Cankar, K., Scheffer, S.J., Beenen, H.G., Shepherd, L.V., Stewart, D., Davies, H.V., Wilkockson, S.J., Leifert, C., Gruden, K., & Kok, E.J. (2009) Transcriptome analysis of potato tubers-effects of different agricultural practices. Journal of Agricultural and Food Chemistry 57, 1612–1623.

van Dijk, J.P, Leifert, C., Barros, E., & Kok, E.J. (2010) Gene expression profiling for food safety assessment, examples in potato and maize. Regulatory Toxicology and Pharmacology 58, 21–25.

Vassiliou, A. (2000) Farm structure optimization of, and the impact of widespread conversion to ecological olive production systems. Institute of Rural Studies, University of Wales, Cardiff.

Vassiliou, A., Kabourakis, E., & Papadopoulos, D. (2008) Traceability and ethical traceability in the Greek olive oil chain. In: Ethical traceability and informed choice, ed. C. Coff, D. Barling, M. Korthals, & T. Nielsen. Springer, Berlin, 167–191.

Volakakis, N. (2009) Alternative strategies for the control of fruit flies in organic olive production. PhD thesis, Newcastle University, UK.

Volakakis, N.G., Eyre, M.D., & Kabourakis, M.E. (2012) Olive fly *Bactrocera oleae* (Diptera, Tephritidae), activity and fruit infestation under mass trapping in an organic table olive orchard in Crete, Greece. Journal of Sustainable Agriculture 36, 683–698.

Yiridoe, E. K., Bonti-Ankomah, S. & Martin, R.C. (2005) Comparison of consumer perceptions and preference toward organic versus conventionally produced foods: a review and update of the literature. Renewable Agriculture and Food Systems 20, 193–205.

4 The influence of growing region and cultivar on olives and olive oil characteristics and on their functional constituents

Joan Tous

4.1 Introduction

The olive tree (*Olea europaea* L.) is one of the most important crops in Mediterranean countries, especially Spain, Italy, Greece, Tunisia, Turkey, and Morocco. In recent decades, the interest in extra virgin olive oil (EVOO) has grown due to its recognized nutritional, sensorial, culinary, and healthy properties and due to its being one of the fundamental components of the "Mediterranean diet." Several functional compounds are included in its minor fraction and play an important role in human health (Visioli & Galli, 1998; Martínez de Victoria & Mañas, 2004; Montedoro *et al*., 2007; García-González *et al*., 2008; Servili *et al*., 2011; Perona & Botham, 2013).

Moreover, the interest in olive trees and olive oil has extended to other countries such as Argentina, Chile, Uruguay, Australia, New Zealand, South Africa, and the United States. This expansion, which is primarily due to new agricultural practices (irrigation, intensive plantation, mechanization, and cultivars) devised by farmers to increase olive oil yield per hectare without parallel loss of sensory and nutritional properties, is based on the adaptation of cultivars to climates as well as latitudes and altitudes different from those of their native Mediterranean regions. It is also known that the composition and quality of EVOO are affected by factors such as cultivar, fruit ripening, cultural practices, processing methods, and agroclimatic conditions (Kiritsakis, 1992; Uceda *et al*., 2004; Tous *et al*., 2005a; Montedoro *et al*., 2007; Inglese *et al*., 2010; Servili *et al*., 2011; García-González, *et al*., 2009, 2012; Aparicio *et al*., 2013).

Many researchers have shown that the cultivar may have a significant effect on the olive's fatty acid profile as well as polyphenol and tocopherol contents (Uceda *et al*., 2004, 2005; Rondanini *et al*., 2011), while others have shown that geographical location has a significant effect on these parameters and other minor components, mainly in some cultivars (Tous & Romero, 1994; Tous *et al*., 1997; Aguilera *et al*., 2005; Ceci & Carelli, 2007; García-González *et al*., 2010; Mailer *et al*., 2010; Romero *et al*., 2016).

This chapter reviews the current situation of olive orchard characteristics in some world olive crop areas (the Mediterranean Basin, North and South America, as well as Australia) and analyzes the influence of the growing regions and cultivars on EVOO's characteristics and functional constituents.

4.2 Overview of olive orchards in some world crop areas

Olive plantation systems occur in a wide variety throughout the Mediterranean. Today significant producers show up in other areas, like North and South America, Australia, New Zealand, and South Africa. The

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Table 4.1 Surface and olive oil production average of several global olive oil countries.

Country	Surface (ha) 2012–2013	Average oil production (tons) 2009–2010 and 2014–2015
European Union		
Spain	2,584,564	1,273,850
Italy	1,350,000	394,650
Greece	1,160,000	284,250
Portugal	358,513	69,900
Magreb countries		
Tunisia	1,839,600	172,800
Morocco	922,000	123,000
Middle East		
Turkey	831,000	164,700
Syria	647,500	153,000
South America		
Argentina	100,000	20,333
Chile	24,000	15,750
Others		
Australia	30,000	15,167
United States (California)	21,300*	10,000**
European Union – total	**5,523,122**	**2,034,300**
World total	**11,188,406**	**2,878,800**

* L. Ferguson, P. Vossen, and D. Flynn (personal communication, 2015); ** Average crops for 2013–2014 and 2014–2015.
Adapted from International Olive Council (IOC); and L. Ferguson, P. Vossen, and D. Flynn, personal communication, 2015.

world's olive crop surface is over 11 million hectares, with major production in Mediterranean countries (95% of 2.87 Mt oil; Table 4.1). According to Tous *et al.* (2014), the typology of planting models distribution in the olive world is as follows:

- 70% of this surface is for traditional (T), marginal, or hilly Mediterranean groves with less than 120 trees/ha.
- 29% is for modern orchards, like intensive (IS) orchards with 200–400 trees/ha as well as high-density (HD) ones with 450–800 trees/ha.
- 1% is for super-high-density (SHD) orchards with over 1500 trees/ha.

Regarding the SHD planting model, currently the worldwide planted area of olive trees in a hedgerow system exceeds 100,000 ha (50% in Spain, 15% in Portugal, 14% in Chile, 8% USA, 4% in Tunisia, and the remaining 9% in other places). All these various planting systems have very different performances regarding economic profit (Pastor *et al.*, 2006; AEMO, 2010; Freixa *et al.*, 2011; Tous, 2011) and environmental and social impacts (Junta de Andalucía, 2003; Fleskens, 2008; Velasco *et al.*, 2011).

The characteristics of some distinguished olive orchards of six Mediterranean countries (Spain, Italy, Greece, Portugal, Tunisia, and Morocco), two South American countries (Argentina and Chile), and two more countries (Australia and the United States) were used for the analysis of variability of EVOO (Figure 4.1). Surface and oil production (average 2009–2010 to 2014–2015 crops) of these countries are shown in Table 4.1. In each country, a classification of the existing olive orchards was made, with main focus on planting models, orchard size, environmental site (rainfall and soils), cultivars, yields, pests, harvest methods, and manpower available (Table 4.2).

Figure 4.1 The global areas of olive orchards in the Mediterranean, South America, Australia, and the United States used for the analysis of extra virgin olive oil (EVOO) characteristics.

4.2.1 European Union (EU)

4.2.1.1 Spain

This is the world's main olive oil and table olives producing and exporting country. The olive tree cultivation area in this country is more than 2.5 million ha, which represents 23% of the world coverage, and the average oil production is about 1.2 million tons. The Andalusia region, which surrounds Jaén and Cordoba, is the most important area, and it contains 62% of Spain's orchards, followed by other regions, such as Castilla-La Mancha (8%), Extremadura (5%), and Catalonia (4%). Of all the cultivars grown in Spain, 24 are classified as major (Barranco, 2004), with 'Picual', 'Hojiblanca', 'Arbequina' (which are described below), 'Cornicabra', 'Manzanilla', and 'Empeltre' being the most important. Regarding the orchard types, cultivars, climate, and soils, Andalusia ('Picual' cv. [cultivar]) and Catalonia ('Arbequina' cv.) are quite different (Table 4.2). In the first region, traditional olives are mostly grown on the hillsides under dry-farming conditions with plantings that have very wide spacing, and every tree has three to four trunks. The altitude of these regions ranges from 100 to 400 m. The orchard sizes vary and depend on the region and the planting model, being 5 ha for T, 10–50 ha for IS, 50–100 ha for SHD, and 200 ha for HD. The main ecological factors that determine the productivity of the olive orchards in rain-fed conditions are erosion, associated with the slope of the lands, rainfall, and frost (Rallo, 2006). The yield potential in modern Andalusia orchards ranges from 5 to 15 t/ha and is considerably higher than in Catalonia (5–10 t/ha). The harvest methods are similar (manual and mechanical by trunk shakers and grape harvesters). With respect to pests and diseases, leaf spot (*Fusicladium oleagineum*), olive fly (*Bactrocera oleae*), bacterial canker (*Pseudomonas savastanoi*), and verticillium wilt (*Verticillium dahlia*) are the most common. The 'Picual' cultivar in Andalusia has a high incidence of this last disease. The Spanish workers' labor costs are high, about 6–6.5 €/hour.

Table 4.2 Comparison of the most outstanding characteristics of several olive crop areas located in the Mediterranean Basin and South America.

Country. Production area. *Hemisphere (H)*	Latitude	Altitude (m)	Average Annual Tª (°C)	Rainfall (mm)	Soil	Orchard types	Oil cultivars
Spain. Catalonia: Reus and Borges Blanques. *N*	41°N and 41°30′N	113–304	15.8–14.4	500–415	Plain-hilly; little fertile; basic pH; calcareous	Traditional Intensive SHD	'Arbequina'
Spain. Andalusia: Córdoba and Jaén (Úbeda). *N*	37°46′N	110–578	16.8–16.0	500–495	Hilly; clay-loamy texture; deep; basic pH; calcareous	Traditional Intensive SHD	'Picual' (42%), 'Hojiblanca', 'Arbequina'
Italy. Toscana: Firenze. *N*	43°48′N	51	14.6	763	Light slope, clay-loamy texture, neutral to slightly alkaline pH	Traditional Intensive	'Frantoio' (48%), 'Moraiolo' (22%), 'Leccino' (16%)
Italy. Puglia: Bari. *N*	41°30′N	40	16.7	500–600	Plain, loamy-sandy texture, moderate to high lime content, basic pH	Traditional Intensive SHD	'Coratina', 'Peranzana'
Greece. Crete: Heraklion	35°N	37	18,3	478	Plain, clay-loamy texture, different soil types (poor, rocky, or fertile)	Traditional Intensive	'Koroneiki'
Portugal. Alentejo: Beja-Ferreira. *N*	38°02′N	245	17.0	450–550	Hilly; acid pH; clay, clay-loamy textures	Intensive HD SHD	'Arbequina' (80%), 'Cobrançosa', 'Picual', 'Arbosana'
Tunisia. North Tunisia. *N*	36°48′N	200–300	18.4	400–500	Plain and hilly; deep; clay-loamy; basic pH; calcareous	Traditional Intensive SHD	'Chetoui', 'Arbequina', 'Arbosana', 'Koroneiki'
Tunisia. Center-south, Sfax. *N*	34°44′N	50	18.6	200–300	Plain areas; sandy texture; basic pH; deep; calcareous	Traditional Intensive SHD	'Chemlali', 'Arbequina'
Morocco. Central region: Marrakech. *N*	32°N	460	20.3	250	Plain, fertile, deep, basic pH; loamy red clay soils	Intensive SHD	'Picholine', 'Marocaine', 'Arbequina', 'Leccino'
United States: California. Sacramento Valley (SV): Sacramento. *N*	38°N	5	17.1	474	Plain, usually deep, fertile, basic pH; calcareous	Intensive SHD	'Manzanilla', 'Mission', 'Arbequina', 'Arbosana', 'Frantoio, 'Koroneiki'

United States: California. San Joaquin Valley (SJV): Bakersfield. *N*	38°N	142	18.7	145	Plain, deep, fertile, basic pH; calcareous	Intensive, SHD	'Manzanilla', 'Gordal', 'Arbequina', 'Arbosana'
Argentina. La Rioja: Chilecito[a]. *S*	29°14′S	850–945	18.5	180	Plain; deep; basic pH; gravel and sandy-silt textures; calcareous; little fertile	Intensive HD	'Arbequina', 'Barnea', 'Picual', 'Coratina', 'Arbosana'
Argentina. San Juan: Cañada Honda[a]. *S*	31°33′S	630–700	18.2	125	Plain; deep; basic pH; little fertile; sandy-loamy; calcareous	Intensive HD	'Arbequina' (50%), 'Picual', 'Coratina', 'Barnea', 'Changlot', 'Hojiblanca', Arbosana'
Chile. Región III: Copiapó, Atacama. *S*	27°36′S	160	17.0	100	Plain; little fertile; basic pH; calcareous; moderately saline	Traditional Intensive SHD	'Arbequina', 'Leccino', 'Frantoio', 'Coratina', 'Arbosana'
Chile. Región IV: Coquimbo. *S*	30°S	200	16.5	150	Plain, basic pH; little fertile; calcareous	Intensive SHD	'Arbequina', 'Arbosana', 'Leccino','Picual', 'Frantoio', 'Koroneiki'
Chile. Región VII: Maule. *S*	38°S	300	14.3	500–600	Hilly; fertile, deep and clay-loamy soils, acid pH.	Intensive SHD	'Arbequina' (over 5%) 'Arbosana', 'Picual', 'Leccino', 'Frantoio'., 'Coratina'
Australia. Victoria: Mildura. *S*	34°11′S	51	17.1	292	Plain, little fertile, neutral to slightly alkaline pH, sandy-loamy soils, moderately saline	Intensive, HD	'Barnea', 'Frantoio', 'Arbequina', 'Arbosana', 'Picual', 'Coratina', 'Leccino'
Australia Western Australia (WA): York. *S*	31°52′S	174	17.6	450	Plain, little fertile, basic pH, sand over shallow clay texture	Intensive	'Barnea', 'Frantoio', 'Picual', 'Leccino', 'Coratina', 'Arbequina'

Source: Based on data from Tous *et al.* (2014) and other country reports.
[z] HD: high density; [y] SHD: super-high density; [x] In other valleys of La Rioja and San Juan provinces the soils are moderately saline.

4.2.1.2 Italy

This is the world's main consuming, importing, and exporting country for olive oil. The olive is cultivated on more than 1.35 million ha (12% of the world total), and the average oil production is about 394,650 tons (Table 4.1). The southern area (Puglia, Calabria, and Sicily regions) is the most important (about 80% of the total production), followed by the north-central zone (Tuscany, Lazio, and Liguria regions, covering the remaining production). There are several cultivars, four of them being outstanding ('Frantoio', 'Leccino', 'Carolea', and 'Coratina') and representing about 33% of the total production. There is a differentiation between the 'Frantoio' and 'Leccino' cv. in Tuscany and the 'Coratina' cv. in Puglia with respect to orchard kinds, climate, and soils, as shown in Table 4.2. The altitude of these regions ranges from 200 to 500 m. The traditional orchard sizes are very small (less than 2 ha), rain fed, and aged, with low planting density; moreover, they are very costly. The modern orchards are usually of the IS planting model (250–500 trees/ha), and currently some SHD plantings have started to appear. Sometimes, low temperatures below 0 °C may occur, and olive trees might be damaged, mainly in the Liguria, Tuscany, and Umbria regions. The yield potential in modern Puglia orchards ranged from 8 to 9 t/ha, considerably higher than in Tuscany (6–7 t/ha). At present, less than 20% of plantations are located on terraces or slopes with an inclination of more than 15% (north-central), while the rest is located in flat, easily manageable areas. The harvest methods are similar to those of Spain (manual and mechanical by trunk shakers), but in Puglia and Sicily some grape harvesters are also used. Pest and diseases, like leaf spot, olive fly, olive knot (*Pseudomonas savastanoi*), and recently new bacterial diseases (e.g., *Xylella fastidiosa*), are the most common. The last disease causes a serious problem to Puglia traditional orchards. The Italian manpower costs are very high (7–10 €/hour), higher than in Spain and other Mediterranean countries.

4.2.1.3 Greece

Greece is an important producing and exporting country of both olive oil and table olives. Olive orchards' total area was estimated to be around 1.16 million ha (10.36% of the world total), and average oil production was about 284,250 tons. Among the most important Greek cultivars are 'Koroneiki', 'Kalamon', 'Chalkidikis', 'Konservolia', 'Throumbolia', 'Tsounati', and several others (Balatsouras, 1986; Therios, 2009). 'Chalkidikis' olives are mainly processed as table olies (10 g per fruit). Generally olive orchards in Greece are mainly located in Peloponnese, the island of Crete, and other islands, as well as in Chalkidiki. Lately, there has been a great spread of olive tree cultivation in northern Greece. The altitude of the olive orchards ranges from 50 to 1000 m with annual rainfall ranging from 200 to 1000 mm/year (Chartzoulakis, 2012). The olive trees grow principally on the hills, where clayey and limestone soils predominate. The orchard sizes are very small with an average of less than 2 ha; they are rain fed, aged, and with high harvest costs. According to Metzidakis and Koubouris (2006), there are mainly three planting models: traditional (less than 100 trees/ha with a yield of about 1250 kg/ha), semi-intensive (100–150 trees/ha, usually rain fed with a yield ranging between 2500–3750 kg/ha), and intensive (irrigated with densities of 200–500 trees/ha, and yield levels up to 5000 kg/ha).

The main ecological factors that determine the productivity of the olive orchards in rain-fed conditions are erosion, associated with the slope of the lands and rainfall. The harvest methods are manual and mechanical by olive pickers. With respect to pests and diseases, olive fly, leaf spot, and bacterial canker (*Pseudomonas savastanoi*) are the most common. The Greek manpower costs are lower than in Spain and Italy, about 4–4.5 €/hour.

4.2.1.4 Portugal

Portugal has experience high olive expansion during the past decade. Its olive cultivation area surface is about 358,513 ha, and the average oil production is 69,900 tons. Alentejo is the most important productive region in Portugal with 64,000 ha (Fevereiro *et al.*, 2011). The Beja Alqueva Dam growing area is outstanding for its high olive expansion during a seven-year period (2003–2010), with about 25,000 ha of new orchards, a unique phenomenon in Europe (Tous *et al.*, 2014). Three planting models are used (IS, HD, and SHD) with 'Arbequina' being the main cultivar, which represents 80% of the total growing area, followed by others, like 'Cobrançosa', 'Picual', and 'Arbosana'. Concerning the terrain, they are quite different from those in

Spain. Normally they are hilly, with an acid pH and clay-loamy textures (Table 4.2). The altitude of this region is about 245 m. The orchard size is variable, according to the planting model, meaning below 250 ha for IS and over 450 ha for HD with a colossus harvester and SHD. The yield potential in Alentejo's modern orchards ranges between 9 and 10 t/ha. Mechanical harvest methods used are similar to those of Andalusia: trunk shakers as well as colossus and grape harvesters. With respect to pests and diseases, leaf spot, olive fly, and olive knot are susceptibilities. Portugal's manpower costs are similar to those of Spain, about 5.5–6 €/hour.

4.2.2 Maghreb countries

4.2.2.1 Tunisia

Tunisia is an important oil-producing and exporting country. The olive tree is implanted on more than 1.8 million ha, which represent 30% of the cultivated land (96.5% traditional and 3.5% modern orchards). The average oil production is about 172,000 t. There are three main growing areas: first, the northern (Tunis) part has a surface of 230,000 ha, a subtropical Mediterranean climate, and rainfall of over 500 mm/year. The second one, located in the central plateau region with an area of about 842,000 ha, has a semi-arid Mediterranean climate with 300–350 mm annual rainfall. The third one is situated in the southern desert region (Sfax area) with a surface of 708,000 ha and a hot subtropical climate, where rainfall is less than 250 mm/year. Table 4.2 shows the most outstanding characteristics of some olive crop areas located in Tunisia, as climate and cultivars ('Chetoui' and 'Chemlali' for traditional and 'Arbequina', 'Arbosana', and 'Koroneiki' for SHD orchards). The altitude of these regions ranges from 50 to 300 m. The orchard dimensions vary and depend on the planting model, with sizes from 5–10 ha for IS to over 30 ha for SHD. The yield potential in modern Tunisian orchards ranges between 4 and 12 t/ha, and it fluctuates more than in the Iberian countries. Harvesting is mostly done manually, but some grape harvesters are used as well. Leaf spot and olive fly are the most common problems. The manpower costs in Tunisia are very low, about 0.6 €/hour (5–6 €/day), lower than in other areas of this study (Tous *et al.*, 2014).

4.2.2.2 Morocco

Morocco is an important olive oil and table olives producing and exporting country. The olive tree is implanted in more than 922,000 ha, which represent 56% of the cultivated land (around 24,000 ha are conducted under drip irrigation), and the oil production average is about 120,000 t. Although olives are grown throughout the country, there are two main areas where cultivation is concentrated (Boulouha & El-Kholy, 2012): one is the northern region (40% of the total, located in Fez, Meknes, Taza, Taounate, Ouezzane, and Chefchaouen), where the soils are fertile and hilly, and the rainfall is between 400 and 1000 mm/year. The other is the central region (32%, in Marrackech, Essaouira, Souss Kelaa, and Beni Mellal), where the soil is fertile, deep, and flat; the rainfall is only between 200 and 370 mm/year; and irrigation is necessary in order to assure sustainable yields. Regarding the cultivars, 'Marocan Picholine' is the most common (nearly 95% of the total olive trees), and there are others, mainly in the new orchards, such as the native selections ('Haouzia' and 'Menara') and some foreign cultivars ('Picual', Manzanilla', and 'Frantoio' in IS models as well as 'Arbequina', 'Arbosana', and 'Koroneiki' in SHD orchards). The altitude of these regions ranges from 50 to 800 m. The orchard dimensions are variable and depend on the planting model, being 1–2 ha for T, 5–10 ha for IS, and over 50 ha for SHD. The yield potential in modern orchards and the harvesting methods are similar to those of the Tunisian groves. With respect to pests and diseases, leaf spot and olive fly are the most common. The manpower costs in Morocco are quite higher than in Tunisia, about 0.83 €/hour (10–12 €/day).

4.2.3 South America

4.2.3.1 Argentina

Argentina has a high productive potential. The olive tree is cultivated in more than 100,000 ha (28% according to traditional models, mainly in Mendoza province, and the rest as IS and HD orchards), and the average oil production is about 20,333 t. Catamarca province is the most important (31,400 ha), followed by La Rioja

(29,900 ha), San Juan (18,600 ha), and Mendoza (14,000 ha) (Gómez del Campo *et al.*, 2010). For this study, two provinces were chosen: La Rioja (Chilecito Valley) and San Juan (Cañada Honda Valley), where new orchard development is currently at its height. The main differences of the olive orchards in these provinces are related to vegetative tree growth, yield potential, oil content, and climate (temperatures, rainfall, frost, and wind). Chilecito Valley is considered the best for high tree growth, yield potential in full production (12–14 t/ha), and irrigation doses (10,000 m^3/ha), but crops there have lower oil content (13%) than in the Cañada Honda Valley. In San Juan, there is normally less yield potential (10–12 t/ha), low rainfall (100 mm), and lower costs (for inputs, water, energy, etc.) than in the Rioja province. The irrigation water used in commercial orchards comes mainly from the aquifers, pumped from a depth of 80–300 m. The altitude of these valleys ranges from 600 to 850 m (Table 4.2), and these changes cause major climatic differences despite the fact that they have similar latitude (Tous *et al.*, 2014). Analysis of thermal and other climatic variables from several olive-growing regions in Argentina (Arid Chaco), and their influence on the olive phenology (as the rate of vegetative growth, flowering and maturation periods, oil content evolution, etc.), indicate important differences with Spain and other world sites (Ayerza & Sibbet, 2001; Gómez del Campo *et al.*, 2010). 'Arbequina' is the most important (50% of the total) cultivar, followed by others, like 'Picual', 'Coratina', 'Barnea', and 'Changlot'. The orchard sizes depend on the technology, normally ranging from 150 to 250 ha, and reaching in some cases 1000 ha. Harvest methods are similar in both provinces (manual and mechanical using trunk or side-by-side shakers and colossus harvesters). There are only a few problems with pests and diseases, because the humidity is very low, which is related to very low rainfall. The manpower costs are medium, about 4.2 €/hour (Tous *et al.*, 2014).

4.2.3.2 Chile

Chile is an emergent olive oil country with promising prospects favored by the climatic conditions. The production is mainly focused on oil exporting. The olive tree is cultivated in more than 24,000 ha, and the average oil production is about 15,750 t. It is mainly located in the Maule region VII (4,800 ha), followed by others such as Coquimbo region IV (4,200 ha), O'Higgins region VI (4,100 ha), and Atacama region III (3,670 ha). The climate of Atacama, Coquimbo, and Maule is very similar to that of the Mediterranean basin. The main differences of the olive orchards in these regions are related to climate, soils, tree growth, yield potential, oil content, and the irrigation doses, with regions III and IV having a higher tree growth, yield potential in full production (12–15 t/ha), and irrigation doses (8000 m^3/ha), but a much lower rainfall (10–150 mm) and more basic pH soils than region VII. In Maule, there is usually lower yield potential (8–12 t/ha) and lower oil content (15%), but higher rainfall (600 mm), frost, and different soil types (e.g., hilly, acid pH, and fertile) than the Atacama-Coquimbo regions (Table 4.2). The altitude of these regions ranges from 160 to 300 m. Several cultivars are used in the modern orchards (Tous *et al.*, 2014): 'Arbequina' (over 50% of the total cultivation) is the most outstanding cultivar, followed by others like 'Frantoio' (18%), 'Arbosana' (8%), 'Picual' (8%), 'Leccino' (5%), and 'Koroneiki' (3%). The orchard sizes are variable and depend on the planting model and harvest methods. IS and HD have a size of 65–200 ha and are harvested with trunk and side-by-side shakers, and SHD are over 210 ha and are harvested with grape harvesters. The geographical isolation of Chile determines the scarce incidence of pest and disease problems (Sebastiani *et al.*, 2006). Sometimes, however, a few incidences of leaf spot appear. The manpower costs of this country are cheaper than Argentina, about 2–2.5 €/hour (Tous *et al.*, 2014).

4.2.4 Other countries

4.2.4.1 Australia

Australia is a new olive oil producing country with high productive potential and promising marketing prospects. The olive tree cultivation covers about 30,000 ha, which is distributed in more than 2000 farms. About 10,000 ha is traditional cultivation, mainly in South Australia, and the rest are IS and HD orchards. Oil production average is 15,166 t. According to Tous (2015), the olive tree cultivation is mainly located in the following six states: Victoria (Vic; 9000 ha, 25.5%), Western Australia (WA; 7400 ha, 21%), New South Wales (NSW; 7250 ha, 20.5%), South Australia (SA; 7000 ha, 19.8%), Queensland (Qld; 4250 ha, 12%), and Tasmania (Tas; 350 ha, 1%). In some areas of these states (Vic, SA, and WA), the climate is very similar

to that of the Mediterranean Basin. In the olive orchards, there are mainly two planting models: traditional (mainly in SA) and IS-HD (adapted to mechanical harvesting with trunk shakers and large over-the-row machines, like a colossus). The main differences with the olive orchards in these regions are related to climate, soils, vegetative tree growth, yield potential, oil content, and the irrigation doses. Vic and SA have higher tree growth, yield potential in full production (8–12 t/ha), and irrigation doses (5000–10,000 m^3/ha), but much lower rainfall (250–300 mm) and more basic pH soils than NSW and Qld. In these states, there is normally a smaller yield potential (5–8 t/ha) and lower oil content (15%), but higher rainfall (600 mm); they suffer from frost periods and have different soil types (hilly, deep, acid pH, fertile, etc.) in comparison with the Vic, SA, and WA states (Table 4.2). The altitude of these olive orchards is less than 200 m. Several cultivars are used in the modern orchards (Sergeeva, 2012; Ravetti & Edwards, 2014; Tous, 2015). 'Barnea' covers over 40% of the total cultivation, mainly in Vic, and is the most outstanding cultivar, followed by 'Frantoio' (26%), 'Picual' (15%), 'Manzanilla' (6%), 'Leccino' (3%), 'Coratina' (2.5%), and 'Arbequina' (2%); 'Koroneiki', 'Hojiblanca', 'Arbosana', and some others cover the remaining portion. The orchard sizes are variable and depend on the planting model and harvest methods. Ninety percent of the growers had less than 10 ha, and only 9% had between 10 and 100 ha. The remaining 1% of Australian growers, with more than 100 ha, have been producing about 90% of the country's olive oil production (Ravetti & Edwards, 2014). Due to very low rainfall, only sometimes black scale, olive knot, leaf spot, and anthracnose appear. The manpower costs in this country are very high, about 11 €/hour, higher than in other growing areas studied in this work.

4.2.4.2 United States (California)

This country represents one of the most interesting olive oil and table olive markets. California is the main state where about 98% of the US olive groves are gathered in. The olive growing area is 21,300 ha (about 58.2% for oil production and 41.8% for table olives), and the average oil production is about 10,000 t (Table 4.1). It is mainly located in three producing areas: the San Joaquin Valley (64%, mainly table olive orchards, and a few SHD oil plantings), Sacramento Valley (34%, IS table and SHD oil orchards), and northern coast (Sonoma and Napa counties, HD oil orchards). The main differences between the olive orchards in these regions are related to climate, soils, vegetative tree growth, yield potential, oil content, and irrigation doses, highlighting the San Joaquin Valley for its higher vegetative tree growth, yield potential in full production (7–14 t/ha), and irrigation doses (7000–10,000 m^3/ha), but much lower rainfall (150–360 mm) and more basic pH soils than in the Sacramento Valley, where there is a similar yield potential (mainly in SHD orchards), lower average temperatures, and higher rainfall (475 mm), although it sometimes suffers frost periods in comparison with the San Joaquin Valley (Table 4.2). The altitude of these regions ranges from 160 to 300 m. 'Manzanilla' (about 70% of the total), 'Sevillano' (Gordal), 'Ascolano', 'Mission', and 'Barouni' are used for table olives, while 'Arbequina' (over 60% of the total), 'Arbosana', 'Frantoio', 'Mission', 'Koroneiki', and so on are the most outstanding for oil production. The orchard sizes vary and depend on the planting model used, on the cultivar chosen (table or oil), and on harvest methods. There are traditional table groves (manual harvested) smaller than 12 ha, IS-HD groves with trunk or side-by-side shakers between 30 and 80 ha, and SHD orchards with grape harvesters over 80 ha. With respect to pests and diseases, olive fly, black scale, olive leaf spot, olive knot, and the soil-borne fungus *Verticillium dahliae* are the most common. The manpower costs of this country are cheaper even from the bordering southern countries, and the workers are mainly Mexican.

4.3 Global olive oil cultivars

There are hundreds of cultivars of the olive tree (*Olea europaea*) in the world. Table 4.2 shows some global oil cultivars ('Picual', 'Arbequina', 'Frantoio', 'Leccino', 'Koroneiki', 'Barnea', etc.) growing in several olive areas in the Northern Hemisphere (Spain, Italy, Greece, Tunisia, Morocco, and the United States) and some Southern Hemisphere countries (Argentina, Chile, and Australia). The typical olive oil composition of these cultivars is presented in Table 4.3, but this composition changes a lot when they are cultivated in other areas (Table 4.4). For example, the oleic acid content of 'Arbequina' ranges from 52% in Catamarca

Table 4.3 Fruit and virgin olive oil characteristics of 12 global cultivars growing in Spain (Catalonia[1] and Andalusia[2]), Italy[3], Israel[4], Australia[5], and California[6].

Cultivar	Origin	Oil content (% dry weight)	C16:0 (%)	C18:1 (%)	C18:2 (%)	C18:3 (%)	Total polyphenols (ppm caffeic acid)	Tocopherol (mg/kg)
'Arbequina'[1]	Catalonia (Spain)	54.4	14.5	69.4	11.1	0.72	234	250
'Arbosana'[1]	Catalonia (Spain)	50.7	13.6	73.0	7.9	0.90	343	200
'Barnea'[4]	Israel	46.2	14.2	65.7	13.7	0.85	284	170
'Coratina'[3]	Puglia (Italy)	54.0	12.4	75.4	7.9	0.72	599	352
'Empeltre'[1]	Ebro Valley (Spain)	50.5	12.3	73.4	9.73	0.73	300	400
'Frantoio'[3]	Toscana (Italy)	52.0	11.1	79.1	6.0	0.61	173	143
'Hojiblanca'[2]	Cordoba (Spain)	44.3	9.8	74.6	8.8	1.04	187	340
'Koroneiki'[1]	Crete (Greece)	52.4	11.4	76.6	6.8	0.93	400	297
'Kalamon'[5]	Peloponnese (Greece)	44.2	10.6	75.8	9.2	0.60	–	–
'Leccino'[3]	Toscana (Italy)	47.3	11.3	79.9	4.90	0.58	146	236
'Mission'[6]	California (United States)	48.2	11.5	75.35	8.1	0.75	High	–
'Picual'[2]	Jaén (Spain)	50.4	10.1	78.3	4.4	0.89	664	350

Source: Based on data from Cimato *et al.* (1996), Mannina *et al.* (2001), Sweeney (2002), Tous *et al.* (2004, 2005b, 2011), Uceda *et al.* (2004, 2005), Pardo *et al.* (2007), Vossen (2007), Lavee (personal communication, 2015), and Wang *et al.* (personal communication, 2015).

Table 4.4 Comparison of some functional compounds of 11 global olive oil cultivars planted in different world growing areas (European Union, Maghreb, South America, Australia, United States, etc.). [y]Thesis data for A. Benavides, University of Coquimbo (Chile). [x]Unpublished results.

Cultivar	Growing area	C16	C16′	C18	C18′	C18″	C18″‴	Total phenols caffeic acid mg/Kg	Tocopherols (mg/Kg)	Source
'Arbequina'	Spain: Catalonia, Garrigues	12.58	1.1	2.04	74.5	9.04	0.55	295	–	Tous *et al.* (1997), Morelló (2004)
	Spain: Catalonia, Priorat-Siurana	14.47	1.51	1.84	70.24	10.08	0.64	188	–	Tous *et al.* (1997), Romero (2011)[x]
	Spain: Catalonia Reus	15.4	–	–	69.4	11.1	0.72	234	–	Tous *et al.* (2011)
	Spain: Catalonia Reus	14.6	1.60	1.60	68.2	12.57	0.61	201	–	Tous *et al.* (2005b)
	Spain: Andalusia Córdoba	15.9	2.33	1.62	65.1	12.5	0.72	182	–	Uceda *et al.* (2005)
	Spain: Cast-La Mancha, C. Real	14.9	2.0	1.7	70.6	9.05	0.70	244	250	Pardo *et al.* (2007)
	Marruecos: Marrakech	17.5	2.86	1.69	63.22	13.30	0.58	–	–	El Antari *et al.* (2003)
	Northern Tunisia, SHD	17.57	2.41	1.88	58.82	12.93	0.63	108.2	165 α	Allalout *et al.* (2009)
	Northern Tunisia, SHD, i·18	17.37	2.08	1.85	61.89	11.58	0.69	196.17	220,23 α	Allalout *et al.* (2009)
	Northwestern Tunisia, IS, mod irrigation	11.64	1.90	1.70	69.14	12.70	0.95	196.2	220.2	Dabbou *et al.* (2010), 500 msl, 450 mm
	California, US: SHD, i·18	15.7	1.7	1.7	66.6	12.2	0.73	86.5	–	Berenguer *et al.* (2006)
	Australia	16.1	2.3	1.7	68.5	9.7	0.5	–	–	Sweeney (2002)
	Australia: NSW/Qld	19.7	3.5	1.2	54.5	19.4	0.7	–	253	Mailer *et al.* (2010)
	Australia: WA	16.4	2.1	1.5	63.4	14.3	0.6	–	213	Mailer *et al.* (2010)
	Australia: Vic	14.8	1.8	1.5	69.7	10.6	0.6	–	315	Mailer *et al.* (2010)
	Australia: Tas	10.4	0.8	1.9	81.0	4.4	0.6	–	286	Mailer *et al.* (2010)
	NW Argentina (Catamarca)	19.2	3.3	1.6	51.8	21.9	1.0	45–223	310	Rondanini *et al.* (2011), Ceci and Corelli (2010)
	Argentina: La Rioja	18.9	2.8	1.6	56.6	18.0	0.77	45–158	328	Ceci and Carelli (2007, 2010), 2004–2005 crops
	Argentina: San Juan	17.9	2.28	3.3	60.2	16.5	0.74	80.2	–	Mattar and Turcato (2006), Cornejo and Bueno (2011), Cobos *et al.* (2014)

(*continued*)

Table 4.4 (*Continued*)

Cultivar	Growing area	C16	C16′	C18	C18′	C18″	C18‴″	Total phenols caffeic acid mg/Kg	Tocopherols (mg/Kg)	Source
	Argentina: Mendoza	15.8	1.6	1.9	63.3	15.5	0.60	130	–	Ceci and Carelli (2007, 2010), 2004 crop
	Argentina: Buenos Aires	12.47	1.08	2.08	71.7	10.72	0.69	63	247	Ceci *et al.* (2009)
	Argentina: Rio Negro	14.39	1.31	1.94	68.71	11.43	0.79	248.2	287	Ceci *et al.* (2009)
	Argentina: Cordoba	17.2	2.54	1.4	61.3	16.0	0.79	223.3	251,2	Torres *et al.* (2009)
	Chile: ≠latitudes (29°-36°): III-VII regions	13.0	0.97	1.77	73.91	8.39	0.33	283.3	179–346 11–221	Troncoso *et al.* (2006), Rodríguez (2007)[y], Anaya (2007)[y], Estay *et al.* (2014), Portilla *et al.* (2014), N. Romero *et al.* (2012)[x]
	Chile: IV (Coquimbo)-VII (Maule-Talca) region,	10.9	0.6	1.9	78.2	6.9	0.5	259/310*	–	Estay *et al.* (2014), 2009–2010 season, García-González *et al.* (2010)*
	Chile: IV (Coquimbo) region	13.4	–	1.76	72.58	8.87	0.53	–	–	Tapia (2009)
	Chile: VII (Maule-Talca) region, SHD	14.5	1.33	1.32	73.9	7.69	0.12	351	–	M.L. Hurtado[x] (2012), 2007–2008 crops
	CV (%)	*16.18*	*39.35*	*21.33*	*10.51*	*32.43*	*26.69*	*43.47*	*168.11*	
'Frantoio'	Central Italy	11.94	0.73	1.68	77.0	7.45	0.53	477	–	Fontanaza *et al.* (1994), Mannina *et al.* (2001), Famiani *et al.* (2002)
	Italy: Central Italy	13.34	1.01	1.65	75.77	8.04	0.55			Mannina *et al.* (2001)
	Italy: Central Italy	12.6	–	–	76.2	7.1		498		Alfei *et al.* (2010), 86 samples
	Italy: Toscana	11.1	–	–	79.1	6.0	0.61	173	143	Cimato *et al.* (1996)
	Spain: Cordoba	12.45	1.23	1.89	73.9	8.43	0.86	382.7	200	Uceda *et al.* (2005), Uceda *et al.* (2004) (tocopherols)

Spain: Tivenys, Catalonia	13.69	1.28	1.70	71.1	10.6	0.79	300	–	Romero Aroca *et al.* (2013)
Spain: Córdoba	10.9	0.89	1.53	78.3	6.79	0.49	726	151	Aguilera *et al.* (2004)
Spain: Jaen	14.5	1.12	1.66	70.9	10.43	0.54	635	286	Aguilera *et al.* (2004)
Australia	14.3	1.2	2.1	69.1	12.0	0.6	–		Sweeney (2002)
Australia: NSW/Qld	15.6	1.7	1.6	65.7	13.8	0.7	–	251	Mailer *et al.* (2010)
Australia: WA	13.8	1.1	1.7	70.3	11.6	0.6	–	180	Mailer *et al.* (2010)
Australia: Vic	12.9	1.0	1.5	72.0	11.1	0.6	–	210	Mailer *et al.* (2010)
Australia: Tas	9.1	0.4	2.0	80.3	6.7	0.6	–	178	Mailer *et al.* (2010)
Argentina: Catamarca	17.19	1.65	1.63	63.55	14.02	1.23	–	–	Mannina *et al.* (2001)
Argentina: La Rioja	15.8	1.65	2.2	63.2	14.95	1.0	347.6	339	Ceci and Carelli (2007, 2010) Rondanini *et al.* (2011)
Argentina: San Juan	13.6	–	2.39	68.40	12.4	0.78	80.15	–	Mattar and Turcato (2006), Ceci and Carelli (2010)
Argentina: San Juan	–	–	–	–	–	–	80.5	259	Cobos *et al.* (2014)
Chile: VII (Maule-Talca) region, IS	13.7	1.1	1.2	74.1	8.8	0.1	468	–	M.L. Hurtado (2012),[x] 2007–2008 crops
Chile: IV (Coquimbo)-VII (Maule-Talca) region,	11.8	0.7	2.0	76.0	7.9	0.5	345	–	Estay *et al.* (2014), 2009–010 season
Chile: ≠latitudes (29°-36°): III-VII regions	12.3	0.83	1.7	76.4	7.1	0.3	264/387*	148–326 110–146	Troncoso *et al.* (2006), García-González *et al.* (2010),* Portilla *et al.* (2014), Rodriguez, 2007),[y] Estay *et al.* (2014), N. Romero *et al.* (2012)[x]
CV (%)	*14.32*	*32.75*	*16.40*	*6.99*	*28.68*	*39.45*	*51.98*	*28.06*	

(*continued*)

Table 4.4 (*Continued*)

Cultivar	Growing area	C16	C16′	C18	C18′	C18″	C18‴″	Total phenols caffeic acid mg/Kg	Tocopherols (mg/Kg)	Source
'Picual'	Spain: Córdoba	10.16	1.08	3.79	78.34	4.4	0.89	664.3	350	Uceda *et al.* (2004, 2005), (tocopherols)
	Spain: Reus	11.90	1.08	3.16	78.28	4.43	0.67	509.9	–	Tous *et al.* (2005b)
	Spain: Tivenys	12.18	1.18	3.14	76.46	5.3	0.86	355	–	Romero Aroca *et al.* (2013)
	Spain: Castilla-La Mancha, C. Real	10.5	0.78	2.9	80.7	3.51	0.62	652	190	Pardo *et al.* (2007)
	Australia	13.8	1.3	2.5	77.2	3.9	0.6	–		Sweeney (2002)
	Australia: NSW/Qld	15.3	2.2	1.8	72.6	6.4	0.9	–	325	Mailer *et al.* (2010)
	Australia: WA	11.7	1.0	2.5	78.7	4.6	0.7	–	233	Mailer *et al.* (2010)
	Australia: Vic	11.8	1.0	2.3	80.4	3.1	0.6	–	263	Mailer *et al.* (2010)
	Australia: Tas	9.6	0.6	2.4	83.4	2.6	0.6	–	267	Mailer *et al.* (2010)
	NW Argentina: Catamarca, La Rioja, S. Juan	13.8	1.7	2.5	71.9	7.7	1.0	22–270	311 (Catamarca)	Rondanini *et al.* (2011), Ceci and Carelli (2010)
	Argentina: La Rioja	14.5	1.93	3.0	71.3	7.4	0.86	36–93	319	Ceci and Carelli (2007, 2010), 2004–2005 crops
	Argentina: San Juan	13.67	–	2.23	75.38	5.54	0.77	212,5	–	Mattar and Turcato (2006)
	Chile: IV Región	10.5	–	2.6	80.4	3.1	–	232	184–267	Troncoso *et al.* (2006), Portilla *et al.* (2014), Anaya, (2007)[y]
	Chile: IV-VII Regions	–	–	–	–	–	–	402	–	García-González *et al.* (2010)
	CV (%)	*14.76*	*38.91*	*19.06*	*4.78*	*34.25*	*18.80*	*60.04*	*69.36*	
'Leccino'	Spain: Tivenys,	14.09	1.62	1.95	72.87	7.96	0.78	273	–	Romero Aroca *et al.* (2013)
	Spain: Córdoba	14.13	1.43	1.85	70.82	9.95	0.85	–	–	Uceda *et al.* (2005)
	Spain: Córdoba	12.6	1.08	1.80	77.8	5.3	0.52	472	205	Aguilera *et al.* (2004)
	Spain: Jaen	15.0	1.24	1.61	74.1	6.73	0.52	718	334	Aguilera *et al.* (2004)
	Italy: Central Italy	13.23	1.25	1.53	77.96	4.54	0.68		–	Mannina *et al.* (2001)
	Italy: Central Italy	13.90	–	–	75.2	6.7	–	397	–	Alfei *et al.* (2010), 73 samples

	Italy: Toscana	11.3	–	–	79.9	4.9	0.58	146	236	Cimato *et al.* (1996)
	Central Italy	–	–	–	79.08		–	157–	–	Pannelli *et al.* (1994)
	Australia	14.2	1.2	2.0	75.0	6.5	0.6	–	–	Sweeney (2002)
	Australia: NSW/Qld	14.7	1.4	1.6	73.1	7.9	0.6	–	462	Mailer *et al.* (2010)
	Australia: WA	13.1	1.1	1.8	75.4	7.3	0.6	–	394	Mailer *et al.* (2010)
	Australia: Vic	13.2	1.1	1.7	77.1	5.5	0.6	–	389	Mailer *et al.* (2010)
	Australia: Tas	11.6	0.8	1.9	77.9	6.4	0.6	–	374	Mailer *et al.* (2010)
	NW Argentina: Catamarca. La Rioja, S. Juan	14.8	1.5	2.4	67.5	11.9	0.9	–	–	Rondanini *et al.* (2011)
	Argentina: Catamarca	17.39	1.16	1.71	68.45	9.19	1.43	–	–	Mannina *et al.* (2001)
	Chile: IV Región	12.54	–	1.89	77.90	4.57	0.60	–	–	Tapia (2009)
	Chile: IV, VI, VII Regions	–	–	–	–	–	–	357	–	García-González *et al.* (2010)
	CV (%)	*10.97*	*17.87*	*12.26*	*4.92*	*29.87*	*33.90*	*55.17*		
'Coratina'	Spain: Tivenys	10.9	0.9	3.73	78.3	4.6	0.8	587	*28.30*	Uceda *et al.* (2005), Romero *et al.* (2013)
	Italy: Apulia	12.3	0.51	2.1	75.43	7.94	0.72	–	–	Mannina *et al.* (2001)
	Italy: South Italy	11.2			77.9	7.1		599		Alfei *et al.* (2010), 61 samples
	Australia	11.6	0.7	2.1	75.6	8.8	0.5	–	–	Sweeney (2002)
	Australia: NSW/Qld	12.3	0.5	2.0	74.0	9.5	0.8	–	481	Mailer *et al.* (2010)
	Australia: WA	12.4	0.4	1.8	76.3	7,2	0.8	–	352	Mailer *et al.* (2010)
	Australia: Vic	11.0	0.4	1.6	78.0	7.3	0.7	–	306	Mailer *et al.* (2010)
	Australia: Tas	8.5	0.3	1.7	81.8	6.1	0.7	–	244	Mailer *et al.* (2010)
	NW Argentina: Catamarca, La Rioja, S. Juan	12.9	0.7	2.1	68.9	12.9	0.9	332 LRioja	–	Rondanini *et al.* (2011), Ceci and Carelli (2010)
	Argentina: Catamerca	12.8	0.6	2.0	68.7	14.3	0.7	263–675	437	Ceci and Carelli (2007, 2010)
	Argentina: Catamarca	16.29	0.67	1.77	71.50	7.99	1.27	–	–	Mannina *et al.* (2001)

(*continued*)

Table 4.4 (*Continued*)

Cultivar	Growing area	C16	C16′	C18	C18′	C18″	C18‴″	Total phenols caffeic acid mg/Kg	Tocopherols (mg/Kg)	Source
	Argentina: San Juan	–	–	–	–	–	–	483.5	386.5	Cobos *et al.* (2014)
	Chile: IV Región	8.14	–	1.80	80.53	7.10	0.56	–	–	Tapia (2009)
	CV (%)	*18.09*	*31.75*	*28.09*	*5.70*	*32.61*	*26.16*	*21.86*	–	
'Barnea'	Israel	14.25	0.97	2.30	65.71	13.74	0.85	284	170	Lavee, S. (2015),[X] Mean 15 oil samples from irrigated orchards.
	Spain: Tivensys	10.03	0.95	2.19	70.71	12.44	0.83	416	–	Romero *et al.* (2013)
	Australia	11.7	0.9	1.9	71.5	12.9	0.6	–	–	Sweeney (2002)
	Australia: NSW/Qld	14.5	1.2	1.8	63.0	18.1	0.7	–	254	Mailer *et al.* (2010)
	Australia: WA	11.7	0.8	2.0	70.8	13.2	0.6	–	244	Mailer *et al.* (2010)
	Australia: Vic	11.4	0.9	1.8	73.2	11.3	0.6	–	290	Mailer *et al.* (2010)
	Australia: Tas	8.0	0.5	2.3	79.6	8.1	0.6	–	293	Mailer *et al.* (2010)
	NW Argentina: Catamarca.La Rioja, S. Juan	13.6	1.3	2.1	61.0	19.8	0.9	43–75 Catamarca	–	Rondanini *et al.* (2011), Ceci and Carelli (2010)
	Argentina: La Rioja	13.7	1.35	2.1	63.15	17.9	0.9	53–131	255	Ceci and Carelli (2007, 2010)
	Chile: IV Región	10.1	–	2.2	77.04	7.93	0.55	–	–	Tapia (2009)
	Chile: IV, VI, VII Regions	–	–	–	–	–	–	310	–	García-González *et al.* (2010)
	CV (%)	*17.87*	*26.94*	*9.09*	*8.95*	*29.83*	*19.85*	*65.38*	*17.75*	

'Empeltre'	Spain: Córdoba	13.0	1.11	1.79	69.29	12.05	0.89	421	400	Uceda *et al.* (2004, 2005) (tocopherols)
	Spain: Reus	13.9	1.47	1.38	68.61	13.05	0.77	239	–	Tous *et al.* (2005b)
	Spain: Ebro Valley	12.27	1.02	1.83	73.46	9.73	0.73	299	–	Tous *et al.* (2004)
	Spain: Tivens	14.44	1.85	1.44	67.52	12.71	0.92	306	–	Romero *et al.* (2013)
	NW Argentina: Catamarca.La Rioja, S. Juan	15.7	1.2	1.9	65.5	12.8	1.2	135 Catamarca	–	Rondanini *et al.* (2011), Ceci and Carelli (2010)
	Argentina: La Rioja	14.5	1.5	1.6	70.6	10.1	0.8	25	299	Ceci and Carelli (2007, 2010)
	Argentina: Mendoza	11.7	1.0	1.8	75.2	8.4	0.7	53	167	Ceci and Carelli (2007, 2010)
	Chile: IV Región	12.6	–	1.46	74.63	7.47	0.65		–	Tapia (2009)
	CV (%)	*9.97*	*23.90*	*12.39*	*4.99*	*20.05*	*20.92*	*68.90*	*8.37*	
'Arbosana'	SpainL Reus	12.95	1.20	1.95	74.77	7.43	0.93	269	–	Tous *et al.* (2005b)
	Spain: Córdoba,	13.5	1.65	1.73	72.8	8.17	0.8	–		León *et al.* (2006), SHD
	Spain: Reus	16.3			73.0	7.9	0.9	343		Tous *et al.* (2011), SDH
	Northern Tunisia	17.78	2.12	2.07	64.79	12.09	0.54	137.84	200.23 α	Allalout *et al.* (2009), SHD
	NW Argentina: Catamarca.La Rioja, S. Juan	16.50	2.8	2.0	61.0	15.6	0.9	–	–	Rondanini *et al.* (2011)
	CV (%)	*13.50*	*35.21*	*7.58*	*8.91*	*34.47*	*19.76*	*41.57*		
'Hojiblanca'	Spain: Córdoba	9.79	0.79	2.96	74.61	8.82	1.04	187	340	Uceda *et al.* (2005), Uceda *et al.* (2004) (tocopherols)
	Spain: Reus	10.8	0.6	2.90	73.70	9.8	1.1	274	–	Tous *et al.* (2005b)
	NW Argentina: Catamarca.La Rioja, S. Juan	15.8	1.4	2.2	69.8	8.0	1.3	–	–	Rondanini *et al.* (2011)
	CV (%)	*26.53*	*44.94*	*15.73*	*3.51*	*10.16*	*11.87*	*26.69*	–	

(*continued*)

Table 4.4 (*Continued*)

Cultivar	Growing area	C16	C16′	C18	C18′	C18″	C18″‴	Total phenols caffeic acid mg/Kg	Tocopherols (mg/Kg)	Source
'Koroneiki'	Spain: Reus	11.4	–	–	76.6	6.89	0.93	400	–	Tous *et al.* (2011), SHD
	Spain: Córdoba	11.32	0.90	2.24	77.66	5.57	0.74	480	200	Uceda *et al.* (2005) Uceda *et al.* (2004) (tocopherol)
	Greece: Crete island, irrigated	12.6	0.77	2.59	75.9	6.1	0.67	302.5	207.2	Stefanoudaki *et al.* (2009)
	Greece: Crete island, **rainfed**	12.9	0.92	3.49	71.3	9.3	0.88	363.7	297.5	Stefanoudaki *et al.* (2009)
	Greece: Crete island, Chania	–	–	–	–	–	–	138–441	–	Kalogeropolos and Tsimidou (2014)
	Greece: Crete island, Heraklion	–	–	–	–	–	–	130–205	–	Kalogeropolos and Tsimidou (2014)
	Greece: Peloponissos (Lakonia)	–	–	–	–	–	–	87–225	–	Kalogeropolos and Tsimidou (2014)
	Northern Tunisia	11.65	1.07	2.15	75.53	8.56	0.26	236.48	294.5 α	Allalout *et al.* (2009), SHD
	Northern Tunisia, Béjaoua	17.3	2.0	1.8	60.0	8.3	0.6	211	424 α	Dabbou *et al.* (2009), Rainfall 500 mm
	Australia	13.4	1.0	2.5	75.9	5.9	0.7	–	–	Sweeney (2002)
	Australia: NSW/Qld	13.8	1.1	2.0	75.4	6.1	0.7	–	204	Mailer *et al.* (2010)
	Australia: WA	10.7	0.8	2.4	76.6	8.0	0.5	–	216	Mailer *et al.* (2010)
	Australia: Vic	11.1	0.8	2.3	79.8	4.5	0.5	–	251	Mailer *et al.* (2010)
	Chile: IV Región	10.5	–	2.47	78.83	4.84	0.76	–	–	Tapia (2009)
	Chile: IV-VII Regions	–	–	–	–	–	–	318	–	García-González *et al.* (2010)
	CV (%)	*15.73*	*36.45*	*18.97*	*7.20*	*23.69*	*28.66*	*38.11*	*5.17*	
'Mission'	US: California, Butte county	12.5	0.7	2.9	74.8	7.4	0.7	–	–	Wang *et al.* (2015)[x]
	US: California, Gerber, oil filtered	10.6	0.5	2.7	75.9	8.8	0.8	–	–	Wang *et al.* (2015)[x]
	CV (%)	*11.6*	*20.5*	*3.8*	*1.0*	*12.4*	*2.8*	–	–	
	International Olive Council limits	***7.5–20***	***0.3–3.5***	***0.5–5***	***55–83***	***3.5–21***	**≤ 1.0**			

(Argentina) to 81% in Tasmania (Australia). In general, the cold areas of Tasmania (Australia) produce olive oils very low in palmitic and linoleic acids and rich in oleic acid. On the contrary, the hot areas in northwest Argentina (Catamarca and La Rioja) produce olive oils very rich in palmitic and linoleic acids and very low in oleic acid. According to existing data, polyphenol content varies a lot (45–650 mg/kg). It seems, however, that north-western Argentina usually produces olive oil of very low polyphenol content, while Spain and Italy produce olive oils richer in these compounds. No trends can be pointed out for tocopherol content, which ranges from 28 to 480 mg/kg. Similar trends were stated by several authors (Tous *et al.*, 1997; Ceci & Carelli, 2007; García-González *et al.*, 2010; Mailer *et al.*, 2010; Romero *et al.*, 2016).

Cultivar performance also varies in the orchards according to the mechanization requirements, tree size, or planting distances. While for IS and HD planting systems (200–700 trees/ha), all the most cited cultivars show good results globally, in the case of SHD (over 1500 trees/ha), there are different results regarding vigor, growth, and balance between productivity and continuous harvest efficiency. Currently, in SHD hedgerow olive plantings, 'Arbequina' (clone i·18 and others) is mostly used (Tous *et al.*, 1999, 2008). Other materials used are 'Arbosana-i·43' (Tous & Romero, 2000), 'Koroneiki', and recently 'Sikitita' (Rallo *et al.*, 2008). The most outstanding characteristics for the major global olive oil cultivars have been studied by several authors (Tous & Romero, 1993; Barranco *et al.*, 2000; Barranco, 2004; Rallo *et al.*, 2005; Vossen, 2007; Tous *et al.*, 2011; Romero-Aroca *et al.*, 2013, 2014).

4.3.1 Spanish cultivars

4.3.1.1 'Picual'

This is the main Spanish cultivar. It is very widespread in the Andalusia region, mainly in the Jaén, Córdoba, and Granada provinces, and in countries such as Argentina, Chile, and Australia (Figure 4.2). It is much appreciated for its early bearing, high productivity, medium fruit size (an average of 3.3 g), high oil yield, and easy cultivation for IS orchards. It has medium vigor with dense canopy and ripens early in the season. It is considered to be olive knot tolerant, but highly susceptible to olive leaf spot and verticillium wilt. The oils are high in polyphenols with medium to high bitterness and pungency flavor, although it stands out for its high stability index and high oleic acid content. It is suitable for blending with oils of low stability.

4.3.1.2 'Arbequina'

This is the most important cultivar in Catalonia (north-eastern Spain). It is also found extensively in Andalusia and Aragón (Figure 4.3). Outside Spain, it is found mainly in Argentina, Chile, the United States

Figure 4.2 'Picual' cultivar.

Figure 4.3 'Arbequina' cultivar.

(California), Tunisia, Morocco, and elsewhere. It is a very productive cultivar, early bearing, and with a regular yield. It is considered to be frost-resistant and adaptable to different climatic and soil conditions (Tous & Romero, 1993). Its low vigor makes it suitable for IS and SHD hedgerow orchards. Its semi-erect growth habit facilitates its training in a central leader. It has some tolerance to verticillium wilt. Small fruit size (an average of 1.7 g) ripens early to midseason, and it has high oil content. It produces EVOO with a medium fruity flavor, balanced in the mouth, where the sweet attribute is outstanding and easily appreciated by new consumers, but it shows low stability. Its commercialization can be monocultivar or after blending it with other oils.

4.3.1.3 'Hojiblanca'

One of the main cultivars of Spain, widely planted in large Andalusian groves, mainly in the province of Córdoba (Figure 4.4). This cultivar is appreciated for its resistance to calcareous soils. It is a double-purpose cultivar, very suitable for Californian black olive pickling, because of its firm texture. Its fruit (average weight of 4.8 g) has low oil content, but it is appreciated for its quality. Its high resistance to fruit removal, however,

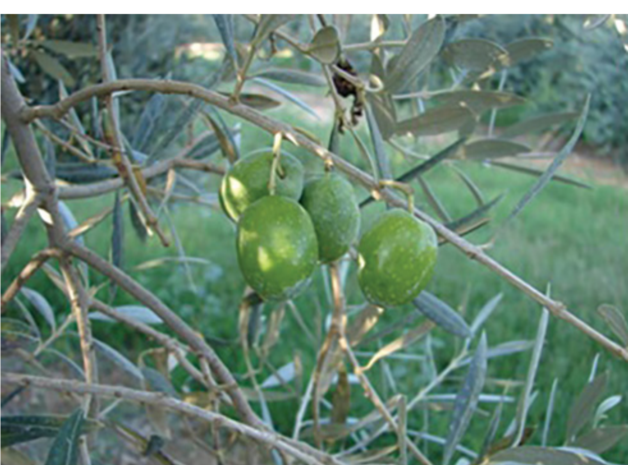

Figure 4.4 'Hojiblanca' cultivar.

Figure 4.5 'Empeltre' cultivar.

makes mechanical harvesting with trunk shakers a problem. It is susceptible to olive leaf spot, olive knot, and verticillium wilt. The oil produced is green, fruity, and of medium to high intensity, balanced with medium pungency and medium to low bitterness.

4.3.1.4 'Empeltre'

This is the most widespread dual-purpose cultivar in the Ebro valley and Balearic Islands. It has also been spread in Argentina (Mendoza, San Juan, and Cordova provinces), Chile (regions III and IV), and Peru (Tous *et al.*, 2004). This cultivar is vigorous with dense canopy, and it is appreciated for its productivity and excellent oil quality. It yields best inside inland areas. It is commercially propagated by grafting, because of its poor root-forming ability. The early ripening of its fruit (average of 2.9 g), the low resistance to fruit removal, and the erect growth habit of its branches make it ideal for mechanical shaker harvesting (Figure 4.5). It is susceptible to winter frost and has fruit set problems. It is tolerant to verticillium wilt. Virgin olive oils from this cultivar are ripe and fruity of half intensity, balanced with medium to light pungency and bitterness.

4.3.1.5 'Arbosana'

This cultivar is originally from Catalonia, but it is now extensively planted in SHD orchards over several Spanish regions and other parts of the world (United States, Chile, Portugal, Tunisia, and so on). It is an early bearing cultivar with high productivity (Figure 4.6). Due to its low vigor, it adapts very well to hedgerow systems for olive growing. It has some tolerance to olive leaf spot, but it is sensitive to frost and olive knot. Its fruit is small (an average of 1.6 g) and ripens several weeks later than the 'Arbequina' cultivar (Tous *et al.*, 2011). It produces intense, green, and fruity oil, spicy with high levels of bitterness and astringency. It is particularly suitable for blending, in order to stabilize and prolong the shelf life of the milder 'Arbequina' oils, due to higher polyphenol content.

4.3.2 Italian cultivars

4.3.2.1 'Frantoio'

This is the main Italian cultivar, grown principally in the central regions (Tuscany, Umbria, etc.). Outside Italy, it has spread in numerous olive-growing countries. It is highly valued for its high and regular productivity, its ability to adapt to different environmental conditions (Figure 4.7), and its high root-forming

Figure 4.6 'Arbosana' cultivar.

ability. It is sensitive to winter cold temperatures, as well as to olive knot and olive fruit fly, but is tolerant to verticillium wilt. The fruit are small to medium in size (average weight of 2.1 g) with medium to high oil content. The fruit are harvested when their skin color is from green to pale yellow, pinkish, or purple. The oil is greatly appreciated for its excellent organoleptic characteristics and stability.

4.3.2.2 'Leccino'

One of the main cultivars planted in Italy, mainly in the Tuscany and Umbria regions. It has also spread to numerous olive-growing countries, such as Australia. This vigorous cultivar is considered rustic because it adapts easily to different soil conditions, and it is especially tolerant to cold temperatures (Figure 4.8). It has a high root-forming ability. Its early bearing and its high and regular productivity are appreciated. It has medium-sized fruit (an average of 3.0 g), early ripening, with low fruit removal force that makes mechanical harvest easy. It is considered tolerant to olive leaf spot and olive knot. It has a low to medium oil content. The oils produced are normally blended to add complexity to 'Frantoio' and the Tuscan oils.

Figure 4.7 'Frantoio' cultivar.

Figure 4.8 'Leccino' cultivar.

4.3.2.3 'Coratina'

This cultivar from the Puglia region has become widespread in recent decades in some olive countries of the Southern Hemisphere. This medium-vigor cultivar starts producing early and has a high and regular yield (Figure 4.9). It produces a medium-sized fruit (average weight 3.8 g) with late ripening and a high fruit removal force that makes mechanical harvesting difficult. It is cold tolerant. The oils are very high in polyphenols and oleic acid content, and very stable, and care must be taken to moderate bitterness in the oil. These virgin oils are often used for blending with other oils.

4.3.3 Greek cultivars

4.3.3.1 'Koroneiki'

The 'Koroneiki' (Figure 4.10) is an olive cultivar from Greece primarily used in olive oil production. This cultivar represents about 60% of the olive grove area in Greece (Therios, 2009). It is in a dominant position throughout the Peloponnese and Crete regions. Recently its cultivation has been extended in central and north Greece and in growing areas all over the world, in SHD growing systems. It is very productive and

Figure 4.9 'Coratina' cultivar.

Figure 4.10 'Koroneiki' cultivar.

early bearing, and it is considered drought-resistant, but frost-sensitive and well-suited to hot growing areas. It is tolerant to olive leaf spot but susceptible to olive knot. The fruit is very small (an average of 0.9 g), ripens after 'Arbequina' but before 'Arbosana', and has a high-quality oil yield. It produces quite stable EVOO, rich in oleic acid and polyphenols, with intense green color and bitterness, and a long shelf life. It has a unique rich fruity flavor.

4.3.3.2 'Kalamon'

The 'Kalamon' cultivar (Figure 4.11) is named after the city of Kalamata, Greece. It is a unique table cultivar and has an international acceptance. It is mainly cultivated in Peloponnese, west-central Greece (Agrinion), and recently northern Greece (Chalkidiki), and on the island of Crete. The fruit has an average weight of 2.6–5.5 g. It is mainly a table cultivar, but it can be used for oil production as well, since it contains, when ripe, an average of 17% oil. This cultivar propagates with difficulty from cuttings, and it is normally grown by grafting. It is vigorous and tolerant to olive knot, but requires rich soil and irrigation for good production. Due to its great importance, today this cultivar is also cultivated in other countries, such as Australia, the United States (California), Argentina, and South Africa. The table olives produced are called Kalamon or

Figure 4.11 'Kalamon cultivar.

Figure 4.12 'Barnea' cultivar.

Kalamata olives. They are processed black and are preserved in wine vinegar and/or olive oil. They have a unique and special taste.

4.3.4 Israeli cultivar: 'Barnea'

This cultivar was bred in Israel and was released about 40 years ago (Lavee, 2012). It is the main oil cultivar for Israeli intensive irrigated orchards, and is widely planted in large Australian, New Zealand, as well as South American groves (Figure 4.12). It has vigorous trees with erect canopy, suited to mechanical harvesting, such as trunk shakers and large over-the-row machines (like a colossus harvester). When irrigated, it becomes an early-bearing cultivar with high and regular productivity. Regarding its adaptability in other countries, it can suffer root rot in wet soils, and it is more prone to anthracnose than other cultivars, as Australia's warm and humid weather is different than the hot and dry weather of Israel (Sergeeva, 2012). The fruits (average of 2.3 g) are considered to be moderate to highly sensitive to chilling injuries and to frost damage before harvest. This cultivar has a medium to high oil content, and it is suitable for blending.

4.3.5 Californian cultivar: 'Mission'

This cultivar probably originates from 'Cañivano Blanco', a diffused cultivar from Seville, Spain that is synonymous with the 'Picholine marrocaine' (Díez *et al.*, 2015), and was introduced to California by the Spanish Mission fathers in the mid-18th century. Today it is mainly planted in some counties of the Sacramento Valley (Butte, Glenn, and Tehama) and also in Australia and South Africa. It is a dual-purpose cultivar with good fruit size and high oil content (Figure 4.13). Trees grow very upright and are cold hardy to a greater degree than many other cultivars, but they are very susceptible to the diseases olive leaf spot and verticillium wilt. Productivity is medium and alternate, while ripening is late. It is suitable for blending with other oils.

4.4 Olive oil composition affected by genetic and environmental factors

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4.4.1 Overview of EVOO variability

Olive oil composition depends on the interaction of genetics × environment (Tous *et al.*, 2005a; Inglese *et al.*, 2010). Several authors (Bianchi *et al.*, 2002; Motisi *et al.*, 2004; Uceda *et al.*, 2004), studying the

Figure 4.13 'Mission' cultivar.

relative effects of cultivars, showed that ripening stage and growing area affect the fatty acids composition in Italian, Sicilian, and Andalusian cultivars.

Tous and Romero (1993, 1994) reported variations in fatty acids and sensory characteristics in 'Arbequina' grown in different Catalan areas. They suggested that such variations were due to soil and climate characteristics and cultural practices as well. Similarly, Ranalli *et al.* (1999) observed variations in olive oil composition and sensorial attributes from three Italian cultivars grown in five places in the Tuscany and Umbria regions. Multivariate statistical analysis pointed out significant effects from factors such as maximum temperature, number of windy days, organic matter content in the soil, and C/N ratio. The genetic factor was the most relevant of all.

Edaphic and climatic effects on olive oil composition were studied by Ben Rouina *et al.* (2002) and Motisi and Gullo (2002). Tombesi and Antaras (1998) reported an increase of oleic acid and total polyphenols content when August and September were rainy and colder than usual. Tura *et al.* (2008) studied 17 olive orchards for four years in north Italy and reported a positive correlation between mean temperature in October and total polyphenol content, tocopherols, and volatiles. They also stated a direct relationship between cumulative temperature degrees during the fruit-growing period and saturated and polyunsaturated fatty acids in the oil. Similar results were reported by Sadeghi and Talaii (2002) studying the behavior of the cultivar 'Zard' in three Iranian areas with different altitudes and rain falls. Bongi (2004) observed a lower ratio of oleic/(linoleic + palmitic) acids in warm areas than in cold areas concerning the 'Arbequina' cultivar.

Interannual climatic fluctuations can also affect the olive oil composition as suggested by Koutsaftakis *et al.* (2000) when 'Koroneiki' cultivar was studied. They observed fluctuations on fatty acids, sterols, and waxes for a particular moment in the harvest season related to climate. This is in agreement with Donaire *et al.* (1975), who suggested that any factor affecting photosynthesis at the beginning of fatty acids biosynthesis can change the total fat content and its fatty acid profile as well. In addition, Failla *et al.* (2002), analyzing the olive oil polyphenol and tocopherol contents in 18 orchards in north Italy, discussed strong interannual fluctuations for tocopherols.

Fruit ripening is a relevant factor affecting olive oil chemical composition and sensorial characteristics. Romero-Aroca *et al.* (2002) reported variations in fatty acids and oil stability related to maturity index and crop load in 'Arbequina' growing in Tarragona (northeastern coastal Spain). Motilva *et al.* (2000), working on irrigation trials in Lleida (northeastern inland Spain), pointed out that polyphenol variations are more related to ripe index than to olive tree water stress, even though drought stress can boost the ripening process (Alegre *et al.*, 2002; Morelló, 2004). In some cultivars, such as 'Galega' from Portugal, it was observed that ripening time can change between places (Ramos *et al.*, 2008). Padilla *et al.* (2009) reported variations in gene expression related to lipoxygenase (LOX) synthesis, in 'Picual' and 'Arbequina' cultivars during the ripening process, explaining differences in the volatiles profile. The advanced fruit ripening reduces the LOX activities, decreasing the quantity of the volatile compounds responsible for the positive EVOO

sensory attributes (Servili *et al.*, 2011). Fernández *et al.* (2002) found significant differences for oil qualitative parameters along the ripening process for 'Picual', 'Picudo', 'Hojiblanca', and 'Arbequina'. Koutsaftakis *et al.* (2000), studying the ripening process in 'Koroneiki', observed an increase in 1-3DAGs due to hydrolytic degradation of the oil that increases free acidity.

The fatty acids evolution along the ripening process is related to specific enzymes activity and the temperatures. The oleic and sometimes the linoleic acid generally increase during the olive maturation, while the stearic and especially the palmitic acid tend to decrease (Servili *et al.*, 2011). Hernández *et al.* (2009), studying gene expression in 'Picual' and 'Arbequina', demonstrated that there are three genes: fatty acid desaturate (FAD2-1), (FAD2-2), and (FAD6). FAD2-2 regulates the content of linoleic acid in mesocarp, and its activity can be increased by irrigation and other growing factors. The same work suggests that gene activity level is different for each cultivar, which could explain different behavior between cultivars reported by several authors in different places. New research on gene activity related to fruit growth stages or biotic and abiotic stressing factors shows a high relationship between environmental factors and olive oil composition (Galla *et al.*, 2009).

Motilva *et al*. (2002) and Romero *et al*. (2002), working on 'Arbequina' in Lleida (northeastern Spain), reported lower pigment and polyphenol contents in higher irrigation doses. Similar results were observed by other researchers (d'Andria *et al.*, 2002; Pastor, 2003). Faci *et al.* (2002a, 2002b), studying irrigation strategies in 'Arbequina' in Aragón, observed some differences in fatty acids when comparing maximum irrigation doses. Vossen *et al.* (2008), studying 'Arbequina IRTA-i·18' clones under several irrigation treatments in SHD orchards in California, observed an increase in PUFAs and a decrease in MUFAs related to higher irrigation doses, parallel to a decrease in polyphenols and oil stability, and higher total sterols content and free acidity.

Simöes *et al.* (2002) suggested that soils poor in N and K increase saturated fatty acids and decrease oil stability in the 'Carrasquenha' cultivar. On the contrary, Fernández-Escobar *et al.* (2002) pointed out a lower polyphenol content and stability in oils from the 'Picual' cultivar grown with higher N doses in an eight-year trial. Finally, Alcubilla *et al.* (2002), studying 'Arbequina' behavior in Zaragoza and 'Empeltre' in Teruel, could not detect any effect of N on the olive oil composition.

Pruning is very relevant in olive tree managing because it increases light penetration into the canopy and, then, photosynthesis (Tombesi *et al.*, 1998). Proietti *et al.* (1999) suggested that fruit orientation and lightening can change its fatty acids synthesis. Gregoriou *et al.* (2004), after shadowing 'Koroneiki' trees, observed a significant delay in fruit ripening that resulted in lower oil content, higher oleic acid, and lower levels in palmitic and linoleic acids. Since photosynthesis is supported by lightened leaves, pruning and orchard density could be relevant factors for olive oil composition (Díaz-Espejo *et al.*, 2002).

Baptista and Pereira (2004) reported in Portugal higher photosynthesis and pigment levels in fruits from non-tilled orchards, where water efficiency is increased (Pinheiro *et al.*, 2004).

4.4.2 Effects of growing region and cultivar on EVOO characteristics

As it has been pointed out, olive oil composition is affected by a great number of factors, but the main sources of variability are related to cultivar and environmental conditions (altitude, latitude, and climate), crop management (e.g., irrigation doses), and harvesting time. Table 4.4 compares the virgin olive oil (VOO) variability of 11 global cultivars growing in several olive areas in the Northern Hemisphere (NH) (Spain, Italy, Greece, Tunisia, Morocco, and the United States) and Southern Hemisphere (SH) countries (Argentina, Chile, and Australia). A high variability in some cultivars is pointed out (CV higher than 30%) related to zones for palmitoleic, linoleic, and linolenic acids and tocopherol, and very high (more than 50%) for total polyphenol contents (Figure 4.14). Table 4.5 shows an additive effect of cultivar, latitude, and altitude in both the NH and SH for fatty acid composition and total polyphenol content.

Latitude, altitude, and mean temperature show significant correlations with fatty acids and total polyphenol content from all cultivars planted in both hemispheres (Table 4.6). There is an inverse linear relationship between oleic and linoleic acids ($r = -0.93$) in both hemispheres (Figure 4.15a). Oleic acid content decreases from 1.3 to 2.1% when mean temperature increases by 1 °C (Figure 4.15d). A certain positive correlation is pointed out between polyphenol and oleic acid contents ($r = 0.52$), despite the fact that their syntheses

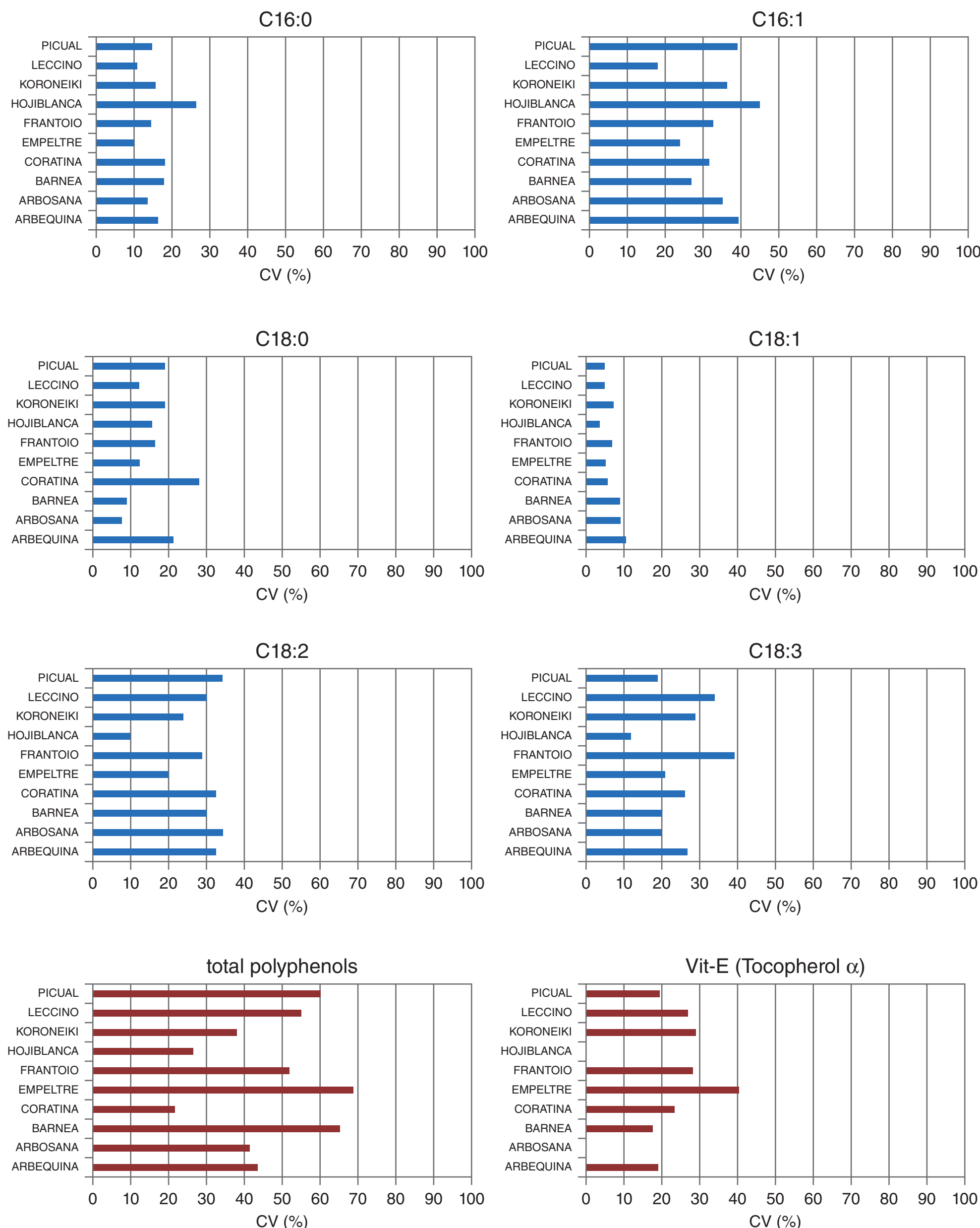

Figure 4.14 CV (%) variability of average main fatty acids, total phenols, and tocopherols related to several zones and cultivars.

are independent from each other, suggesting that particular environmental conditions can favor both compounds (Figure 4.15c). No relationship can be observed, however, between polyphenols and tocopherols (Figure 4.15b). Table 4.6 shows a positive correlation between oleic acid and latitude ($r = 0.35$) that is even higher in the NH ($r = 0.55$), where it is colder due to higher latitudes. Oleic acid content seems inversely related to altitude ($r = -0.43$), but this is only significant in the SH and could be explained by the high temperatures of the highlands of northwest Argentina. These findings are in agreement with Rondanini *et al.* (2011), who studied a wide range of olive oil samples from South America.

Table 4.5 Genetic and environmental effects on main fatty acid composition, polyphenol content, and tocopherols values for several olive cultivars ('Arbequina', 'Frantoio', 'Picual', etc.) grown at different world growing areas of Northern and Southern Hemispheres. Average data from bibliographic review.

			Significance and % of total variance explained				
			Fatty acids				
Hemisphere	**Source**	**Degrees of freedom**	***Palmitic***	***Oleic***	***Linoleic***	**Phenol content**	**Vitamin E**
NORTH	Cultivar	8	50.3**	50.9**	71.5**	49.4**	44.5, ns
	Altitude[1]	1	0.0	1.3, ns	1.4*	8.2**	4.4, ns
	Latitude[2]	5	10.7, ns	21.1**	11.1**	11.9**	10.4, ns
	C × A	4	4.3, ns	2.5, ns	3.1, ns	3.8, ns	27.6*
	C × L	10	8.2, ns	4.3, ns	4.3, ns	9.6, ns	0.9, ns
	Total	48					
	R^2		0.734	0.801	0.912	0.828	0.878
SOUTH	Cultivar	7	27.3 **	29.7**	66.2**	43.9*	57.9**
	Altitude[1]	2	21.8**	26.1**	19.9**	20.6**	8.4**
	Latitude[2]	4	18.8**	14.8**	9.2**	10.5, ns	2.5, ns
	C × A	11	4.0, ns	5.8, ns	6.1, ns	2.5, ns	3.9, ns
	C × L	19	6.4, ns	5.3, ns	5.1, ns	15.8, ns	18.6, ns
	Total	69					
	R^2		0.781	0.816	0.842	0.933	0.914

ns, Not significant; * $p = 0.05$; ** $p = 0.01$. R^2 = total explained variability by the interpolated model.
[1] Altitude (intervals every 300 m).
[2] Latitude (intervals every 2.5° above 28°).

The fatty acid compositions of 'Arbequina' cultivar oils from Argentina (La Rioja and San Juan) and Australia (NSW and Qld) differ greatly from those of Spain and Chile. Oleic acid content averaged 54.2% in La Rioja, 54.5% in NSW and Qld, and 60.2% in San Juan. These values are lower than those observed in Andalusia (65%), Catalonia (69–74%), Chile (73%), and Tasmania (81%). These differences also appear in 'Frantoio', 'Barnea', and 'Picual' from both hemispheres, mainly for C16:1, C18:2, and C18:3 (Table 4.4 and Figure 4.14). These changes may be partly explained by the effect of high temperatures during fruit development and harvesting time that would enhance the plant lipid metabolism, especially the oleic desaturation (Ceci & Carelli, 2007; Torres *et al.*, 2009; Gómez Campo *et al.*, 2010; Rondanini *et al.*, 2011). In La Rioja and San Juan provinces, the fatty acid synthesis takes place in summer and early autumn, when the

Table 4.6 Correlations coefficients (*r*) between fatty acid composition and total phenols versus latitude, altitude, and average temperatures for olive oil samples from Northern and Southern Hemispheres. Data based on bibliographic review.

Parameter	**Latitude**	**Altitude**	**Average temperature**
Fatty acid			
Palmitic	–0.317**	0.365**	0.436**
Palmitoleic	–0.251**	0.209**	0.338**
Stearic	0.011 ns	0.055 ns	0.099 ns
Oleic	0.354**	–0.434**	–0.495**
Linoleic	–0.310**	0.406**	0.388**
Linolenic	–0.248**	0.319**	0.520**
Total phenols	0.360**	–0.296**	–0.322**
Tocopherols	–0.219 ns	0.118 ns	0.166 ns

ns, Not significant; * $p = 0.05$; ** $p = 0.01$.

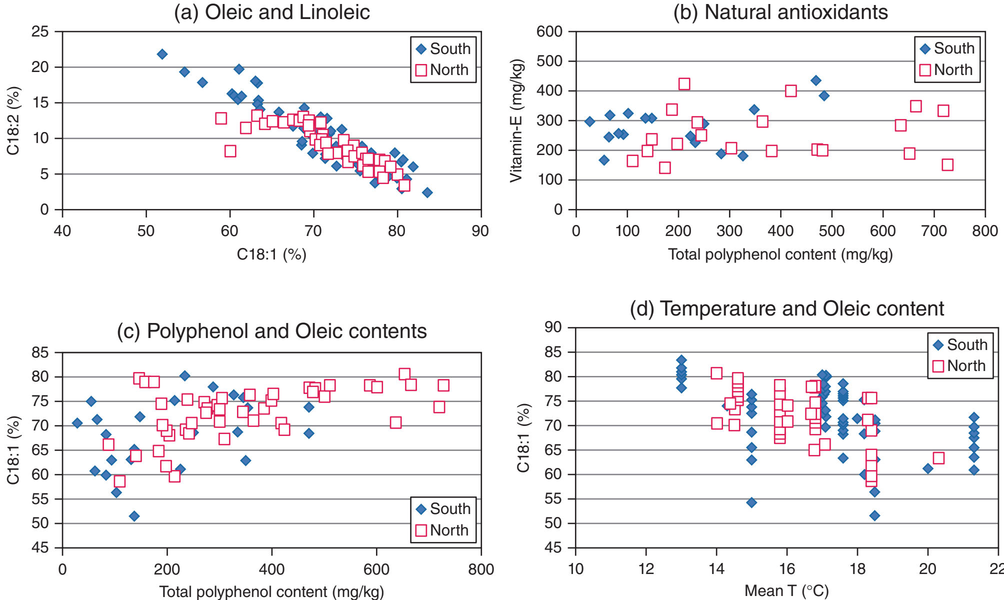

Figure 4.15 Relationships between (a) oleic and linoleic fatty acid contents, (b) polyphenol and tocopherol contents, (c) total polyphenols and oleic acid contents, and (d) mean annual temperature and oleic acid. Relationships are equivalent in both Northern Hemisphere (solid rhombuses) and Southern Hemisphere (empty squares) (n = 132).

temperatures are very high (24 °C average); in contrast, in Spain, oil synthesis occurs in the autumn, when temperatures are lower, which may best explain the variations in fatty acid composition. Angerosa *et al.* (1996) and Romero *et al.* (2003), on the other hand, observed that more wet summers in the NH produce low oleic acid contents, suggesting that the fatty acid synthesis is also regulated by other environmental factors. VOOs of the cultivar 'Picual' are similar in both Spanish regions (Andalusia and Catalonia), as well as in Australia and Chile. Regarding the cultivar 'Frantoio', which originates from central Italy (Tuscany), it has a fatty acid composition very similar in Chile and Spain, while in northwestern Argentina this cultivar produces more polyunsaturated oils. In Andalusia (southern Spain), Aguilera *et al.* (2005) determined a higher content of oleic acid and higher stability when this cultivar was grown in hilly areas (Córdoba, 440 m altitude) rather than on flat areas (Jaén, 280 m). Global fatty acid variability is presented in Figure 4.16 for some olive cultivars, which spread internationally, found 'Arbequina', 'Frantoio', and 'Barnea' oils more variable than others, like 'Picual', 'Coratina', and 'Leccino'.

According to Salas *et al.* (1997) and Torres *et al.* (2009), it is difficult to compare the phenolic content from different origins, because these phenols are greatly influenced by the fruit-ripening stage, the crop, the irrigation doses, and the conditions applied in the olive oil mill. Table 4.4 shows that the polyphenol content of olive oil in Argentinean provinces is lower than that in Spain or Chile. The total polyphenol content ranged from 45 to 700 mg/kg, but in 'Frantoio', 'Picual', 'Coratina', and 'Koroneiki' showing the highest values for their oils mainly in the NH. On the other hand, 'Arbequina' seems less variable (45–295 mg/kg). It can be pointed out that some cultivars, mainly in the SH, have a high percentage of linoleic acid and a low content in polyphenols (Ravetti *et al.*, 2002).

Table 4.5 shows significant differences in tocopherol content between cultivars and altitude, mainly cultivated in the SH. Cultivar variability ranges between 10% in 'Barnea' and 40% in 'Empeltre'. The minimum tocopherol content is related with 'Frantoio' produced in Tuscany (140 mg/kg), while the maximum is for 'Empeltre' produced in Andalusia and Coratina from NSW and Qld (400 mg/kg). There is not any relationship on total polyphenol content (Figure 4.15b).

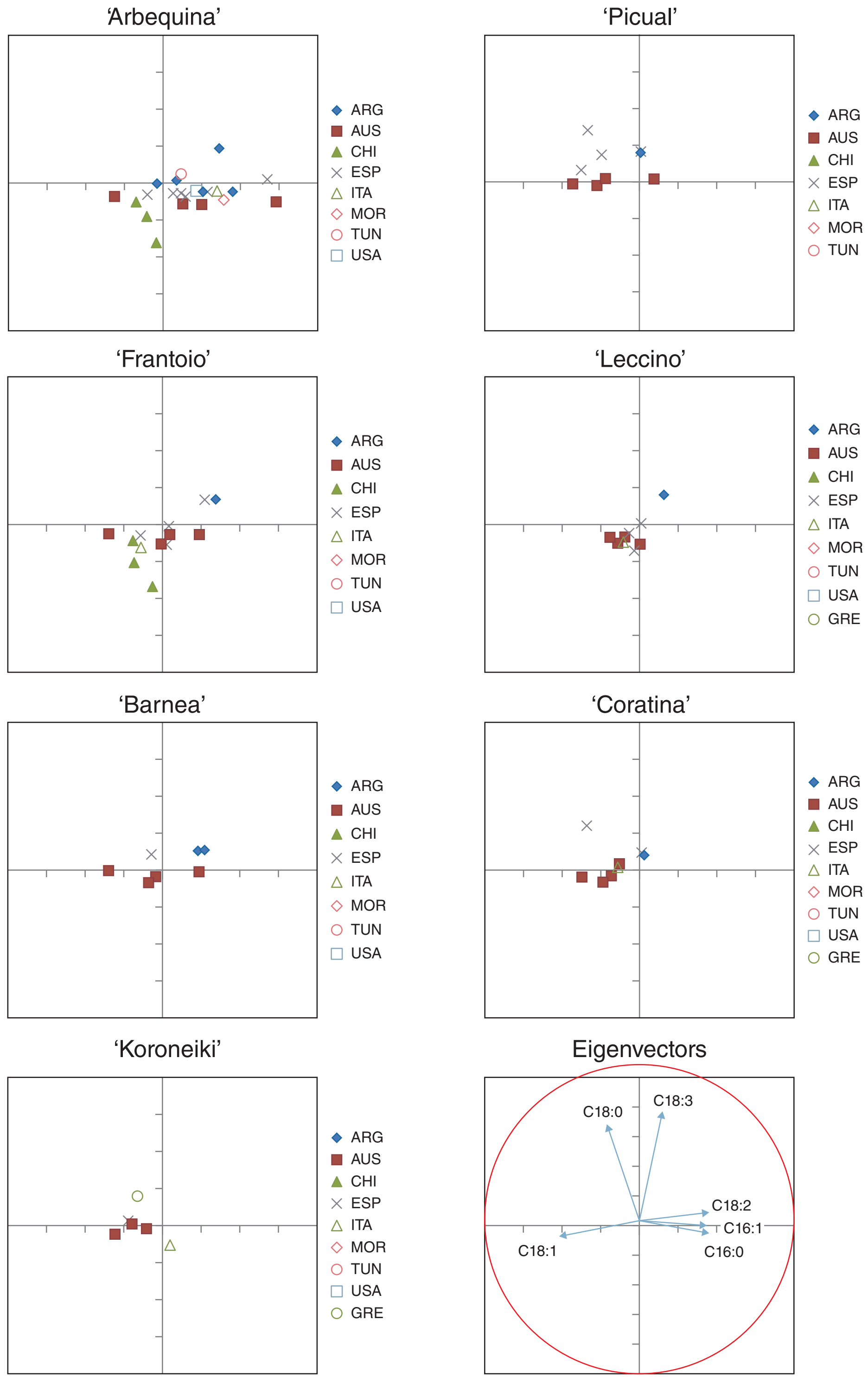

Figure 4.16 Within cultivar fatty acids variability related to the country of origin. Each graph shows principal components PRIN1 (horizontal axis) and PRIN2 (vertical axis) for a single cultivar. The eigenvector figure shows the correlation between each fatty and PRIN1 and PRIN2. The circle is for $r = 1$.

4.5 Conclusion

During recent decades, olives and olive oil have received a great deal of attention in the world markets due to their functional compounds and their positive effects on human nutrition and health. This global acceptance resulted in new markets far away from the Mediterranean Basin. New olives and olive oil producing countries appeared, such as South and North America, South Africa, and Australia, competing with traditional Mediterranean olive countries. The orchards in the new growing areas of South America are sustainable in relatively cheap and flat soils with large farm size, low manpower cost, and mechanical harvesting. These characteristics are similar to those of Australia, but the manpower cost is higher. On the contrary, the Mediterranean countries are less competitive, since olive orchards very often occupy marginal hill slopes or mountainous areas or are grown in adverse environmental conditions (drought). Regarding the Maghreb region, it must be pointed out that its very low manpower cost can increase their competition against the European countries (Spain, Italy, Portugal, etc.).

A comparative study of global cultivars, growing in both the Northern and Southern Hemispheres, reveals significant differences in olive oil characteristics. The olive oil composition of some Mediterranean cultivars is better in some Chilean, Australian, and Mediterranean growing areas and worst in the arid valleys of northwest Argentina, Maghreb countries, and some states of Australia (NSW and Qld). The 'Arbequina' olive cultivar shows a good performance under very different agro-ecological conditions, but it provides oils with different chemical traits than those from Catalonia. The same is true for the Greek cultivars 'Koroneiki' and 'Kalamon', which produce oil and olives of better quality and more functional compounds in Greece than in other countries. On the contrary, cultivars such as 'Picual' and 'Coratina' are less affected. These variations may be explained by the particular environmental conditions, such as mean temperature, day–night amplitude, and drought stress.

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References

Asociación Española de Municipios del Olivo (AEMO). (2010) Aproximación a los costes del cultivo del olivo. AEMO, Córdoba, Spain.

Aguilera, M.P., Beltrán, G., Ortega, D., Fernández, A., Jiménez, A., & Uceda, M. (2005) Characterisation of virgin olive oil of Italian olive cultivars: 'Frantoio' and 'Leccino', grown in Andalusia. Food Chemistry 89, 387–391.

Alcubilla, M., Broca, A., Sáez, E., Negueruela, A.I., Monge, E., Val, J., Espada, J.L., & Gracia, M.S. (2002) Medio agrológico y calidad del aceite de oliva en Aragón: relaciones entre el estado nutricional del olivo y su composición polifenólica. In: Jornadas de investigación y transferencia de tecnología al sector oleícola, Junta de Andalucía. Univ. Córdoba 20–21 Noviembre 2002, ed. Dir. Gral. de Investigación y Formación Agraria y Pesquera, 320–324.

Alegre, S., Marsal, J., Mata, M., Arbonés, A., Girona, J., & Tovar, M.J. (2002) Regulated deficit irrigation in olive trees (*Olea europaea* L.) for oil production. Acta Horticulturae 586(1), 259–262.

Angerosa, F., Di Giancinto, L., Basti, C., & Serraiocco, A. (1996) Influenza della variabile 'ambiente' sulla composizione degli oli vergini di olive. Riv. Ital. Sost. Grasse 63, 461–467.

Aparicio, R., Conte, L.S., & Fiebig, H-J. (2013) Olive oil authentication. In R. Aparicio & J.L. Harwood (Eds.), Handbook of olive oil: analysis and properties (pp. 589–653). New York: Springer.

Ayerza, R., & Sibbett, G.S. (2001) Thermal adaptability of olive (*Olea europaea*, L.) to the Arid Chaco of Argentina. Agriculture, Ecosystems and Environment 84, 277–285.

Balatsouras, G. (2009) The olive tree, private ed. N.p., Athens, Greece, 366–377. (In Greek)

Baptista, P., & Pereira, J.A. (2004) Influence of soil management (natural vegetation and tillage) on physiological parameters in *Olea europaea* cv. Cobrançosa. In: 5th Int. Symp. on olive growing, Izmir, 27 September–2 October 2004, ISHS Abstracts CM-40, ed. M. T. Özkaya.

Barranco, D. (2004) Variedades y patrones. In: El Cultivo del Olivo, ed. D. Barranco, R. Fernández-Escobar, & L. Rallo. Junta de Andalucía and Ediciones Mundi-Prensa, Madrid, 61–91.

Barranco, D., Cimato, A., Fiorino, P., Rallo, L., Touzani, A., Castañeda, C., Serafini, F., & Trujillo, I. (2000) World catalogue of olive varieties. International Olive Oil Council, Madrid.

Ben Rouina, B., Trigui, A., & Boukhirs, M. (2002) Effect of the climate and the soil conditions on crops performance of the 'Chemlali de Sfax' olive trees. Acta Horticulturae 586(1), 285–289.

Bianchi, G., Di Vincenzo, D., & Giansante, L. (2002) Chemometrics for the classification of POD olive oils. Acta Horticulturae 586(2), 599–602.

Bongi, G. (2004) Modelli produttivi in olivicltura. Olivo e Olio, 9, 8–15.

Boulouha, B., & El-Kholy, M. (2012) Following olive footprints in Morocco. In: Following olive footprints (*Olea europaea* L): cultivation and culture, folklore and history, traditions and uses, ed. M. El-Kholy, D. Avanzato, J.M. Caballero, K.S. Chartzoulakis, F. Vita, & E. Peri. AARINENA, IOC, and ISHS, Córdoba, Spain, 266–275.

Ceci, L., & Carelli, A. (2007) Characterization of monovarietal Argentinian olive oils from new productive zones. Journal of the American Oil Chemists' Society 84, 1125–1136.

Chartzoulakis, K.S. (2012) Following olive footprints in Greece. In: Following olive footprints (*Olea europaea* L): cultivation and culture, folklore and history, traditions and uses, ed. M. El-Kholy, D. Avanzato, J.M. Caballero, K.S. Chartzoulakis, F. Vita, & E. Peri. AARINENA, IOC, and ISHS, Córdoba, Spain, 137–153.

Cimato, A., Baldini, A., Caselli, S., Marranci, M., & Marzi, L. (1996) Observations on Tuscan olive germplasm. 3: analytical and sensory characteristics of single-variety olive oils. Olivae 62, 46–51.

D'Andria, R., Morelli, G., Patumi, M., & Fontanazza, G. (2002) Irrigation regime affects yield and oil quality of olive trees. Acta Horticulturae 586, vol.1, 273–276.

Díaz-Espejo, A., Hafidi, B., Fernández, J.E., Palomo, M.J., & Sinoquet, H. (2002) Transpiration and photosynthesis of the olive tree: a model approach. Acta Horticulturae 586, vol.2, 457–460.

Díez, C.M, Trujillo, I., Martínez-Urdiroz, N., Barranco, D., Rallo, L., Marfil, P., & Gaut, B.S. (2015) Olive domestication and diversification in the Mediterranean Basin. New Phytologist, 206, 436–447.

Donaire, J.P., Sánchez, A.J., López, J., & Recalde, L. (1975) Metabolic changes in fruit and leaf during ripening in the olive. Phytochemistry 14, 1167–1169.

Faci, J.M., Berenguer, M.J., Espada, J.L., & Gracia, S. (2002a) Effect of variable water irrigation supply in olive (*Olea europaea* L.) cv. Arbequina in Aragon (Spain). II. Extra virgin oil quality parameters. Acta Horticulturae 586(2), 649–652.

Faci, J.M., Berenguer, M.J., Gracia, M.S., & Espada, J.L. (2002b) Influencia del riego variable en la producción y calidad del aceite de un olivar adulto de cv. 'Arbequina' (Olea *europaea* L.). In: Jornadas de investigación y transferencia de tecnología al sector oleícola, Junta de Andalucía. Univ. Córdoba 20–21 Noviembre 2002, ed. Dir. Gral. de Investigación y Formación Agraria y Pesquera, 316–319.

Failla, O., Tura, D., & Bassi, D. (2002) Genotype-environment-year interaction on oil antioxidants in an olive district of northern Italy. Acta Horticulturae 586(1), 171–174.

Famiani, F., Proietti, P., Farinelli, D., & Tombesi, A. (2002) Oil quality in relation to olive ripening. Acta Horticulturae 586, 671–674.

Fernández-Escobar, R., García, J.M., Sánchez, M.A., Uceda, M., Beltrán, G., & Aguilera, M.P. (2002) Efecto del abonado nitrogenado en la producción y la calidad del aceite de oliva. In: Jornadas de investigación y transferencia de tecnología al sector oleícola, Junta de Andalucía. Univ. Córdoba 20–21 Noviembre 2002, ed. Dir. Gral. de Investigación y Formación Agraria y Pesquera, 300–302.

Fevereiro, P., Leitao, F., Potes, F., Gemas, V., Alves, M., & Favoretto, P. (2011) The Portuguese olive (*Olea europaea*) germplasm. Acta Horticulturae 924, 291–298.

Fleskens, L. (2008) A typology of sloping and mountainous olive plantation systems to address natural resources management. Annals of Applied Biology 153, 283–297.

Freixa, E., Tous, J., Gil, J.M., & Hermoso, J.F. (2011) Comparative study of the economic viability of high and super-high-density olive orchards in Spain. Acta Horticulturae 924, 247–254.

Galla, G., Barcaccia, G., Ramina, A., Collani, S., Alagna, F., Baldoni, L., Cultrera, N., Martinelli, F., Sebastiani, L., & Tonutti, P. (2009) Computational annotation of genes differentially expressed along olive fruit development. BMC Plant Biology 2009, 9:128.

García-González, D.L., Aparicio-Ruiz, R., & Aparicio, R. (2008) Virgin olive oil: chemical implications on quality and health. European Journal of Lipid Science and Technology, 110, 602–607.

García-González, D.L., Morales, M.T., Luna, G., & Aparicio, R. (2009) Stepwise geographical traceability of virgin olive oils by chemical traits modeled on ANN. European Journal of Lipid Science and Technology, 111, 1003–1013.

Garcia-González, D.L., Romero, N., & Aparicio, R. (2010) Comparative study of virgin oil quality from single varieties cultivated in Chile and Spain. Journal of Agricultural and Food Chemistry 58, 12899–12905.

García-González, D.L., Tena, N., & Aparicio, R. (2012) Describing chemical singularity of Spanish protected designations of origin of virgin olive oil. Grasas y Aceites, 63, 26–34.

Gómez del Campo, M., Morales-Sillero, A., Vita, F., Rousseaux, M.C., & Searles, P.S. (2010) Olive growing in the arid valleys of Northwest Argentina (Provinces of Catamarca, La Rioja, and San Juan). Olivae 114, 23–45.

Gregoriou, C., Ponitikis, K., & Vemmos, S.N. (2004) Effect of shading on yield, mophological characteristics of the fruit, oil content and ratio of fatty acids of oil of olive variety 'Koroneiki'. In: 5th Int. Symp. on olive growing, Izmir, 27 September–2 October 2004, ISHS Abstracts BP-215, ed. M. T. Özkaya

Hernández, M.L., Padilla, M.N., Mancha, M., & Martínez-Rivas, J.M. (2009) Expression analysis identifies FAD2-2 as the olive oleate desaturase gene mainly responsible for the linoleic acid content in virgin olive oil. Journal of Agricultural and Food Chemistry 57(14), 6199–6208.

Inglese, P., Famiani, F., Galvano, F., Servili, M., Esposto, S., & Urbani, S. (2010) Factors affecting extra-virgin olive oil composition. In: Horticultural review, vol. 38, ed. J. Janick. John Wiley & Sons, Hoboken, NJ, 83–147.

Junta de Andalucía (2003) El olivar andaluz, ed. Junta de Andalucía. Consejeria de Agricultura y Pesca, Sevilla.

Kiritsakis, A. (1992) El Aceite de Oliva. A. Madrid Vicente Ediciones, Madrid, 157–161.

Koutsaftakis, A., Kotsifaki, F., Stefanoudaki, E., & Cert, A. (2000) Estudio sobre variaciones de determinados parámetros químicos y de los componentes menores de los aceites de oliva virgen obtenidos de aceitunas recolectadas en distintas fases de maduración. Olivae 80 (2), 22–27.

Lavee, S. (2012) Following olive footprints in Israel. In: Following olive footprints (*Olea europaea* L): cultivation and culture, folklore and history, traditions and uses, ed. M. El-Kholy, D. Avanzato, J.M. Caballero, K.S. Chartzoulakis, F. Vita, & E. Peri. AARINENA, IOC, and ISHS, Córdoba, Spain, 164–175.

Mailer, R.J., Ayton, J., & Graham, K. (2010) The influence of growing region, cultivar and harvest timing on the diversity of Australian olive oil. Journal of the American Oil Chemists' Society 87, 877–884.

Mannina, L., Fontanazza, G., Patumi, M., Ansanelli, G., & Segre, A. (2001) Italian and Argentine olive oils: a NMR and gas chromatographic study. Grasas y Aceites 52(6), 380–388.

Martínez de Victoria, E., & Mañas, M. (2004) El aceite de olive en la dieta y salud humanas, p. 733–754. In: El Cultivo del Olivo, ed. D. Barranco, R. Fernández-Escobar, & L. Rallo. Junta de Andalucía and Ediciones Mundi-Prensa, Madrid.

Metzidakis, I.T., & Koubouris, G.C. (2006) Olive cultivation and industry in Greece. In: Recent advances in olive industry, Second Int. Sem. OliveBioteq, ed. T. Caruso, A. Motisi, & L. Sebastiani. Marsala-Mazara Vallo, Italy, 133–139.

Montedoro, G.F., Taticchi, A., Esposto, S., Selvaggini, R., Urbani, S, & Servili, M. (2007) Antioxidants in virgin olive oil. Olea (FAO Olive Network) 26(7), 5–13.

Morelló, J.R. (2004) Caracterización de la fracción fenólica del fruto y del aceite de oliva virgen de la variedad "Arbequina" y evaluación de la influencia de algunos factores agronómicos y tecnológicos. Doctoral thesis, UdL, E.T.S.E.A., Dept. Tecnologia d'Aliments.

Motilva, M.J., Tovar, M.J., Alegre, S., & Girona, J. (2002) Evolution of oil accumulation and polyphenol content in fruits of olive tree (*Olea europaea* L.) related to different irrigation strategies. Acta Horticulturae 586(1), 345–348.

Motilva, M.J., Tovar, M.J., Romero, M.P., Alegre, S., & Girona, J. (2000) Influence of regulated deficit irrigation strategies applied to olive trees (Arbequina cultivar) on oil yield and oil composition during the fruit ripening period. Journal of the Science of Food and Agriculture 80, 2037–2043.

Motisi, A., Campisi, G., Caruso, T., Sacchi, R., Ambrosino, M.L., & Finoli, C. (2004) Variability of olive oil quality as related to cultivar and agronomic factors in Sicily. In: 5th Int. Symp. On olive growing, Izmir, 27 September–2 October 2004, ISHS Abstracts OO-171, ed. M. T. Özkaya.

Motisi, A., & Gullo, G. (2002) Effect of soil texture on root hydraulic conductivity in *Olea europaea* L. Cv. "Nocellara del Belice" potted trees. Acta Horticulturae 586(2), 503–506.

Padilla, M.N., Hernández, M.L., Sanz, C., & Martínez-Rivas, J.M. (2009) Functional characterization of two 13-lipoxigenase genes from olive fruit in relation to the biosynthesis of volatile compounds of virgin olive oil. Journal of Agricultural and Food Chemistry 57(19), 9097–9107.

Pardo, J.E., Cuesta, M.A., & Alvarruiz, A. (2007) Evaluation of potential and real quality of virgin olive oil from the designation of origin "Aceite Campo de Montiel" (Ciudad real, Spain). Food Chemistry 100, 977–984.

Pastor, M. (2003) Influencia del riego sobre la calidad del aceite de oliva. Revista Oleo, 92, 76–87.

Pastor, M., Vega, V., & Hidalgo, J. C. (2006) Densidades de plantación en olivar de regadío. El caso de las plantaciones superintensivas de Andalucía. Fruticultura Profesional (Especial Olivicultura IV) 160, 27–42.

Perona, J.S., & Botham, K.M. (2013) Olive oil as a functional food: nutritional and health benefits. In: Handbook of olive oil: analysis and properties, ed. R. Aparicio & J.L. Harwood. New York: Springer, 677–714.

Pinheiro, A.C., Sampaio, E., Piçarra, I.A., Santos, L.S., Peça, J.O., & Barriguinha, A., (2004) Soil conservation practices in olive orchards. In: 5th Int. Symp. On olive growing, Izmir, 27 September–2 October 2004, ISHS Abstracts CM-29, ed. M. T. Özkaya.

Proietti, P., Famiani, F., & Tombesi, A. (1999) Gas exchange in olive fruit. Photosynthetica 36(3), 423–432.

Rallo, L. (2006) The olive industry in Spain. In: Recent advances in olive industry, Second Int. Sem. OliveBioteq, ed. T. Caruso, A. Motisi, & L. Sebastiani. Marsala-Mazara Vallo, Italy, 151–162.

Rallo, L., Barranco, D., Caballero, J.M., Del Río, C., Martin, A., Tous, J., & Trujillo, I. (2005) Variedades de olivo en España, ed. Junta de Andalucía. MAPA and Ediciones Mundi-Prensa, Madrid.

Rallo, L., Barranco, D., De la Rosa, R., & León, L. (2008) 'Chiquitita' olive. HortScience 43 (2), 529–531.

Ramos, A., Peres, F., Simöes, P., & Pinheiro, MC. (2008) Olive ripening and harvest time in protuguese cultivars of "Beira Interior" región. Acta Horticulturae 791, 693–696.

Ranalli, A., De Mattia, G. Patumi, M., & Proietti, P. (1999) Quality of virgin olive oil as influenced by origin area. Grasas y Aceites 50(4), 249–259.

Ravetti, L., & Edwards, M. (2014) Olive oils from Australia and New Zealand. In: Olive oil sensory science, ed. E. Monteleone and S. Langstaff. John Wiley & Sons, Ltd., Chichester, 313–336

Ravetti, L., Matías, A.C., Patumi, M., Rocchi, P., & Fontanazza, G. (2002) Characterization of virgin olive oils from Catamarca and La Rioja, Argentina. General characteristics. Acta Horticulturae 586, 603–606.

Romero, M.P., Tovar, M.J., Motilva, M.J., Luna, M., & Girona, J. (2002) Effect of irrigation strategies applied on olive tree (Olea europaea L.) on oil pigment content and colour. Acta Horticulturae 586 vol.1, 325–328.

Romero, M.P., Tovar, M.J., Ramo, T., & Motilva, M.J. (2003) Effect of crop season of the composition of virgin olive oil with Protected Designation of Origin "Les Garrigues". Journal of the American Oil Chemists' Society 80, 423–430.

Romero, N., Saavedra, J., Tapia, F., Sepúlveda, B., & Aparicio, R. (2016) Influence of agroclimatic parameters on phenolic and volatile compounds of Chilean virgin olive oils and characterization based on geographical origin, cultivar and ripening stage. Journal of the Science of Food and Agriculture 96, 583–592.

Romero-Aroca, A., Claret, A., & Guerrero, L. (2014) Olive oils from Spain. In: Olive oil sensory science, ed. E. Monteleone and S. Langstaff. John Wiley & Sons, Ltd., Chichester, 229–246.

Romero-Aroca, A., Díaz, I., & Tous, J. (2002) Optimal harvesting period for 'Arbequina' olive cultivar in Catalonia (Spain). Acta Horticulturae 586(1), 393–396.

Romero-Aroca, A., Hermoso, J.F., & Tous, J. (2013) Caracterización de los aceites de catorce variedades de olivo en el sur de Cataluña. Vida Rural. 358, 66–72.

Rondanini, D.P., Castro, D.N., Searles, P.S., & Rousseaux, MC. (2011) Fatty acid profiles of varietal virgin olive oils (*Olea europaea* L) from mature orchards in warm arid valleys of Northwestern Argentina (La Rioja). Grasas y Aceites 62 (4), 399–409.

Sadeghi, H., & Talaii, A.R. (2002) Impact of environmmental conditions on fatty acids combination of olive oil in an Iranian olive, cv. Zard. Acta Horticulturae 586 vol.2, 579–581.

Salas, J., Pastor, M., Castro, J., & Vega, V. (1997) Influencia del riego sobre la composición y características organolépticas del aceite de oliva. Grasas y Aceites 48(2), 74–82.

Sebastiani, L., d'Andria, R., Motisi, A., & Caruso, T. (2006) The olive industry outside the Mediterranean. In: Recent advances in olive industry, Second Int. Sem. OliveBioteq, ed. T. Caruso, A. Motisi, & L. Sebastiani. Marsala-Mazara Vallo, Italy, 183–195.

Sergeeva, V. (2012) Following olive footprints in Australia. In: Following olive footprints (*Olea europaea* L): cultivation and culture, folklore and history, traditions and uses, ed. M. El-Kholy, D. Avanzato, J.M. Caballero, K.S. Chartzoulakis, F. Vita, & E. Peri. AARINENA, IOC, and ISHS, Córdoba, Spain, 32–47.

Servili, M., Esposto, S., Tatichi, A., Urbani, S., Veneziani, I., Di Maio, I., Selvaggini, R., & Gucci, R. (2011) From the orchard to the virgin olive oil quality: a critical overview. Acta Horticulturae 924, 364–378.

Simöes, P., Pinheiro, C., Cordeiro, A.M., & Marcelo, M.E. (2002) Effect of the nitrogen and potassium fertilization on fatty acids composition and oxidative stability for 'Carrasquenha' cultivar olive oil at different harvest periods: preliminary study. Acta Horticulturae 586(1), 337–340.

Sweeney, S. (2002) National Olive Variety Assessment Project update. The Olive Press, Summer, 14–25.

Therios, J. (2009) Olives. Crop Production Science in Horticulture series. CABI, Cambridge, MA, 255–270.

Tombesi, A., & Antaras, T. (1998) La techniche colturali e la qualità dell'olio d'oliva. Rivista di Frutticoltura 7–8, 25–30.

Tombesi, A., Boco, M., & Pilli, M. (1998) Microclima del frutto: influenza della luce sull'accrescimento e sulla sintesi dell'olio. Rivista di Frutticoltura 7–8, 63–67.

Torres, M.M., Pierantozzi, P., Cáceres, M.E., Lalombarda, P., Fontanazza, G., & Maestri, D.M. (2009) Genetic and chemical assessment of Arbequina olive cultivar grown in Córdoba province, Argentina. Journal of the Science of Food and Agriculture 89, 523–530.

Tous, J. (2011) Olive production systems and mechanization. Acta Horticulturae 924, 169–184.

Tous, J. (2015) Situación del olivo en Australia. Mercacei 83, 188–202.
Tous, J., Del Río, C., Caballero, J., & Rallo, L. (2005a) Libro II. Variabilidad y selección. In: Variedades de olivo en España, ed. L. Rallo, D. Barranco, Juan M. Caballero, C. del Río, A. Martín, J. Tous, and I. Trujillo. Junta de Andalucía, MAPA y Ediciones Mundi-Prensa, Madrid.
Tous, J., & Romero, A. (1993) Variedades de olivo. Con especial referencia a Catalunya. Ed. Fundación 'La Caixa' y AEDOS S.A., Barcelona.
Tous, J., & Romero, A. (1994) Cultivar and location effects on olive oil quality in Catalonia, Spain. Acta Horticulturae 356, 323–326.
Tous, J., & Romero, A. (2000) Variedad 'Arbosana IRTA-i·43'. Olint (2)8, 13–15.
Tous, J., Romero, A., & Díaz, I. (2005b) Composición del aceite (Banco de Germoplasma de Cataluña). In: Variedades de olivo en España (Libro II: Variabilidad y selección), ed. L. Rallo, D. Barranco, Juan M. Caballero, C. del Río, A. Martín, J. Tous, and I. Trujillo. Junta de Andalucía, MAPA y Ediciones Mundi-Prensa, Madrid.
Tous, J., Romero, A., Hermoso, J.F., Msallem, M., & Larbi, A. (2014) Olive orchard design and Mechanization: Present and future. Acta Horticulturae 1057, 231–246.
Tous, J., Romero A., Hermoso, J.F., & Ninot, A. (2011) Mediterranean clonal selections evaluated for modern hedgerow olive oil production in Spain. California Agriculture 65(1), 34–38.
Tous, J. Romero, A., & Plana, J. (1999) 'Irta-i·18', a clone of the 'Arbequina' olive variety. Olivae 77(6), 50–52.
Tous, J., Romero, A., Plana, J., Espada, J.L., Gracia, M.S., Lizar, B., Rallo, J., Martorell, A., Iñiguez, A, García, J., & Elguea, J.A. (2004) Varietal fact card: cv 'Empeltre'. Olivae 102, 31–32.
Tous, J., Romero, A., Plana J., Guerrero, L., Díaz, I., & Hermoso, J.F. (1997) Características químico-sensoriales de los aceites de oliva 'Arbequina' obtenidos en distintas zonas de España. Grasas y Aceites 48 (6), 415–424.
Tous, J., Romero, A., Plana, J., & Hermoso, J.F. (2002) Behaviour of ten Mediterranean olive cultivars in the northeast of Spain. Acta Horticulturae 586, 113–116.
Tous, J., Romero, A., Plana, J., & Hermoso, J.F. (2008) Olive oil cultivars suitable for very-high density planning conditions. Acta Horticulturae 791, 403–408.
Tura, D., Failla, O., Pedó, S., Bassi, D., Gigliotti, C., & Serraiocco, A. (2008) Effects of seasonal weather conditions on olive oil quality in Northern Italy. Acta Horticulturae 791, 769–776.
Uceda, M., Beltrán, G., & Jiménez, A. (2005) Composición del aceite (Banco de Germoplasma de Córdoba). In: Variedades de olivo en España (Libro II: Variabilidad y selección), ed. L. Rallo, D. Barranco, Juan M. Caballero, C. del Río, A. Martín, J. Tous, and I. Trujillo. Junta de Andalucía, MAPA y Ediciones Mundi-Prensa, Madrid.
Uceda, M., Hermoso, M., & Aguilera, MP. (2004) La calidad del aceite de oliva. In: El cultivo del olivo, 5th ed., ed. D. Barranco, R. Fernández-Escobar, & L. Rallo. Junta de Andalucía and Ediciones Mundi-Prensa, Madrid, 659–684.
Velasco, M., Vilar, J., & Puentes, R. (2011) PAC 2014–2020: implicaciones y estrategias para el olivar tradicional español. Olivae 115, 48–60.
Visioli, F., & Galli, C. (1998) The effect of minor constituents of olive oil on cardiovascular disease: new findings. Nutrition Teviews 56, 142–147.
Vossen, P. (2007) Olive oil: history, production, and characteristics of the world's classic oils. HortScience 42 (5), 1093–1100.
Vossen, P.M., Berenguer, M.J., Grattan, S.R., Connell, J.H., & Polito, V.S. (2008) The influence of different levels of irrigation on the chemical and sensory properties of olive oil. Acta Horticulturae 791, 439–444.

5 Olive fruit and olive oil composition and their functional compounds

Fatima Paiva-Martins and Apostolos Kiritsakis

5.1 Introduction

Fresh, ripe olives are mainly composed of water, oil, sugars, proteins, and cellulose. Other important components are pectin; phenolic compounds; organic acids, such as citric, oxalic, malonic, fumaric, tartaric, acetic, and triterpenic acids; tannins; and inorganic salts (Fedeli & Jacini, 1971). Certain minerals such as iron, calcium, potassium, phosphorus, manganese, magnesium, and copper are found in the pulp of olive fruit. The copper content of the olive fruit increases substantially during the ripening of the fruit. Olive fruit from the 'Mastoides' cultivar contains more mineral elements than the fruit from the 'Koroneiki' cultivar (Androulakis, 1987). Nevertheless, the olive fruit composition varies according to the cultivar, the environment, and the degree of ripeness (Kiritsakis & Markakis, 1987; Tura *et al.*, 2009; Garcia *et al.*, 2012; Fernández-Cuesta *et al.*, 2013; Jiménez *et al.*, 2013).

Several researchers have shown that olive and olive oil constituents contribute to their beneficial effects on health. The prevention of cardiovascular diseases (CVDs) and of certain cancers by extra virgin olive oil (EVOO) consumption has been related to its well-balanced fatty acid (FA) composition/profile, where oleic acid is the main component, and to the presence of minor compounds, such as polar phenols, tocopherols, and sterols. These characteristics make olive oil a premium functional food and, therefore, a product of major economic and social importance in the Mediterranean region. The first indirect evidence indicating that consuming olive oil, within the Mediterranean diet, might increase longevity originated in the Seven Countries Study (Keys *et al.*, 1986), followed by the MONICA study (Tunstall-Pedoe *et al.*, 1999), which showed lower CVD incidence and mortality rates in Mediterranean countries compared with other European regions or the United States. These health benefits were attributed to their Mediterranean dietary pattern, which includes regular olive oil consumption.

Recent results from the EPIC-Spain cohort have provided some of the clearest evidence to date about olive oil consumption, independent of the Mediterranean dietary pattern, that is related to a reduction in overall mortality (Buckland *et al.*, 2011). Olive oil consumption was negatively related to CVD mortality after following 40,622 initially healthy volunteers from Spain for 13.4 years. Compared with non-consumers (15% of the sample), the top quartile of olive oil consumption (participants consuming over 24.9 g per day) was associated with a 26% decrease in overall mortality, and an incredible 44% decrease in CVD mortality, after multivariate adjustment including components of the Mediterranean diet.

This chapter describes olives and olive oil composition and their functionality.

5.2 The olive fruit

The olive fruit, or olive, is a drupe (like the cherry or plum), oval in shape, and it consists of two main parts: the pericarp and the endocarp. The pericarp represents 66–85% of the weight of the fruit and is composed of the epicarp (or skin) and the mesocarp (or pulp). The endocarp, also called the pit or kernel, contains

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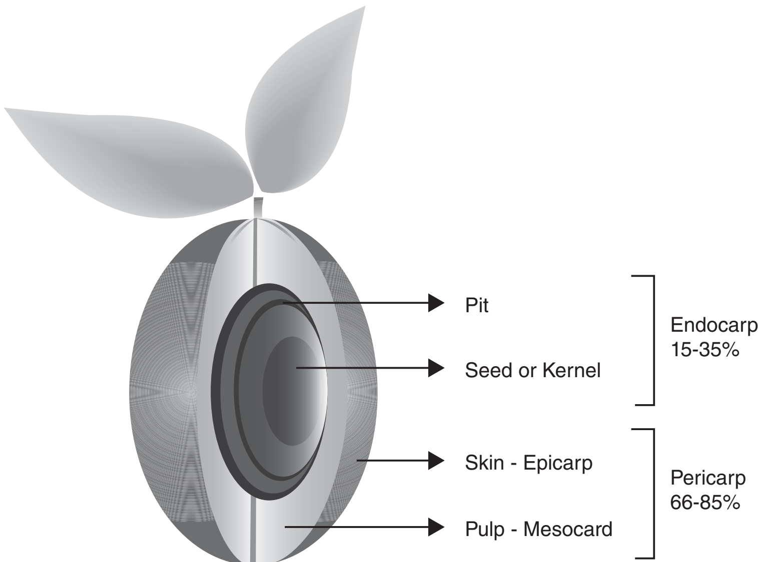


Figure 5.1 Parts of the olive fruit.

the seed, which represents <3% of the fruit weight (Figure 5.1). The pericarp contains 96–98% of the total amount of oil; the remaining 2-4% of the oil is in the endocarp (Kiritsakis, 1998a).

5.3 Description of olive fruit and olive oil constituents

5.3.1 Water

Water is one of the main constituents of the olive fruit and accounts for about 70% of its weight. Water serves as a solvent for the organic acids, tannins, oleuropein, and other water-soluble constituents of the fruit. The amount of water in the fruit depends on the stage of maturity, the cultivar, and other factors such as climate, irrigation, and soil moisture.

5.3.2 Sugars

The main sugars of the pulp are glucose and fructose. Sucrose, maltose, isomaltose, mannose, and galactose have also been found in certain cultivars (Fedeli, 1977; Lanza *et al.*, 2014). Glucose and in smaller quantities fructose are also found in the kernel of the fruit. A decrease in the sugar content of olive fruit with maturation is related to an increase in the oil content (Kiritsakis, 1998a).

5.3.3 Proteins

The olive fruit mesocarp contains 1.5 to 3.0% protein depending on the cultivar and the maturity stage. All of the amino acids present in the other plant proteins are found in the olive fruit alone (Manoukas *et al.*, 1973). The protein content is relatively low, but its nutritional value is high. Free nitrogen content varies considerably and constitutes less than 1% of total nitrogen. Aspartic and glutamic acids are usually the main amino acids found in the fruit of several olive cultivars, followed by leucine and valine (Fedeli, 1977; Martín-Garcia *et al.*, 2003). Arginine and phenylalanine may also be found in some cultivars in high percentages (Lanza *et al.*, 2014). Table 5.1 shows the amino acid composition of three Greek olive cultivars (Manoukas *et al.*, 1973).

The olive stone has been proposed as a cheap source of protein. In fact, olive seed proteins could be of interest for the production of valuable substances such as bioactive peptides that can demonstrate a wide

Table 5.1 Amino acid composition of three Greek olive cultivars (g of amino acid/16 g of N).

Amino acid	'Koroneiki'	'Throumbolia'	'Megaritiki'
Arginine	10.17	9.38	9.71
Histidine	2.74	2.99	2.34
Lysine	5.13	1.85	7.02
Methionine	1.29	1.52	1.16
Cystine	Trace	Trace	Trace
Phenylalanine	3.00	2.76	3.14
Tyrosine	2.42	2.52	2.22
Leucine	5.25	4.71	5.55
Isoleucine	3.04	2.96	3.28
Threonine	2.60	2.51	3.30
Valine	4.07	3.79	4.03
Alanine	5.45	6.49	3.60
Aspartic acid	10.99	11.11	12.28
Glutamic acid	10.72	12.54	11.85
Glycine	6.54	8.79	3.50
Proline	4.31	7.44	2.70
Serine	3.05	3.48	4.30

variety of bioactivities, including antihypertensive and antioxidant properties (Rodríguez *et al.*, 2007). Enzymatic hydrolysates of olive seed protein isolate were prepared by treatment with five different proteases, and all hydrolysates presented antioxidant properties and antihypertensive capacity. Furthermore, these fractions also maintained their bioactivities after being subjected to *in vitro* gastrointestinal digestion. This shows that olive seed proteins constitute a cheap and valuable source of antioxidant and antihypertensive peptides.

5.4 Olive oil

Minor components constitute about 1–2% of the total amount of virgin olive oil (VOO) (Kiritsakis, 1998a). After saponification of olive oil, the remaining unsaponifiable fraction contains many minor components, including hydrocarbons, like squalene and β-carotene; sterols, such as β-sitosterol, campesterol, and 4-methylsterols; fatty alcohols; triterpenic alcohols, such as uvaol and erythrodiol; triterpenic acids, such as oleanolic and maslinic acids; other terpenic compounds; tocopherols, mainly α-tocopherol; and pigments, like chlorophylls and pheophytins. In olive oil, minor components are also included: phenolic compounds such as oleuropein aglycons, tyrosol, and hydroxytyrosol, as well as waxes, sterol esters, mono- and diacylglycerols, phosphatides, unusual glycero-glycolipids, and other unidentified constituents (Kiritsakis, 1991; Harwood & Aparicio, 2000; Karantonis *et al.*, 2002; Covas *et al.*, 2006).

5.4.1 Olive oil acylglycerols and fatty acids

The olive oil constituents are primarily triacylglycerols (TAGs) (~99%) and secondly free fatty acids, monoacylglycerols (MAGs), and diacylglycerols (DAGs). FAs esterified in olive oil acylglycerols are palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3). Myristic (C14:0), heptadecanoic, and eicosanoic acids are found in trace amounts. The International Olive Oil Council (IOOC) also sets limit for trans fatty acids in each commercial category. For edible VOO categories, the levels for C18:1t and the sum of C18:2t and C18:3t isomers are extremely low (<0.05% in each case). Expansion of olive tree cultivation in countries such as Australia, Argentina, New Zealand, and South Africa as well as in California has shown that olive oil data deviated to a certain degree from those currently accepted by the Codex (Rondanini *et al.*, 2011). Diversity in environment and cultivar characteristics resulted in wide ranges for the four major FAs, usually showing higher percentages of linoleic and linolenic acids (Faouzia *et al.*, 2008).

Fedeli (1977) reported the major triacylglycerols of olive oil as POO (18.4%), SOO (5.1%), POL (5.9%), OOO (43.5%), and OOL (6.8%), where P = palmitic, O = oleic, S = stearic, L = linoleic, and Ln = linolenic acids. Stearic and palmitic acids are absent from the 2-position of unsaturated species, the tri- and tetraunsaturated, or from molecules with more than five double bonds. Trilinolein or ECN 42 triacylglycerol content, which is used as an authenticity marker by the European Union (EU), is the sum of the amounts of LLL, PPP, SLnLn, PPL, PPLn, OLLn, PLLn, and POLn (positional isomers included).

The presence of partial acylglycerols in olive oil is due to either incomplete triacylglycerol biosynthesis or hydrolytic reactions. The level of DAGs in VOO ranges from 1 to 2.8% (Mariani *et al.*, 1991; Kiosseoglou *et al.*, 1993; Lercker, 2011; Spyros *et al.*, 2004). When DAGs are present in higher concentrations, olive oil is of low quality (Kiritsakis, 1998a). Storage conditions affect the distribution of FAs. 1,2-DAGs present in fresh oil tend to isomerize to the more stable 1,3-DAGs. This rearrangement gives information about the age of the oil and storage conditions. The ratio of 1,3-DAGs/1,2-DAGs is considered as a useful criterion to monitor quality (Spyros *et al.*, 2004).

MAGs are present in much smaller quantities (less than 0.25%), whereas 1-species are considerably higher than the respective 2-MAGs. The ratio of MAGs to DAGs depends on oil acidity (Paganuzzi, 1999). Olive trees, like other crop plants, are sensitive to environmental conditions. Therefore, olive oil FA composition may differ from sample to sample, depending on the zone of production, latitude, climate, cultivar, and stage of fruit maturity (Kiritsakis & Markakis, 1987; Tsimidou *et al.*, 1987; Kiritsakis, 1991; Salvador *et al.*, 2003; Mailer *et al.*, 2007; Gómez-Rico *et al.*, 2008; Diraman *et al.*, 2010; Taamalli *et al.*, 2010; Rondanini *et al.*, 2011; Garcia *et al.*, 2012). Most Greek, Portuguese, Italian, and Spanish olive oils are low in linoleic and palmitic acids and have a high percentage of oleic acid. This is in accordance with the current biochemical evidence which indicates that, in olive and other plant species, the polyunsaturated fatty acids (PUFAs) C18:2 and C18:3 are produced by the consecutive desaturation of oleic acid. In contrast, most Tunisian olive oils are high in linoleic and palmitic acids and lower in oleic acid (Oueslati *et al.*, 2009). Elevated linoleic acid content negatively affects the technological properties of the oil; therefore, the oxidative stability and the low levels of α-linolenic acid are essential for aroma biogenesis during the milling and malaxation processes in the olive oil mill (Olías *et al.*, 1993).

5.4.1.1 Effects of fatty acids on the biosynthesis of biologically active signaling molecules

There are two classes of fatty acids derived from essencial fatty acids (EFAs), the n-3 series derived from the EFA α-linolenic acid present in low concentrations in olive oil (<1%), and the n-6 series derived from the EFA linoleic acid, also present in olive oil and usually in higher concentrations (up to 21%). These EFAs are mainly used as a source of metabolic energy, but they are both needed as membrane components and as precursors of biologically active signaling molecules. However, there has been a continuous debate about the amounts and the ratio of n-3/n-6 PUFAs that are required (Ramsden *et al.*, 2010; Michas *et al.*, 2014). Linoleic (n-6) and α-linolenic acids (n-3) are the main dietary components leading to the formation of 20 carbon metabolites, arachidonic acid (AA) and eicosapentaenoic acid (EPA), respectively, which are the direct precursors of the biologically active mediators, eicosanoids (Baum *et al.*, 2012) (Figure 5.2).

AA is the major precursor for eicosanoids. It is generally agreed that AA has to be released from membrane lipids as a first step, and this release seems to be facilitated in the presence of low tocopherol concentration at the membranes. Once released, the FA can be oxidized via a cyclooxygenase, a lipoxygenase, or a cytochrome P-450 system to produce, ultimately, various biologically active classes of compounds, named prostaglandins, thromboxanes, leucotrienes, and prostacyclins, which have different roles in the body (Ridgway *et al.*, 2016). Therefore, the quantity of eicosanoids produced has important health implications since they play important roles as inflammatory mediators, in cell adhesion, in the clotting process, and as signaling molecules (Ridgway *et al.*, 2016).

The effects of oleic acid on eicosanoid metabolism are less clear. The metabolism of n-9 FAs does not give rise to eicosanoid precursors, but whether they have any effect or not on the synthesis of eicosanoids from other precursors is not known. However, in healthy young adults a Mediterranean-based diet for 4 weeks led to a significant decrease in the omega-6/omega-3 FA ratio (n-6/n-3) to about 2:1, which was associated with an improvement in the inflammatory markers (Ferruci *et al.*, 2006).

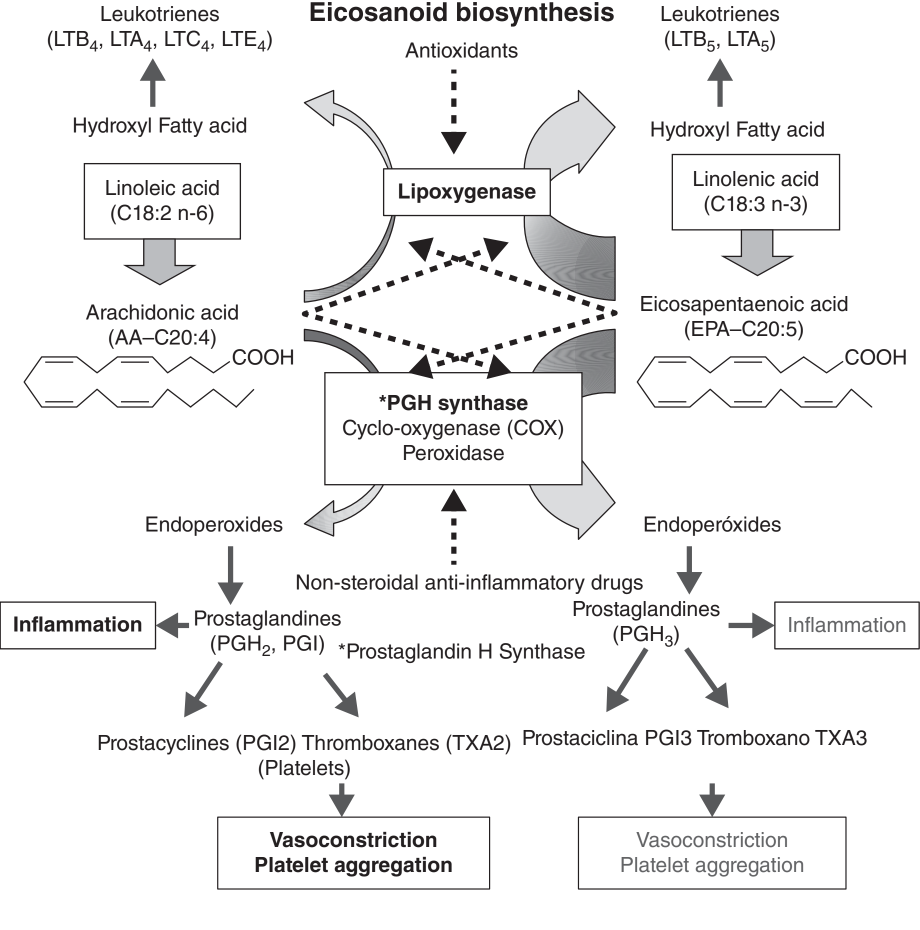


Figure 5.2 Influence of dietary n-3/n-6 polyunsaturated fatty acids and drugs on eicosanoid production. Effects in bold signify strong effects. Effects in gray signify weak effects. Wide arrows signify that a larger concentration of subtract is needed to activate enzymes.

For many years, several studies have consistently shown that n-6 PUFAs enhance tumor growth and that n-3 PUFAs suppress tumor growth (Liu & Ma, 2014). Several studies have demonstrated that the levels of prostaglandins are higher in various cancers, including breast cancer, compared to the normal tissues (Lupulescu, 1995).

It has also been demonstrated that a monounsaturated fatty acid (MUFA)-rich diet also decreases plasma levels of plasminogen activator inhibitor type I, von Willebrand factor, E-selectin, and thrombomodulin (Rose, 1997) and reduces the coagulation cascade factor VII activation.

5.4.1.2 Effects of fatty acids on the biosynthesis and metabolism of lipoproteins

The quantitative relationship between plasma cholesterol and the amount and type of fat in the diet was demonstrated initially by Keys *et al.* (1957, 1965), Hegsted *et al.* (1993), and later others (Denke & Grundy, 1992; Yu *et al.*, 1995). It has been concluded that dietary cholesterol has a modest plasma cholesterol-raising effect, dietary saturated fatty acids (SFAs) have potent plasma cholesterol-raising effects, and dietary PUFAs

have a plasma cholesterol-lowering effect, but the cholesterol-raising effect of dietary SFAs, in particular of meristic and palmitic acids, is more potent than the lowering effect of PUFAs. Therefore, reducing dietary SFA intake has remained the cornerstone of public health nutrition policy for reducing CVD risk (Lichtenstein *et al.*, 2006; Perk *et al.*, 2012).

Assuming that the ratio of n-3/n-6 PUFAs is important for optimal health, this ratio is too high in the current Western diet because of the relatively high level intake of vegetable oils other than olive oil in the market. In earlier diets, this ratio might have been <1, whereas in many modern "Western" diets it reaches 15 or more (Simopoulos, 2003, 2008; Blasbalg *et al.*, 2011).

Intake of linoleic acid, up to 12% of dietary energy, does not affect high-density lipoprotein (HDL) cholesterol (Mensink & Katan, 1992); higher levels of n-6 PUFAs, however, lower HDL cholesterol, which is undesirable (Mattson & Grundy, 1986). Furthermore, n-6 PUFA-rich oils are prone to oxidation, which may have negative consequences, including the increased oxidation of low-density lipoprotein (LDL) and its uptake by macrophages. High intake of saturated and n-6 FAs together with a low intake of oleic acid and n-3 FAs promotes the development of metabolic syndrome, while the inverse combination prevents its occurrence (Kremmyda *et al.*, 2011).

Several studies have shown that MUFA consumption is as effective in lowering total cholesterol and LDL cholesterol as n-6 PUFAs when they replace saturated fats without affecting or actually increasing the cardioprotective HDL cholesterol fraction (Gardner & Kraemer, 1995). Interestingly, when MUFAs replace carbohydrates in the diet, the levels of very-low-density lipoprotein are increased (Mensink *et al.*, 2003; Appel *et al.*, 2005; Berglund *et al.*, 2007). Yu *et al.* (1995) explored the results of 18 studies in healthy and normocholesterolemic participants and, using meta-regression, found that MUFAs increased HDL cholesterol and decreased both total and LDL cholesterol.

Despite the fact that no great differences existed in lipoprotein content between the consumption of MUFAs and PUFAs, both oleate-rich LDL (Reaven *et al.*, 1993) and HDL (Solà *et al.*, 1997) are less susceptible to oxidation than linoleate-rich particles (Lapointe *et al.*, 2006). Evaluating the results of 14 studies comparing the resistance of LDL to oxidation, it was found that 12 studies indicated that MUFA-rich diets did promote a higher resistance of LDL to oxidation than PUFA-rich diets (Steinberg *et al.*, 1989). Furthermore, results of the EUROLIVE study (Cicero *et al.*, 2008) demonstrated that, after olive oil ingestion, oleic acid concentration in LDL increased and those of linoleic and arachidonic acid decreased. An inverse relationship also was observed between the oleic to linoleic acid ratio and biomarkers of oxidative stress. In addition, high oleic acid intake has been associated with reduced blood pressure (Terés *et al.*, 2008; Schwingshackl *et al.*, 2011). Since MUFA-containing oils are more stable and less susceptible to oxidation than those containing n-6 PUFAs, they have emerged as the candidates of choice for replacing saturated fats. Consumption of a MUFA-rich diet by humans also decreased the expression of adhesion molecules on peripheral blood mononuclear cells (PBMCs) and might, therefore, have specific anti-inflammatory effects (van Dijk *et al.*, 2012).

An olive oil rich diet, consumed for five weeks, was demonstrated to increase the resistance of LDL to oxidation and to decrease LDL-induced adhesion of monocytes to endothelial cells (Mata *et al.*, 1996). Dietary studies in humans have also shown that when exposed to oxidative stress, LDL enriched in oleic acid promotes less monocyte chemotaxis compared with LDL enriched with linoleic acid (Tsimikas *et al.*, 1999). Animal studies suggest that an olive oil-rich diet may decrease the expression of scavenger receptors on macrophages, thus reducing the extent of foam cell formation (Miles *et al.*, 2001).

Oxidation of HDL reduces the HDL functionality (Helal *et al.*, 2013). Oleic acid consumption has been shown to reduce *in vivo* HDL oxidation in humans (Solà *et al.*, 1997). Moreover, paraoxonase 1 (PON1), a protein associated with HDL that modulates the antioxidant and anti-inflammatory role of HDL, significantly increases its anti-inflammatory activities following consumption of EVOO.

5.4.1.3 Effects of fatty acids on the synthesis of isoprostanes and neuroprostanes

Lipids that undergo peroxidation represent major targets for free radical attack. Besides the biosynthesis of eicosanoids, free radical non-enzymatic oxidation of AA (C20:4 n-6) can take place *in vivo* with the production of an isoprostane, F_2-IsoPs, although there is some evidence to suggest that F_2-IsoPs can be

15-F_{2t}-IsoP 17-F_{2t}-Dihomo-IsoP ent-7-epi-F_{2t}-diomoisoP

4(RS)-4F_{4t}-NeuroP 16-B_1-PhytoP

Figure 5.3 Representative IsoP, Dihomo-IsoP, NeuroP, Diomo-isoP, and PhytoP in biological systems.

derived, in part, via a cyclooxygenase-induced pathway (Milne *et al.*, 2013). There are numerous reports demonstrating that IsoPs are the most reliable biomarkers of oxidative stress *in vitro* and in animal models (Morrow *et al.*, 1990), as well as in humans (Barden *et al.*, 2009). Oxidation products of the n-3 FAs alpha-linolenic acid (ALA, C18:3 n-3), eicosapentaenoic acid (EPA, C20:5 n-3), and docosahexaenoic acid (DHA, C22:6 n-3) yield the phytoprostanes (Morrow *et al.*, 1992), F_3-IsoPs and F_4-IsoPs or neuroprostanes (NeuroPs) (EFSA, 2011), respectively (Figure 5.3). More recently, dihomo-isoprostanes (Dihomo-IsoPs) derived from adrenic acid (AdA, C22:4 n-6) have been reported (Taber & Roberts, 2005). DHA is located mainly in brain grey matter and AdA in brain white matter. Several IsoPs have been shown to be biologically active (Jahn *et al.*, 2008). In fact, isoprostanes are not only biomarkers of lipid peroxidation but also mediators of oxidant injury.

The oxidation of docosahexaenoic acid (DHA) to 4(RS)-4-F_{4t}-NeuroP is necessary to prevent ischemia-induced arrhythmias. Under oxidative stress conditions such as ischemic diseases, non-enzymatic oxygenated metabolites of DHA formed by peroxidation of cardiac membrane lipids, notably 4(RS)-4-F_{4t}-NeuroP, are responsible for the potentially antiarrhythmic properties of DHA by countering the cellular stress by reactive oxygen species (ROS). Importantly, it appears that nonenzymatically oxygenated metabolites of ω3 PUFAs can communicate and exert a physiological role (Roy *et al.*, 2015). This highlights potential beneficial effects of increased ROS production dependent on the cellular environment and supports previous reports that have shown that n-3 fatty acids reduce oxidative stress, in part, via attenuation of inflammation.

5.4.1.4 Effects of fatty acid on the membrane fluidity and behavior of membrane proteins

Oleic acid is a key component of membrane lipids (Hodson & Fielding, 2013). Importantly, oleic acid is the most common FA in nature and the most abundant FA in the human body (Fraser *et al.*, 2010; Kotronen *et al.*, 2010). The degree of unsaturation of cell membranes regulates their fluidity and can influence the behavior of membrane proteins. The linked lipid chain of oleic acid reduces the order within the lipid bilayer, helps to maintain the hydration level, and increases membrane fluidity (Weijers *et al.*, 2012).

Earlier studies have demonstrated that the ingestion of VOO reduces blood pressure (Escriba *et al.*, 2003) as well as the need for antihypertensive medication (Ferrara *et al.*, 2000) in humans. oleic acid is also important for preserving the homeoviscous state of membranes in neuronal cells. Oleic acid decreases the levels of the toxic amyloid-β peptide, which reciprocally interacts with cholesterol in lipid rafts (Yang *et al.*, 2010), and amyloid plaques in a mouse model of Alzheimer's disease. The incorporation of oleic acid into the brain mitochondrial membranes after the administration of VOO could also reduce oxidative stress associated with age (Ochoa *et al.*, 2011). A neurotrophic lipid factor formed by binding oleic acid to albumin *in vitro* induces significant motor recovery (40%) in rats with spinal cord injury (Rodríguez-Rodríguez *et al.*, 2004), ameliorating both spasticity and pain by inducing significant changes in the membrane structure, and indicating that the action of oleic acid is mediated by structural effects.

Oleoylethanolamide (OEA)

N-Arachidonoylethanolamine (anandamide, AEA)

Figure 5.4 Chemical structures of some ethanolamides.

5.4.1.5 The role of ethanolamides and the effect of fatty acids on their biosynthesis

The absorptive epithelium of the proximal small intestine converts oleic acid released during fat digestion into oleoylethanolamide (OEA) (Figure 5.4), an endogenous high-affinity agonist of peroxisome proliferator-activated receptor-α (PPAR-α) (Fu *et al.*, 2008). OEA interacts with this receptor to cause a state of satiety characterized by prolonged intermeal intervals and reduced feeding frequency. OEA signaling may be a key component of the physiological system devoted to the monitoring of dietary fat intake, and its dysfunction might contribute to overweight and obesity (Pavón *et al.*, 2010). It seems that this endogenous ethanolamide is a lipid mediator that inhibits food intake and body weight gain, and has hypolipemiant action *in vivo*, as well as a lipolytic action *in vitro* (Lauffer *et al.*, 2009). In fact, the administration or consumption of fats rich in cis-MUFA, oleic acid, is associated with lower body mass index (BMI) values (Nigam *et al.*, 2014). Moreover, in rats with ad libitum access to food, a daily supplement of olive oil (in which oleic acid constitutes about 70–80% of all FAs) induces body weight reductions (Nigam *et al.*, 2014). In contrast, araquinodonyl etanolamide (anadamide), biosynthesized from the n-6 arachidonic acid, increases food uptake (Vögler *et al.*, 2008).

5.5 Pigments

Initially, the color of olives is green, later turning purple to bluish and becoming black in overripe olives. Usually, this color development from green to purple is modulated by an accumulation of anthocyanins (Ryan *et al.*, 2002) together with the degradation of chlorophylls and carotenoids (Kiritsakis, 1991; Minguez-Mosquera & Gallardo-Guerrero, 1995). The green color is due to chlorophylls, the purple and blue colors are due to anthocyanins, and the black color is formed by the oxidation of phenolic compounds including oleuropein. Anthocyanins and specifically the glycosides of cyanidin are responsible for the purple and blue colors of ripe olives (Markakis, 1982). Cyanidin 3-rutinoside and cyanidin 3-glucoside account for more than 90% of the total anthocyanins. Anthocyanins increase rapidly during the development of olives, reaching a maximum and then decreasing when the fruit is overripe (Vlahov & Solinas, 1993). In VOO, two main classes of natural pigments have been identified: chlorophylls and carotenoids. The former encompass chlorophylls a and b and their derivatives (pheophytins a and b, and pheophorbides). Among pheophytins, pheophytin α is predominant (Gandul-Rojas & Mínguez-Mosquera, 1996). Chlorophyll a can only be found in freshly produced oils. Under light exposure green pigments degrade, causing oil bleaching (Psomiadou & Tsimidou 2002), and photooxidation occurs (Kiritsakis &Dugan, 1985).

In VOO, the content of major carotenoids ranges from 4 mg/kg for β-carotene to 10 mg/kg for lutein, although VOO also contains β-cryptoxanthin and 5,6-epoxyxanthophylls, including neoxanthin, violaxanthin, antheraxanthin, and their furanoxides (Gandul-Rojas & Mínguez-Mosquera, 1996; Gallardo-Guerrero, 2002). Their concentration decreases progressively during ripening, but less drastically than chlorophylls. Carotenoids are susceptible to reactions, including isomerization (trans to cis) and oxidation, which decrease the average shelf life of VOO (Aparicio-Ruiz & Gandul-Rojas, 2012). Carotenoid levels in human blood and tissues are critical for maintaining human health as they are converted to retinol (vitamin A) by retinol dehydrogenases in the intestine and liver. Lutein is the most abundant carotenoid in human triacylglycerol-rich lipoproteins after the ingestion of VOO (Perez-Galvez *et al.*, 2005). In biological membranes, carotenoids

are present as carotenoid–protein complexes and as direct components of the lipid phase, demonstrating an ability to form self-assemblies (Sujak *et al.*, 2000).

The conformation of carotenoids in membranes is of great importance because the energy associated with spectroscopic transitions is directly related to the antioxidant properties exhibited by carotenoids in lipid environments. Differences in the membrane spatial distribution of β-carotene and lutein, as well as subsequent differences in their antioxidant properties, may constitute an advantage to synergistically trap in-tandem (lutein > β-carotene) triplet-excited molecules of sensitizers, singlet oxygen, or free radicals and to prevent the propagation cycle of lipid peroxidation from the interface of the membrane to the inner region of the membrane. Lutein exhibits high efficiency in protecting against lipid peroxidation in membranes composed of raft-forming mixtures as well as in models of photoreceptor outer segments (Wisniewska-Becker *et al.*, 2012). There is clinical evidence that lutein and β-carotene, together with vitamins C and E, reduce the risk of age-related macular degeneration (Han *et al.*, 2012; Aslam *et al.*, 2013; Yonova-Doing *et al.*, 2013). These carotenoids have also been proposed to favor optical density of the macular pigment (Feeney at al., 2013) and protect against age-related cataracts (Cui *et al.*, 2013), cognitive decline, atrial fibrillation (Karppi *et al.*, 2013), osteoporotic fractures (Dai *et al.*, 2014), and vascular aging (Wolak & Paran, 2013).

5.6 Phenols

VOO can be differentiated from all other vegetable oils due to its very particular phenolic composition (Krichene *et al.*, 2007; Gómez-Rico *et al.*, 2008; García-González *et al.*, 2010; Taamalli *et al.*, 2010; Pardo *et al.*, 2011; Ghanbari *et al.*, 2012; Ben Hassine *et al.*, 2014; del Monaco *et al.*, 2015). The olive drupe contains high concentrations of phenolic compounds that can range between 1 and 3% of the fresh pulp weight. The polar phenol classes present in olives are phenolic alcohols such as hydroxytyrosol (HT) and tyrosol (T), phenolic acids, secoiridoids, flavonoids, lignans, and hydroxyl-isochromans. Secoiridoids are the most representative class, with oleuropein being present at levels up to 14% of the dry weight (Amiot *et al.*, 1989). During VOO extraction, secoiridoid glycosides such as oleuropein and ligstroside present in the fruit are hydrolyzed by β-glucosidases, producing more lipophilic secoiridoid derivatives that are released into the oil during the mechanical extraction process. In contrast, several simple phenols and glycosides, such as oleuropein, being much more hydrophilic, diffuse into the aqueous phase during the oil fruit processing and are mostly lost in the wastewater phase (Kiritsakis, 1988a). Consequently, the quantitative phenolic composition of oil is quite different from the one found in olive fruit.

EVOO usually contains phenolic compounds that can range between 50 and 1000 ppm (mg/kg) depending on the cultivars, pedoclimatic conditions, maturity stage of the fruit, and extraction conditions (Kiritsakis,1998b), but usual values in commercial VOO range between 100 and 400 mg/kg. According to literature, the monocultivar olive oil from ‘Arbequina’, ‘Picual’, and ‘Cornicabra’ (Spain); ‘Moraiolo’ and ‘Leccino’ (Italy); ‘Verdeal’, ‘Carrasquinha’, and ‘Cordovil’ (Portugal); ‘Koroneiki’ (Greece); and ‘Picholine’ (Morocco) have usually shown a very high concentration in total phenols (Salvador *et al.*, 2003; Gómez-Rico *et al.*, 2008; García-González *et al.*, 2010; Garcia *et al.*, 2012). On the other hand, cultivars such as ‘Arbequina’, ‘Manzanilla’, ‘Morisca’, and ‘Picolimon’ (all from Spain); ‘Queslati’, ‘Nebjmel’, ‘Swabaâ Algia’, and ‘Semni’ (all from Tunisia); or even ‘Ayvalik’ and ‘Nizip’ (a cultivar from the south-eastern part of Turkey with a very high oil productivity) contain lower amount of phenols (Krichene *et al.*, 2007; Gómez-Rico *et al.*, 2008; Taamalli *et al.*, 2010; del Monaco *et al.*, 2015). The main phenols present in VOO are presented in Table 5.2.

5.6.1 Phenol classes present in olives and olive oil

5.6.1.1 Hydroxybenzoic acids

Hydroxybenzoic acids have a general structure of the C6-C1 type derived directly from benzoic acid. Variations in structure occur due to hydroxylations and methoxylations of the aromatic ring. From the quantitative point of view, the overall hydroxybenzoic acids content is generally low in fruits and olive oil. Protocatechuic acid, vanillic acid, gallic acid, syringic acid, and *p*-hydroxybenzoic acid have been found in olive oil

Table 5.2 Main phenols present in virgin olive oil (mg/kg).

Phenols	Range
Hydroxytyrosol	0.0–25.4
Hydroxytyrosol acetate	21.4–131.0
Tyrosol	0.10–123.1
Vanillic acid	0.09–0.8
Caffeic acid	0.0–1.0
Syringic acid	0.0–2.3
p-Cumaric acid	0.04–0.6
Ferulic acid	0.0–2.4
3,4-DHPEA-EDA	74.7–840
4-HPEA-EDA	13.0–86.4
Tyrosol derivative	0.0–113.4
Lignans	112–275
3,4-DHPEA-EA	25.6–310
Luteolin	0–10

in concentrations lower than 3.5 mg/kg (Montedoro *et al.*, 1993). Gallic acid, syringic acid, and vanillic acids have been also reported in olive fruits. Vanillic acid was found in olive leaves at a 0.63% (dry basis) concentration (Mousa *et al.*, 1996).

5.6.1.2 Hydroxycinnamic acids

Phenolic acids with the basic chemical structure of C6-C3 (cinnamic acid) have been found in olive fruits (Benavente-García *et al.*, 2000), namely caffeic, p-coumaric, o-coumaric, protocatechuic, and sinapic acids (Mousa *et al.*, 1996; Garcia *et al.*, 2001). These acids have been found at low concentrations in olive oil (0.3–1.7 mg/kg), olive fruits, and olive leaves (Montedoro *et al.*, 1993; Mousa *et al.*, 1996). The major caffeic acid derivative in olives is verbascoside (Figure 5.5) a heterosidic ester of caffeic acid, rutinose, and 3,4-dihydroxyphenylethanol. This compound is present in all of the constitutive parts of the olive fruit, and, depending on the cultivar, its content can vary between 0.2 and 22 mg per g of the dry weight of olive fruits (Andary *et al.*, 1982). Despite its high content in olives, this compound has not been reported in olive oil.

5.6.1.3 Flavonoids

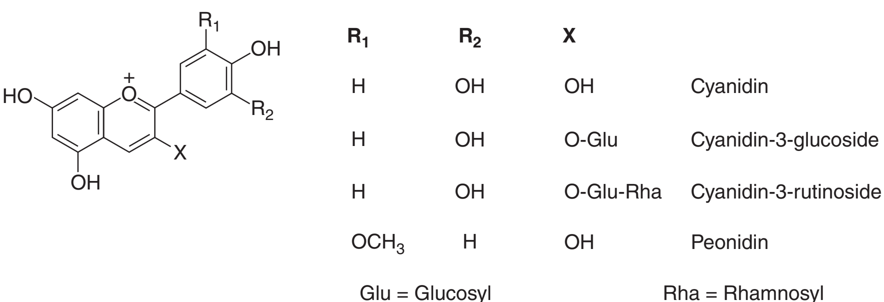
Rutin, a flavonol, and luteolin-7-glucoside, a flavone, are the most common flavonoids found in olives (Amiot *et al.*, 1989). The content of flavonoids in olives and olive oil is very low. However, the concentration of these compounds in the fruits during maturation can reach 150 mg per g of fresh weight. Especially in the last months of maturation of the fruits, other glycosides including apigenin-7-glucoside, cyanidine-3-glucoside, and cyanidine-3-rutinoside (Figure 5.6) can also be found. In olive oil, the flavonoids luteolin (2–8.4 mg/kg) and apigenin (0.4–2 mg/kg) have been detected (Figure 5.6) (Mateos *et al.*, 2001).

Verbascoside

Figure 5.5 Chemical structure of verbacoside.

	X	R_1	R_2	R_3	
Flavonols:	OH	OH	OH	H	Quercetin
	O-Glu-Rha	OH	OH	H	Rutin
Flavones:	H	H	OH	H	Apigenin
	H	OH	OH	H	Luteolin
	H	OH	O-Glu	H	Luteolin-7-glucoside

Anthocyanidins:



R_1	R_2	X	
H	OH	OH	Cyanidin
H	OH	O-Glu	Cyanidin-3-glucoside
H	OH	O-Glu-Rha	Cyanidin-3-rutinoside
OCH_3	H	OH	Peonidin

Glu = Glucosyl Rha = Rhamnosyl

Figure 5.6 Structure and classification of the major olive flavonoids.

5.6.1.4 *Phenyl alcohols*

These compounds include molecules containing the C_6-C_2 structure corresponding either to 2-(4-hydroxyphenyl) ethanol (*p*-HPEA, tyrosol, Ty) or to 2-(3,4-dihydroxyphenyl) ethanol (3,4-DHPEA, hydroxytyrosol, HT) (Figure 5.7) (del Monaco *et al.*, 2015).

Hydroxytyrosol and tyrosol derivatives other than glycosides represent the major phenolic fraction of almost all VOOs and olive fruits. Bianchi and Pocci (1994) reported the presence of 3,4-dihydroxyphenylglycol in olives and in vegetation waters. 2-(3,4-dihydroxyphenyl) ethyl acetate (hydroxytyrosol acetate) (Figure 5.7) has also been identified as a component of the phenolic fraction of olive oil (up to 42 mg/kg) (Bernes *et al.*, 2001; Garcia *et al.*, 2012).

R_1	R_2	
H	H	Tyrosol
OH	H	Hydroxytyrosol
OH	OH	3,4-Dihydroxyphenylglycol

Hydroxytyrosol acetate

Glu =

Figure 5.7 Chemical structures of tyrosol, hydroxytyrosol, its glycoside derivatives, and 3,4-dihydroxyphenyl glycol.

Figure 5.8 Metabolic pathway leading to the biosynthesis of secoiridoids. *Source*: Silva *et al.* (2010a). Reproduced with permission of Elsevier.

Glycosides of hydroxytyrosol were found in olive fruits, olive leaves, and fresh olive oil obtained from cold-processing olives (Bianco *et al.*, 1998).

5.6.1.5 Secoiridoids

The prevalent phenolic compounds in olives and VOO are the secoiridoid derivatives (Montedoro *et al.*, 1993). The secoiridoids are a subclass of iridoids, which is a class of regular monoterpenoid lactones containing a cyclopentanodihydropyran ring system, the iridane skeleton (see in Figure 5.8 in bold).

The secoiridoids are believed to be formed from carboxylic iridoids by the cleavage of the cyclopentane ring followed by functionalization (Bianco *et al.*, 1998). Examples of this class of compounds are secologanin and elenolic acid (Figure 5.8).

These compounds themselves are not phenolic compounds, but in *Olea europaea* they are found as esters of tyrosol and hydroxytyrosol. Oleuropein, a heterosidic ester of elenolic acid with hydroxytyrosol, is clearly predominant among phenolic compounds in olives. Ligstroside, which is a heterosidic ester of elenolic acid with tyrosol, demethyloleuropein, and oleoside, a non-phenol, and nüzhenide are also found in high concentration in the fruit (Figure 5.9) (Inouye & Uesato, 1986).

Oleuropein and demethyloleuropein have been found in all the constitutive parts of fruits such as peel, pulp, and seed, but they are most frequent in the pulp. In contrast, nüzhenide, (Gariboldi *et al.*, 1986), a tyrosol derivative, has been found only in the seeds (Figure 5.10).

Figure 5.9 Structure of the major secoiridoids found in olive fruit.

Figure 5.10 Structure of nuzhenide.

Oleuropein is mainly responsible for the bitter taste of immature olives, which contain approximately 2% (of the fruit weight) oleuropein. As the fruit reaches maturity, the oleuropein content diminishes. Oleuropein and ligstroside are water soluble and diffuse in the aqueous phase during the processing of olive fruit and, therefore, they can only be detected as minor hydrophilic phenols in VOO (Owen *et al.*, 2000). During olive oil extraction, they are hydrolyzed by glucosidases. Therefore, the prevalent phenols of VOO are the respective aglycones of oleuropein and ligstroside and compounds arising from them by loss of the carboxymethyl moiety (Figure 5.11) (Montedoro *et al.*, 1993; Mateos *et al.*, 2001), with a much more lipophilic character. In VOO, the most abundant secoiridoids are the dialdehydic form of elenolic acid linked to 3,4-DHPEA or p-HPEA (3,4-DHPEA-EDA or p-HPEA-EDA) and isomers of the oleuropein and ligstroside aglycons (3,4-DHPEA-EA and p-HPEA-EA, respectively) (Figure 5.11) (Salvador *et al.*, 2003; Gómez-Rico *et al.*, 2008; García-González *et al.*, 2010; Garcia *et al.*, 2012; del Monaco *et al.*, 2015). Usually, the dialdehydic form of decarboxymethyl oleuropein aglycone, 3,4-DHPEA-EDA, is the major secoiridoid found in EVOO (Gómez-Rico *et al.*, 2008; del Monaco *et al.*, 2015), followed by 3,4-DHPEA-EA and 4-HPEA-EDA. Oleuropein aglycone and 3,4-DHPEA-EDA have also been reported in olive fruits (Gariboldi *et al.*, 1986; Servili *et al.*, 1999a, 1999b).

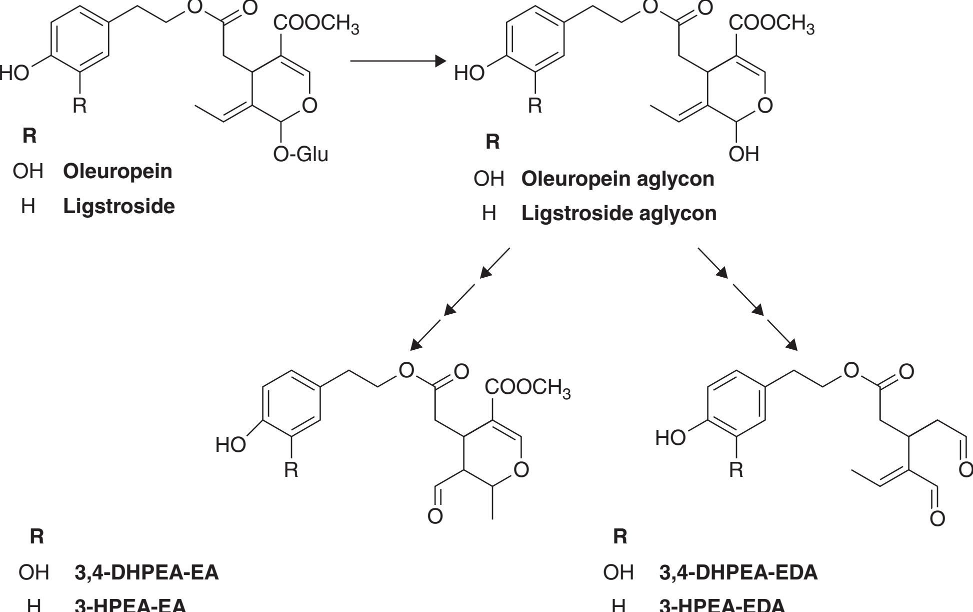


Figure 5.11 Structure of oleuropein and ligstroside aglycones and their derivatives, the major secoiridoids found in olive oil.

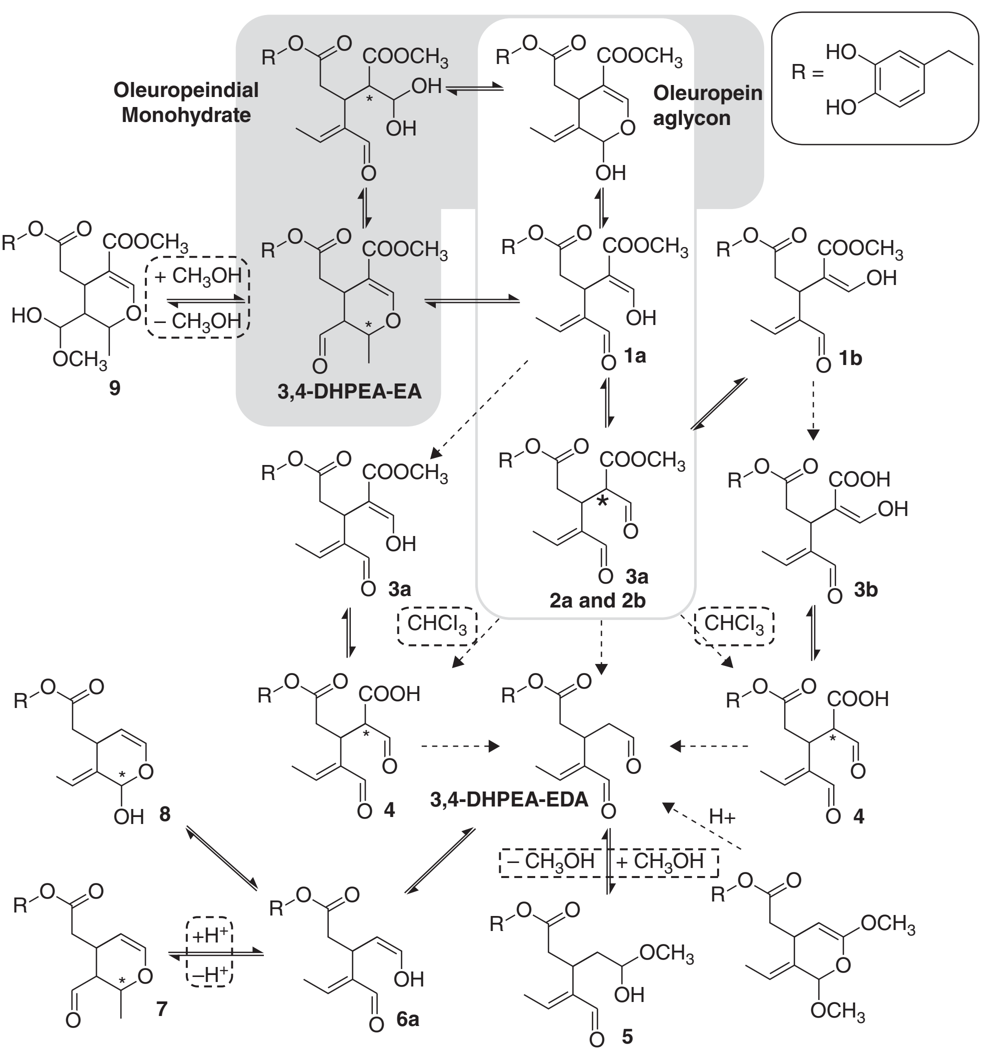


Figure 5.12 Transformation pathway of oleuropein in chloroform-d/deuterium oxide (1:1) (gray) and in deuterium oxide (white). Adapted from Bianco *et al.* (1999) and Paiva-Martins and Gordon (2001).

Since the concentration of oleuropein and ligstroside derivatives rises largely during the mechanical extraction of olive oil, nuclear magnetic resonance (NMR) studies on enzymatic hydrolysis of oleuropein by β-glucosidase have been performed in chloroform/water (1:1) and in water in order to understand the mechanism of synthesis/biosynthesis of these derivatives (Paiva-Martins & Gordon, 2001). The results are summarized in Figure 5.12.

5.6.1.6 Lignans

Lignans are products of the dimerization of two phenylpropene or phenylpropene precursors (C6-C3 dimers). The lignans pinoresinol and 1-acetoxypinoresinol (Figure 5.13) have been found in EVOO (Garcia *et al.*, 2001; Mateos *et al.*, 2001). Pinoresinol concentration ranged from 19 to 65 mg per kg of olive oil, and 1-acetoxypinoresinol concentration ranged from 5 to 97 mg per kg of olive oil. Lignans seem to be among the most stable phenolics during oil storage and processing (Silva *et al.*, 2010a, 2010b).

R	
H	Pinoresinol
$OCOCH_3$	1-Acetoxypinoresinol

Figure 5.13 Chemical structures of pinoresinol and 1-acetoxypinoresinol.

5.6.1.7 Hydroxyl-isochromans

Hydroxyl-isochromans compounds derive from a reaction between hydroxytyrosol and aromatic aldehydes (benzaldehyde and vanillin). Such a reaction also occurs in a natural matrix, with oleic acid acting as a catalyst. Two compounds of this class (Figure 5.14), 1-phenyl-6,7-dihydroxyisochroman and 1-(3′-methoxy-4′-hydoxy)phenyl-6,7-dihydroxy-isochroman, were identified.

The levels of these compounds in samples of EVOO are very low and extremely variable, ranging from 8 to 1400 ng/kg for 1-phenyl-6,7-dihydroxy-isochroman and from 20 to 390 ng/kg for 1-(3-methoxy-4-hydroxy-phenyl)-6,7-dihydroxy-isochroman (Bianco *et al.*, 2001).

5.6.2 Contribution of polar phenols to oil quality

5.6.2.1 Sensory contribution

Polar phenols are responsible for the key sensory characteristics of bitterness, pungency, and astringency (Angerosa *et al.*, 2003; Gutierrez-Rosales *et al.*, 2003). However, individual olive oil polar phenols have shown bitter, astringent, and burning sensations to different degrees (Cerretani *et al.*, 2008). Deacetoxy-ligstroside aglycon (*p*-HPEA-EDA), for example, is the key source of the burning sensation found in many olive oils (Cerretani *et al.*, 2008). In contrast, deacetoxy-oleuropein aglycon (3,4-DHPEA-EDA, the HT analog), tested at an equivalent concentration, produced very little burning sensation but is more responsible for the bitterness. Nevertheless, all together, the dialdehydic and aldehydic forms of decarboxymethyl-oleuropein aglycone (3,4-DHPEA-EDA and 3,4-DHPEA-EA) and the dialdehydic form of decarboxymethyl-ligstroside aglycone (4-HPEA-EDA) are responsible for the bitterness.

5.6.2.2 Contribution of polar phenols to the oxidative stability of oil

Different authors reported that the concentration of phenolic compounds, expressed as total phenols, was highly correlated to the shelf life of VOO (Shahidi *et al.*, 1994; Brenes *et al.*, 2001; Ninfali *et al.*, 2001; Shahidi & Zhong, 2007; Shahidi & Ambigaipalan, 2015). Most of the phenols present in olive oil act as antioxidants, but the components that are mainly responsible for the remarkable resistance of olive oil to oxidation are the molecules with catechol moiety in their structure: the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA), decarboxymethyl oleuropein aglycon, and hydroxytyrosol

1-Phenyl-6,7-dihydroxy-isochroman	R_1 = H; R_2 = Phenyl
1-(3-methoxy-4-hydroxy)-phenyl-6,7-dihydroxy-isochroman	R_1 = H; R_2 = 4-hydroxy-3-methoxy-phenyl

Figure 5.14 Chemical structures of the main hydroxyl-isocromans found in olive oil.

(Baldioli *et al.*, 1996; Gordon *et al.*, 2001; Gomez-Alonso *et al.*, 2003; Carrasco-Pancorbo *et al.*, 2006; Romani *et al.*, 2007). Tyrosol, lignans, and ligstroside aglycon are weaker antioxidants.

The antioxidant activity of phenols is enhanced by the presence of tocopherols (Blekas *et al.*, 1995; Pellegrini *et al.*, 2001). This synergism is more evident when the level of phenols is relatively low. Polar phenols reduce the oxidized forms of alpha-tocopherol and, therefore, tocopherols are more quickly consumed in olive oils with low content in ortho-diphenols (Silva *et al.*, 2010a, 2010b).

The evaluation of the antioxidant activity of several olive polar phenols in some lipidic systems containing olive oil – namely, bulk olive oil, olive oil-in-water emulsions, and liposomes – have shown that, depending on the system of assay, HT, 3,4-DHPEA-EA, and 3,4-DHPEA-EDA had antioxidant capacity 3 to 10 times higher than that of α-tocopherol (Paiva-Martins & Gordon, 2002; Paiva-Martins *et al.*, 2003, 2006).

In olive oil in water (O/A) emulsions containing Fe^{3+} or Cu^{2+}, both 3,4-DHPEA-EDA and 3,4-DHPEA-EA showed antioxidant activity, but hydroxytyrosol (HT) showed pro-oxidant activity (Paiva-Martins *et al.*, 2006). Since traces of iron and copper may originate from equipment and food, these results enhance the importance of oleuropein aglycones for the oxidative stability of VOO during processing. In contrast to tocopherols, all polar phenolic components decrease with thermal treatment, but this decrease is dramatic in the presence of foods (Silva *et al.*, 2010a, 2010b). The elemental content of foods may make a huge contribution to the loss of phenolic compounds. In fact, many foods are very rich in important elements, such as iron. For example, iron can be found in potatoes in a concentration of higher than 25 ppm (dry matter), and copper can also be present in a concentration of up to 10 ppm (dry matter) (Bethke & Jansky, 2008). Small concentrations, such as 0.4 ppm, of these ions have been shown to have dramatic effects on the antioxidant activity of these polar phenolic compounds in emulsions and bulk oils because they increase the rate of phenolic compound destruction by oxidation, and this rate varies depending on the compound (Paiva-Martins & Gordon, 2002, 2005; Paiva-Martins *et al.*, 2006). In most studies, hydroxytyrosol actually showed the worst stability in the presence of foods because hydroxytyrosol forms the least stable complex with metals, has the highest reducing capacity, and increases the rate of ferrous ion oxidation to ferric ions by air oxygen (Paiva-Martins & Gordon, 2005), which leads to a fast ferrous ion formation in the media and to a higher rate of phenolic compound destruction.

5.6.2.3 Bioactivity of polar phenols

On November 2011, the European Food Safety Authority (EFSA) released a claim concerning the benefits of daily ingestion of olive oil rich in phenolic compounds (EFSA, 2011). The mechanisms by which VOO can exert its beneficial effects are (1) increasing HDL cholesterol, (2) reducing the oxidative damage to lipids, (3) decreasing inflammation, (4) improving endothelial function, (5) decreasing blood pressure.

The results of the EUROLIVE study on the effect of olive oil consumption on oxidative damage in European populations, a cross-over, multicenter, clinical trial performed with 200 individuals from five European countries (Cicero *et al.*, 2008), have provided conclusive evidence of the *in vivo* protective role of phenolic compounds from olive oil on lipid oxidative damage in humans, at real-life olive oil doses. Participants were randomly assigned to receive 25 ml/day of three similar olive oils, but with increasing phenolic content (from 27 to 366 mg/kg of olive oil), in intervention periods of 3 weeks preceded by 2-week washout periods. All olive oils increased the HDL cholesterol and the ratio between the reduced and oxidized forms of glutathione, while the consumption of medium- and high-phenolic content olive oil also decreased lipid oxidative damage biomarkers such as plasma oxidized LDL, conjugated dienes, and hydroxy fatty acids. The increase in HDL cholesterol and a decrease in the lipid oxidative damage were linear with the phenolic content of the olive oil consumed. The results of the EUROLIVE study have provided first-level evidence that olive oil is more than a MUFA fat. Moreover, the high-phenolic-content olive oil increased the HDL-mediated cholesterol efflux from macrophages compared with low-phenolic-content olive oil (Hernáez *et al.*, 2014). A subsample ($n = 990$) of the PREDIMED study has also shown that a Mediterranean diet, only when enriched in VOO with high phenolic content (316 mg/kg), decreases the LDL oxidation in a significant manner compared with the control group (low-fat diet) (Fitó *et al.*, 2014), confirming previously obtained data (Fitó *et al.*, 2007). In fact, it has been observed that the total polar phenol content bound to human LDL increases in a dose-dependent manner with the phenolic content of the olive oil (Covas *et al.*, 2006).

The cardiovascular protective activity of VOO polar phenol (PP) extracts has been extensively investigated, and most clinical studies (Cicerale *et al.*, 2010; Servili *et al.*, 2014; Covas *et al.*, 2015) have reported that VOO with high phenolic content is effective in reducing the eicosanoid inflammatory mediators derived from arachidonic acid, with reductions in the concentration of inflammation and oxidative stress markers such as oxLDL, F2-isoprostanes, lipid peroxides, TXB2, LTB4, interleukin-6 (IL-6), and C-reactive protein; in decreasing coagulation factors and platelet aggregation (Delgado-Lista *et al.*, 2011); and in reducing blood pressure (Valls *et al.*, 2015). Olive oil PPs have also been shown *in vitro* to inhibit endothelial adhesion and platelet activity and to decrease plasminogen activator inhibitor-1 and factor VII, both linked to the development of CVD (Cicerale *et al.*, 2010).

Current evidence indicates that, besides reducing oxidative stress markers, inflammation, and atherosclerotic risk factors (e.g., ox-LDL, diabetes, and hypertension), olive oil PPs induce specific favorable changes in the expression profile of genes involved in atherosclerosis, inflammation, and oxidative stress (Camargo *et al.*, 2010; Konstantinidou *et al.*, 2010). In fact, the intake of an olive oil with high polar phenol content (40 mL) modified the expression of pro-inflammatory genes linked to atherosclerosis, obesity, dyslipidemia, and type 2 diabetes mellitus, thereby promoting a lesser inflammatory profile in peripheral blood mononuclear cells. Olive oil PPs, including HT and oleuropein at nutritionally relevant concentrations, downregulate the gene expression of adhesion molecules, chemoattractants, matrix metalloproteinase, and proinflammatory enzymes (Zrelli *et al.*, 2011; Scoditti *et al.*, 2014). Other studies have shown that HT upregulates the gene expression of antioxidant/detoxifying enzymes in endothelial cells as well as of the histone deacetylase sirtuin-1 (SIRT-1), an epigenetic modifier, in the heart tissue of a mouse model of accelerated senescence (Helal *et al.*, 2013).

Olive oil phenols have also proven to be effective in protecting against the general mechanisms of neurodegenerative diseases, such as free radical scavenging/antioxidant actions, anti-inflammatory properties, and anti-apoptotic properties, in cell culture and animal models. Olive oil phenols have been shown to be neuroprotective against cerebral ischemia (Mohagheghi *et al.*, 2010), brain hypoxia-reoxygenation (González-Correa *et al.*, 2008), aging (Pitozzi *et al.*, 2010), Alzheimer's disease (Monti *et al.*, 2011), Huntington's disease (Tasset *et al.*, 2011), brain damage after hypoxia-reoxygenation in diabetic rats (De la Cruz *et al.*, 2010), multiple sclerosis (Weinstock-Guttman *et al.*, 2005), Parkinson's disease (Jones, 2011), peripheral neuropathy (Ristagno *et al.*, 2012), and spinal cord injury (Impellizzeri *et al.*, 2012). Some findings also suggest that olive oil has beneficial effects on learning and memory deficits found in aging by reversing oxidative damage in the brain (Farr *et al.*, 2012). Further pharmacological activities of olive oil phenolics include their role in neuroprotection, increase of neurotrophic factor expression such as glial cell-derived neurotrophic factor, hyperpolarization of basal mitochondrial membrane potential as a cytoprotection effect (Schaffer *et al.*, 2007), nerve Na (+), K (+)-ATPase activity reduction (Ristagno *et al.*, 2012), and decrease of DNA strand breaks (Pitozzi *et al.*, 2010) as a marker of oxidative damage.

5.7 Hydrocarbons

The main hydrocarbon present in VOO is squalene. It is an unsaturated terpene, intermediate in the biosynthesis of sterols, and a precursor of phytosterols and cholesterol formation. Squalene accounts for more than 50% of the unsaponifiable matter of olive oil (Kiritsakis, 1998a). It can be found in high concentration among vegetable oils and varies from 1400 to 12,000 mg/kg of oil (Bondioli *et al.*, 1993; Murkovic *et al.*, 2004). The hydrocarbon sterol content is higher in unripe than in ripe olives (Fernández-Cuesta *et al.*, 2013). When the olive oil is refined, the squalene level is dramatically reduced.

The effect of squalene on plasma lipids in humans and animals, however, has been controversial. While some authors did not observe any increase in plasma cholesterol (Ostlund *et al.*, 2002; Liu *et al.*, 2009), others did (Scolastici *et al.*, 2004). A less explored hypothesis is that squalene may have an anti-inflammatory effect since it inhibited the myeloperoxidase activity induced in auricular edema of mice exposed to either arachidonic acid or 12-*O*-tetradecanoylphorbol acetate (de la Puerta *et al.*, 2000). Overall, these studies indicate that squalene administration may play a cardioprotective role.

Squalene plays a major role in maintaining the electrochemical gradient that moves ions across membranes (Hauss *et al.*, 2002). The natural enrichment of squalene in skin surface lipids suggests that this terpene

molecule protects against external chemical, physical, or microbial signals and stressors on the skin surface. Squalene decreases UV-induced DNA damage and prevents photo-aging in human skin (Cho *et al.*, 2009). In addition to its radical scavenging capacity either alone or in VOO in membranes and skin disorders (Viola & Viola, 2009; Wolosik *et al.*, 2013), squalene is effective against several degenerative diseases (Reddy & Couvreur, 2009; Bhilwade *et al.*, 2010) and has been used as a vaccine adjuvant (Tegenge & Mitkus, 2013).

5.8 Triterpenoids

Triterpenoids are polycyclic compounds derived from the linear hydrocarbon squalene, are widely distributed in edible and medicinal products and are an integral part of the human diet. Triterpenoids are being evaluated for use in new functional foods, drugs, cosmetics, and healthcare products. These compounds are present in high concentrations in the epicarp of olive fruit, forming part of the waxes that cover them, and in other parts of olive fruit (Bianchi & Vlahov, 1994). The main triterpenes of VOO are maslinic acid (2α,3β-dihydroxyolean-12-en-28-oic acid), oleanolic acid (3β-hydroxyolean-12-en-28-oic acid), uvaol (Δ12-ursen-3β,28-diol), and erythrodiol (homo-olestranol,5-olean-12-ene-3β,28-diol) (Allouche *et al.*, 2009) (Figure 5.15). Small amounts of ursolic acid (3β-hydroxyurs-12-en-28-oic acid), and betulinic acid (3β-hydroxylup-20(29)-en-28-oic acid) have also been occasionally described in olive products.

Uvaol and erythrodiol possess two hydroxyl groups in remote positions and differ with regard to the methyl group location. According to Pérez-Camino and Cert (1999), natural black olives show concentrations of these compounds up to 2000 mg/kg in the olive flesh. The concentrations of maslinic and oleanolic acids in olive oil depend on the olive cultivar and the oil quality: extra virgin oils contain 200 mg/kg of these triterpenoids, their content exceeds 300 mg/kg in virgin oil, and crude pomace oils have up to 10 g/kg of these compounds (Romero *et al.*, 2010). The total amount of the triterpenic alcohols, erythrodiol plus uvaol, is a quality parameter to detect adulterations in VOOs with "orujo" olive oil (olive pomace oil) because olive pomace oil contains higher amounts of erythrodiol and unaol than VOO. As an example, erythrodiol is present in olive oil in concentrations of around 90 mg/kg but up to 690 mg/kg (Perez-Camino & Cert, 1999) in olive pomace (orujo) olive oil.

Nevertheless, the profile and the content of different triterpenoids in olive fruit seem to be significantly influenced by the fruit development stage. The total triterpene content is usually higher in the unripe fruits (0.21–0.23% of fruit dry weight, depending on the cultivar) and diminished as ripening proceeded, with a decrease of 20% between the green mature and the black ripe stages (final content: 0.17–0.19% of dry weight). Treatment with NaOH, which is employed to debitter the fruits, was shown to cause the loss of triterpene acids due to their solubilization (after sodium salt formation) in alkaline solutions (Romero *et al.*, 2010).

Figure 5.15 Chemical structure of the main triterpenoids found in virgin olive oil.

5.8.1 Anticancer activity of triterpenoids

Several studies have shown that triterpenoids seem to play a role in the prevention of cancer. It is clear that triterpenoids affect tumorigenesis and key factors for its development, such as angiogenesis (Lin *et al.*, 2011). Apart from this, various studies showed the antitumor activities of triterpenoids in different cancers such as hepatocellular carcinoma, skin cancer, colon cancer, lung cancer, breast cancer, myelogenous leukemia, and pancreatic cancer (Li *et al.*, 2010; Lucio *et al.*, 2011; Shan *et al.*, 2011; George *et al.*, 2012; Rufino-Palomares *et al.*, 2013; Wang *et al.*, 2013). The antiangiogenic effects of oleanolic and maslinic acids in human liver cancer cell lines have been studied, and it was found that they reduced cell invasion and migration and decreased ROS (Lin *et al.*, 2011).

Maslinic acid inhibited cancer cell growth without necrotic effects and activated caspase-3, a prime apoptosis protease, at nontoxic concentrations. Thus, maslinic acid has been proposed as a promising new compound for the chemoprevention of colon cancers (Juan *et al.*, 2008a, 2008b). Erythrodiol and uvaol were also found to be capable of crossing the blood–brain barrier and therefore may have the potential for use in the prevention and treatment of brain cancers (Martín *et al.*, 2009).

5.9 Tocopherols

Vitamin E is the generic name for a group of lipid-soluble compounds encountered in plants that includes four tocopherols (alpha, beta, gamma, and delta) and four tocotrienols (alpha, beta, gamma, and delta). α-Tocopherol is the most common form of vitamin E, and it has the highest biological activity among the tocopherols and tocotrienols (Woollard & Indyk, 2003). α-Tocopherol comprises 90% of the total tocopherol content in olive oil.

A wide range of α-tocopherol, depending on the cultivar potential and technological factors, has been reported. The levels currently found in olive oils from Portugal, Spain, Italy, and Greece are usually in the range of 50–380 ppm (Fedeli & Cortesi, 1993; Esti *et al.*, 1996; Ranalli & Angerosa, 1996; Manzi *et al.*, 1998; Lo Curto *et al.*, 2001; Grigoriadou *et al.*, 2007), but in olive oils obtained from olives of early stages of maturation, this value can go up to almost 500 ppm (Garcia *et al.*, 2012). Low amounts of β-tocopherol (~10 mg/kg), δ-tocopherol (~10 mg/kg), and γ-tocopherol (~20 mg/kg) are usually reported.

The tocopherol content of olive oil depends not only on the cultivar but also on several other factors involved during transportation, storage, and processing of the fruit (Grigoriadou *et al.*, 2007). The content of α-tocopherol sharply decreases in the earlier stages of maturation, but then maintains its concentration more or less constantly during ripening, with a further decrease at the latest ripening stage. The content of β-tocopherol seems to follow the same trend as α-tocopherol. γ-Tocopherol however, seems to exhibit a slight increase during maturation (Aguilera *et al.*, 2005). Data on the influence of the extraction system vary (Psomiadou & Tsimidou, 1998); refining or hydrogenation causes loss of tocopherols (Andrikopoulos *et al.*, 1989). Table 5.3 shows the tocopherol content in different vegetable oils.

In a similar fashion, tocopherols, just like phenols, act as antioxidants by trapping the hydroperoxyl radicals and stopping the autoxidation chain reaction. Together with phenolic compounds, they are

Table 5.3 Tocopherol content of certain vegetable oils.

	Tocopherol (mg/100 g)			
Oil	α	β	γ	δ
Olive oil	24	2	5	0.4
Sunflower oil	56	2	4	0.1
Soybean oil	18	3	60	37
Corn oil	27	0.2	38	0.5
Peanut oil	14	0.4	13	1

Source: Values from Belitz *et al.* (2009).

responsible for the oxidative stability of olive oil and, therefore, for its shelf life (Kiritsakis & Osman, 1995). α-Tocopherol affects human nutrition and health aspects, while γ-tocopherol mostly shows a strong activity in seed-protecting compounds, like fatty acids.

Although the daily amount of α-tocopherol supplied by the consumption of olive oil is rather low, it may contribute to antioxidant status in humans. α-Tocopherol is the most bioactive tocopherol and is correlated with preventive action against ROS in biological systems such as plasma, membranes, and tissues (Woollard & Indyk, 2003). γ-Tocopherol has been considered as a cancer chemopreventive agent (Stone *et al.*, 2014).

5.10 Aliphatic alcohols and waxes

Saturated straight-chain aliphatic alcohols with even carbon atoms (C18 to C28) are found in olive oil. The main linear aliphatic alcohols present in olive oil are hexacosanol, octacosanol, tetracosanol, and docosanol. Also tricosanol, pentacosanol, and heptacosanol may be present in traces. Solvent-extracted oil (olive pomace oil) contains more aliphatic alcohols than pressed olive oil (Mariani *et al.*, 1991). The total alcohol content varied for pressed and solvent-extracted oils, from 10 to 70 (mg/100 g) and from 224 to 434 (mg/100 g), respectively (Grob *et al.*, 1990). Fatty alcohol content is also affected by olive cultivar, crop year, fruit ripeness, and processing (Cert *et al.*, 1999; Aparicio & Luna, 2002).

Esters of fatty alcohols with fatty acids (waxes) are important minor olive oil constituents because they can be used as a criterion to differentiate various olive oil categories (EU Regulation 2568, 1991). The main waxes detected in olive oil are esters of oleic or palmitic acid with 36, 38, 40, 42, 44, and 46 carbon atoms (Reiter & Lorbeer, 2001). VOOs contain waxes at levels lower than 150 mg/kg, while raw and refined solvent-extracted olive oils have wax content higher than 2000 mg/kg (Grob *et al.*, 1990).

Phytol and geranylgeraniol (Figure 5.16) are two acyclic diterpenoids (diterpene alcohols) present in the aliphatic alcohol fraction of olive oil in the free and esterified forms (Cert *et al.*, 1999; Reiter & Lorbeer, 2001). Geranylgeraniol levels are used in the calculation of the alcoholic index, a useful parameter for detecting solvent-extracted olive oil in VOO. Esters identified in the wax fraction of EVOO are oleate, eicosanoate, eicosenoate, docosanoate, and tetracosanoate (Reiter & Lorbeer, 2001). They are mainly phytyl derivatives.

5.11 Sterols

While cholesterol is the major sterol in animals, β-sitosterol, Δ^5-avenasterol, and campesterol, in addition to brassicasterol and stigmasterol, are the most commonly occurring sterols (phytosterols) in plants. These sterols contain four hydrophobic hydrocarbon rings (cyclopentanperhydrophenanthrene steroid nucleus), a hydroxyl group at the 3-carbon atom of the A-ring, and an alkyl tail (side chain) (Figure 5.17).

Total sterol content of VOOs varies mainly between 1000 mg/kg, which is the lower limit set by the European Union Commission (EU Regulation 2568, 1991) and 3000 mg/kg. β-Sitosterol is the most abundant sterol in VOO, comprising 80 to 92% of the total sterols, followed by Δ^5-avenasterol (4–14%) and campesterol (0.5–4%) but stigmasterol, cholesterol, 24-methylene-cholesterol, Δ-5,23-stigmastadienol, Δ^7-campesterol, $\Delta^{5,24}$-stigmastadienol, sitostanol, Δ^7-stigmastenol, and Δ^7-avenasterol can also be found in smaller quantities (Aparicio & Luna, 2002). Olive oil also contains methyl sterols such as 4α-methyl-24-methylene-Δ-7-cholesten-3β-ol (gramisterol), 4α,14α-dimethyl-24-methylene-Δ-8-cholesten-3β-ol (obtusifoliol), 4α,14α-dimethyl-9,19-cyclopropane-24-methylene-cholesten-3β-ol (cycloeucalenol),

Figure 5.16 Structural formulae of the olive oil diterpene alcohols.

Figure 5.17 Structural formulae of the main sterols found in olive oil.

and 4α-methyl-(24Z)-24-ethylidene-Δ-7-cholesten-3β-ol (citrostadienol) (Kiosseoglou *et al.*, 1987). The levels of total 4α-methylsterols are lower than those of common sterols and triterpene alcohols and vary between 50 and 360 mg/kg (Cert *et al.*, 1999), but in solvent-extracted olive oils these levels are higher. The levels of Δ5- and Δ7-avenasterol, Δ7-stigmastenol, stigmasterol, and cholerosterol differentiate in virgin, refined, and solvent-extracted olive oils (De Blas & del Valle González, 1996). All these constituents help to maintain VOO stability by inhibiting polymerization reactions during heating at frying temperature.

The sterol profile is affected by the olive fruit cultivar and the degree of maturation (Lukic *et al.*, 2013), crop year, storage time of fruits prior to oil extraction, processing, as well as geographic factors (Aparicio & Luna, 2002), and it is considered a fingerprint for authentic VOO (Cert *et al.*, 1999; Reiter *et al.*, 2001). Approximately 10 to 40% of total sterols are present as steryl esters. Diunsaturated Δ5-sterols (Δ5-avenasterol, stigmasterol, and brassicasterol) are present at relatively higher levels in free than in esterified form. The case of diunsaturated Δ7- sterols, however, is opposite. Solvent-extracted olive oils have been found to contain higher levels of sitosteryl-C18-esters than EVOOs (Grob *et al.*, 1990). Since EVOO contains higher amounts (about 85–90%) of free sitosterol than refined olive oil, the percentage of free β-sitosterol in total β-sitosterol can be used as a key parameter for assessing the quality and genuineness of a VOO.

5.11.1 Bioactivity of sterols

Over the past decade, the possibility of using phytosterols as ingredients in functional foods has led to numerous research studies in relation to their ability to reduce blood cholesterol (Chan *et al.*, 2006). The main conclusion is that the effective doses were between 1.5 and 3 g/day, leading to reductions between 8% and 15% in LDL cholesterol (Gupta *et al.*, 2011). It thus seems unlikely that the quantity found in olive oil alone is sufficient to affect cholesterol uptake from the intestine, but it will contribute to the daily intake of phytosterols from diet. A typical Western diet includes between 200 and 400 mg of plant sterols (Ortega *et al.*, 2006), and, therefore, the amounts of sterols in a normal diet would not have a therapeutic effect. Nevertheless, it was observed that VOO intake induces an increase in the plasma levels of β-sitosterol,

Table 5.4 Volatile constituents of virgin olive oil.

Aldehydes	**Esters**
Acetaldehyde	
Butanal	Methyl pentanoate
2-Methylbutanal	Methyl hexanoate
3-Methylbutanal	Methyl heptanoate
Propanal	Methyl octanoate
Pentanal	Methylbutyl acetate
Hexanal	Methylpropyl acetate
Heptanal	2-Methylpropyl 2-methylpropanoate
Octanal	
Nonanal	Methyl salicylate
E-penta-2-al	Ethyl acetate
Z-hexen-3-al	Ethyl butanoate
E-hexen-2-al	Ethyl benzoate
E-hepten-2-al	Ethyl heptanoate
E-octen-2-al	Ethyl octanoate
E-nonen-2-al	Ethyl nonanoate
E-decen-2-al	Ethyl decanoate
E-undecen-2-al	Ethyl phenyl acetate
Hexa-2,4-dienal	Ethyl cyclohehanoate
Hepta-2,4-dienal	Hexyl acetate
Nona-2,4-dienal	Z-hexen-3-enyl acetate
Deca-2,4-dienal	Octyl acetate
Benzaldehyde	

Alcohols
Methanol
Ethanol
Hexanol
Isopropanol
Heptanol
Octanol
Nonanol
E-hexen-1-ol
Z-hexen-3-ol
2-Phenylethanol
Isobutanol
3-Methylbutanol
2-Methylbutanol
Pentanol

Aliphatic hydrocarbons	**Aromatic hydrocarbons**
Hexane	Ethyl benzene
Octane	Methoxybenzene
Nonane	Dimethoxybenzene
Isopentane	Naphthalene
2-Methylpentane	Ethyl naphthalene
	Dimethylnaphthalene
	Acenaphthene

Furan derivatives	**Ethers**
2-Propylfuran(two isomers)	Methoxybenzene
2-Pentyl-3-methylfuran	Dimethoxybenzene

(*continued*)

Table 5.4 (*Continued*)

Cetones	Thiophene derivatives
3-Methylbutan-2-one	2-Isopropenylthiophene
Acetone	2-Ethyl-5-hexylthiophene
3-Methylbutan-2-one	2,5-Diethylthiophene
Pentan-3-one	2-Ethyl-5-hexyldihydrothiophene
Hexan-2-one	2-Ethyl-5-methyldihydrothiophene
Octan-2-one	2-Octyl-5-methylthiophene
Nonan-2-one	
2-Methyl-2-hepten-6-one	
Acetophenone	

Source: Fedeli (1977), Kiritsakis and Min (1989), Blekas and Guth (1993), Aparicio and Morales (1998), Kiritsakis (1998b), Kanavouras *et al.* (2005), Kiralan *et al.* (2012), Ben Hassine *et al.* (2015).

which is inversely correlated with the intestinal absorption of dietary cholesterol and the plasma levels of total cholesterol and LDLs (low-density lipoproteins) in asymptomatic elderly people at high cardiovascular risk (Escurriol *et al.*, 2009).

The cholesterol-lowering effects of phytosterols (mainly β-sitosterol) in plasma and cell membranes have been shown to maintain cardiovascular health (Choudhary & Tran, 2011). Sterols may also have other important biological properties related to the reduction of reactive oxygen species produced by regulating enzymes and inflammatory effects.

β-Sitosterol can modulate the expression of genes involved in lipid uptake and metabolism. This sterol also enhances mitochondrial function by promoting inner mitochondrial membrane fluidity in hippocampal cells (Shi *et al.*, 2013). These anti-amyloidogenic and brain ATP-preserving/boosting effects of β-sitosterol suggest its potential for use in the prevention and management of neurodegenerative diseases.

High levels of serum campesterol or stigmasterol are correlated with a personal or family history of cardiovascular disease, suggesting that hyperphytosterolemia might be a familial marker for the hyperabsorption of sterols. The absorption levels of cholesterol, campesterol, and β-sitosterol from the body are 31.2%, 9.6%, and 4.2%, respectively. Thus, consumption of food with a significant amount of phytosterols would result in a greater increase in serum campesterol than in serum sitosterol, the most abundant sterol in VOO.

5.12 Flavor compounds

Aroma and flavor, which are distinctive features of olive oil compared to other edible oils, are generated by a number of volatile compounds present at extremely low concentrations (Kiritsakis & Min, 1989; Kiritsakis, 1998b; Kirala *et al.*, 2012; Ben Hassine *et al.*, 2015). Approximately 280 compounds have been identified in the volatile fraction of VOOs (Fedeli, 1977; Solinas *et al.*, 1988; Blekas & Guth, 1993; Guth & Grosch, 1993; Kiritsakis, 1993; Olias *et al.*, 1993; Kiritsakis *et al.*, 2002; Kanavoura *et al.*, 2005).

Volatile constituents found in VOOs are classified into hydrocarbons (more than 80 compounds), alcohols (45 compounds), aldehydes (44 compounds), ketones (26 compounds), acids (13 compounds), esters (55 compounds), ethers (5 compounds), furan derivatives (5 compounds), thiophene derivatives (5 compounds), pyranones (1 compound), thiols (1 compound), and pyrazines (1 compound). From this large number of compounds, only 67 were found to be present at levels higher than their odor threshold. Some of them contribute only to the flavor of VOOs with sensory defects (Morales *et al.*, 2005). Hexanal, E-hexen-2-al, hexan-1-ol, and 3-methylbutanol are the major volatile compounds of olive oil (Fedeli, 1977; Guth & Grosch, 1993; Olías *et al.*, 1993; Kiritsakis, 1998b; Angerosa *et al.*, 2003; Morales *et al.*, 2005). Table 5.4 shows the main volatile compounds of VOO.

Volatile compounds are formed in the olive fruit by enzymatic reactions. These reactions proceed at a high rate depending on both pH and temperature. The C6 and C5 compounds are quantitatively the most important fraction of volatile compounds of high-quality EVOOs. C6 and C5 compounds are enzymatically

Table 5.5 Aroma threshold and description of some volatile compounds found in virgin olive oil.

Compound	Odor threshold*/ mg/ kg	Sensory attributes
Ethyl acetate	0.94	Sweet, aromatic, ethereal
Z-hex-3-enal	0.003	Green, cut grass
Pent-1-en-3-one	0.001	Pungent, mustard
Hexanal	0.075	Green, apple, green fruit
E-hex-2-enal	0.42	Green apple, tomato, pungent
Hexyl acetate	1.04	Fruity, sweet
Z-hex-3-enyl acetate	0.2	Fruity, green leaves
Z-pent-2-en-1-ol	0.25	Banana
Hexan-1-ol	0.4	Fruit, banana, soft
Z-hex-3-en-1-ol	1.1	Grass, banana
E-hex-2-en-1-ol	5	Green, grassy, sweet

*Values reported from Aparicio and Morales (1998), and Morales *et al.* (2005).

produced from PUFAs through the so-called lipoxygenase (LOX) pathway, and, therefore, their concentrations will depend on the level and activity of each enzyme involved in this LOX pathway and on the fatty acid composition of the oil, which are both cultivar dependent. The most abundant compounds are hexanal, *E*-hex-2-enal, *Z*-hex-3-enal, hexan-1-ol, -3-hexen-1-ol, hexyl acetate, and Z-3-hexenyl acetate. These volatiles are responsible for the green and fruity perception of the unique VOO aroma (Table 5.5). C5 aldehydes, ketones and alcohols, and pentene dimers also arise from a cleavage reaction of 13-hydroperoxide of linolenic acid and are usually present at levels lower than their odor threshold. The formation of these C6 and C5 compounds is affected by cultivar, degree of fruit ripeness, storage time of fruits prior to oil extraction, and processing conditions (Kiritsakis & Min, 1989; Lercker *et al.*, 1999; Aparicio & Luna, 2002; Angerosa *et al.*, 2003).

Some olive oil aroma compounds are strongly related to the olive oil cultivar. The octanal, nonanal, and hexen-2-al content of the oil is a cultivar characteristic. Also, the volatile aliphatic alcohols of the oil, such as hexan-2-ol, propanol, amylic alcohols, hexen-2-ol, and heptanol, are related to the olive cultivar. Generally, the cultivar and the cultivation area play important roles in influencing the quality and the flavor and aroma characteristics of olive oil (Kiritsakis, 1998b; Kiralan *et al.*, 2012; Ben Hassen *et al.*, 2015), which facilitate the absorption of bioactive compounds of olive oil by our body. In fact, climatic and soil conditions, cultivation practices, maturity of fruit, and storage conditions influence the flavor quality of the oil. Processing techniques and storage conditions also influence the flavor compounds of olive oil. Therefore, olive oils with a great variety of flavor quality may be the outcome.

5.13 Conclusion

Today, producers as well as consumers are truly concerned about the taste aspects of virgin olive oil. However, taste aspects have not yet been studied in depth. We do not yet know to what extent the differences in aroma and flavor of olive oil are related to cultivar, environment, and other factors. Although much work has been carried out to separate and identify the volatile components of olive oil, more research is needed to determine which factors are responsible, and to what extent, for the unique and delicate taste and flavor of good-quality olive oil.

Olive fruit and olive oil contain a variety of both lipophilic and non-lipophilic compounds with a specific effect on their quality and functional value. The presence of these compounds is affected by several factors and mainly by cultivar. Several studies have shown the functionality of some of these compounds in different aspects. Meanwhile, clinical trial research utilizing olive oil dietary supplementation to assess its potential in prevention and treatment of neurodegenerative and other disorders remains scarce.

It is important to take the necessary attention to preserve the functional compounds present in olives and olive oil in order to totally increase their great positive contribution to human health.

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References

Aguilera, M.P., Beltran, G., Ortega, D., Fernández, A., Jiménez, A., & Uceda, M. (2005) Characterization of virgin olive oil of Italy olive cultivars: 'Frantoio' and 'Leccino', grown in Andalusia. Food Chemistry 89, 387–391.

Allouche, Y., Jimenez, A., Uceda, M., Aguilera, M. P., Gaforio, J. J., & Beltran, G. (2009) Triterpenic content and chemometric analysis of virgin olive oils from forty olive cultivars. Journal of Agricultural and Food Chemistry 57, 3604–3610.

Amiot, M.J., Fleuriet, A., & Macheich, J.J. (1989) Accumulation of oleuropein derivatives during olive maturation. Phytochemistry 28, 67–69.

Andary, C., Wylde, R., Laffite, C., Privat, G., & Winternitz, F. (1982) Structure of verbascoside and orobancoside, caffeic acid sugar esters from *Orobanche rapum-genistae*. Phytochemistry 21(1982), 1123–1127.

Andrikopoulos N., Hassapidou, M., & Manoukas, A. (1989) The tocopherol content of Greek olive oils. Journal of the Science of Food and Agriculture 46, 503–509.

Androulakis, I. (1987) Studies on growth, flowering and mineral content of leaves of the olive (*Olea europea* L.) in relation to biennial bearing and mineral nutrition in Crete. PhD thesis, University of London.

Angerosa, F., Mostallino, R., Basti, C., & Vito, R. (2003) Virgin olive oil odour notes: their relationships with volatile compounds from the lipoxygenase pathway and secoiridoid compounds. Food Chemistry 68, 283–287.

Aparicio, R., & Luna, G. (2002) Characterisation of monovarietal virgin olive oils. European Journal of Lipid Science and Technology 104, 614–627.

Aparicio, R., & Morales, M.T. (1998) Characterization of olive ripeness by green aroma compounds of virgin olive oil. Journal of Agricultural and Food Chemistry 46, 1116–1122.

Aparicio-Ruiz, R., & Gandul-Rojas, B. (2012) Thermal degradation kinetics of neoxanthin, violaxanthin, and antheraxanthin in virgin olive oils. Journal of Agricultural and Food Chemistry 60, 5180–5191.

Appel, L.J., Sacks, F.M., & Carey, V.J. (2005) Effects of protein, monounsaturated fat, and carbohydrate intake on blood pressure and serum lipids: results of the Omni Heart randomized trial. Journal of the American Medical Association 294, 2455–2464.

Aslam, T., Delcourt, C., Silva, R., Holz, F.G., Leys, A., Garcia-Layana, A., & Souied, E. (2013) Micronutrients in age-related macular degeneration. Ophthalmologica 229, 75–79.

Baldioli, M., Servili, M., Perretti, G., & Montedoro, G.F. (1996) Antioxidant activity of tocopherols and phenol compounds of virgin olive oil. Journal of the American Oil Chemists' Society 73, 1589–1593.

Barden, A.E., Croft, K.D., Durand, T., Guy, A., Mueller, M.J., & Mori, T.A. (2009) Flaxseed oil supplementation increases plasma F1-phytoprostanes in healthy men. Journal of Nutrition 139, 1890–1895.

Baum, S.J., Kris-Etherton, P.M., & Willett, W.C. (2012) Fatty acids in cardiovascular health and disease: a comprehensive update. Journal of Clinical Lipidology 6, 216–234.

Ben Hassine, K., El Riachy, M., Taamalli, A., Malouche, D., Ayadi, M., Talmoudi, K., Aouini, M., Jlassi, Y., Benincasa, C., Romano, E., Perri, E., Kiritsakis, A., Hamdi, M., Grati-Kammoun, N., & Hammami, M. (2014) Consumer discrimination of Chemlali and Arbequina olive oil cultivars according to their cultivar, geographical origin, and processing system. European Journal of Lipid Science and Technology 116, 812–824.

Ben Hassine, K., Taamalli, A., Ben Slama, M., Khouloud, T., Kiritsakis, A., Benincasa, C., Perri, E., Malouche, D., Hammami, M., Bornaz, S., & Grati-Kammoun, N. (2015) Characterization and preference mapping of autochthonous and introduced olive oil cultivars in Tunisia. European Journal of Lipid Science and Technology 117, 112–121.

Benavent-García, O., Castillo, J., Lorente, J., Ortuño, A., & Del Rio, J. A. (2000) Antioxidant activity of phenolics extracted from *Olea europaea* L. leaves. Food Chemistry 68, 457–462.

Berglund, L., Lefevre, M., & Ginsberg, H.N. (2007) Comparison of monounsaturated fat with carbohydrates as a replacement for saturated fat in subjects with a high metabolic risk profile: studies in the fasting and postprandial states. American Journal of Clinical Nutrition 86, 1611–1620.

Bethke, P.C., & Jansky, S. H. (2008) The effects of boiling and leaching on the content of potassium and other minerals in potatoes. Journal of Food Science 75, H80–H85.
Bhilwade, H.N., Tatewaki, N., Nishida, H., &Konishi, T. (2010) Squalene as novel food factor. Current Pharmaceutical Biotechnology 11, 875–880.
Bianchi, G., & Pocci, N. (1994) 3,4-Dihydroxyphenylglycol, a major C6–C2 phenolic in *Olea europaea* fruits. Phytochemistry 35, 1335–1337.
Bianchi, G., & Vlahov, G. (1994) Composition of lipid classes in the morphological parts of the olive fruit, cv. Coratina (*Olea europaea* L.). Fat Science Technology 96, 72–77.
Bianco, A., Coccioli, F., Guiso, M., & Marra, C. (2001) The occurrence in olive oil of a new class of phenolic compounds: hydroxy-isochromans. Food Chemistry 77, 405–411.
Bianco, A., Mazzei, R. A., Melchioni, C., Romeo, G., Scarpati, M. L., Soriero, A., & Ucella, N. (1998) Microcomponents of olive oil – III. Glucosides of 2(3,4-dihydroxy-phenyl) ethanol. Food Chemistry 63, 461–464.
Bianco, A.D., Piperno, A., Romeo, G., & Uccella, N. (1999) NMR experiments of oleuropein biomimetic hydrolysis. Journal of Agricultural and Food Chemistry 47, 3665–3668.
Blasbalg, T.L., Hibbeln, J.R., Ramsden, C.E., Majchrzak, S.F., & Rawlings, R.R. (2011) Changes in consumption of omega-3 and omega-6 fatty acids in the United Stated during the 20th century. American Journal of Clinical Nutrition 93, 950–962.
Blekas, G., & Guth H. (1993) Evaluation and quantification of potent odorants of Greek virgin olive oils. In: Food flavors, generation analysis and process influence, ed. G. Charalampous. Elsevier Science, Amsterdam, 419–427.
Blekas, G., Tsimidou, M., & Boskou, D. (1995) Contribution of alpha-tocopherol to olive oil stability. Food Chemistry 52, 289–294.
Bondioli, P., Mariani, C., Lanzani, A., Fedeli, E., & Muller, A. (1993) Squalene recovery from olive oil deodorization distillates. Journal of the American Oil Chemists' Society 70, 763–766.
Brenes, M., Garcia, A., Garcia, P., & Garrido, A. (2001) Acid hydrolysis of secoiridoid aglycons during storage of virgin olive oil. Journal of Agricultural and Food Chemistry 49, 5609–5614.
Buckland, G., Agudo, A., & Travier, N. (2011) Adherence to the Mediterranean diet reduces mortality in the Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Spain). British Journal of Nutrition 106, 1581–1591.
Carrasco-Pancorbo, A., Cerretani, L., Segura-Carretero, A., Gallina-Toschi, T., Lercker, G., & Fernandez-Gutierrez, A. (2006) Evaluation of individual antioxidant activity of single phenolic compounds on virgin olive oil. Progressive Nutrition 8, 28–39.
Cerretani, L., Salvador, M.D., Bendini, A., Lercker, G., & Fregapane, G. (2008) Relationship between sensory evaluation performed by Italian and Spanish official panels and volatile and phenolic profiles of virgin olive oils. Chemosensory Perception 1, 258–267.
Cert, A., Alba, J., & Pérez-Camino, C. (1999) Influence of extraction methods on the characteristics and minor components of extra virgin olive oil. Olivae 79, 41–50.
Chan, Y.M., Varady, K.A., Lin, Y., Trautwein, E., Mensink, R.P., & Plat, J. (2006) Plasma concentrations of plant sterols: physiology and relationship with coronary heart disease. Nutrition Reviews 64, 385–402.
Cho, S., Choi, C.W., Lee, D.H., Won, C.H., Kim, S.M., Lee, S., Lee, M.J., & Chung, J.H. (2009) High-dose squalene ingestion increases type I procollagen and decreases ultraviolet-induced DNA damage in human skin in vivo but is associated with transient adverse effects. Clinical and Experimental Dermatology 34, 500–508.
Choudhary, S.P., & Tran, L.S. (2011) Phytosterols: perspectives in human nutrition and clinical therapy. Current Medicinal Chemistry 18, 4557–4567.
Cicerale, S., Lucas, L., & Keast, R. (2010) Biological activities of phenolic compounds present in virgin olive oil. International Journal of Molecular Sciences 11, 458–479.
Cicero, A.F.G., Nascetti, S., & Lopez-Sabater, M.C. (2008) Changes in LDL fatty acid composition as a response to olive oil treatment are inversely related to lipid oxidative damage: the EUROLIVE study. Journal of the American College of Nutrition 27, 314–320.
Covas, M.I., de la Torre, K., & Farre-Albaladejo, M.L. (2006) Postprandial LDL phenolic content and LDL oxidation are modulated by olive oil phenolic compounds in humans. Free Radical Biology and Medicine 40, 608–616.
Covas, M.I., de la Torre, R., & Fitó, M. (2015) Virgin olive oil: a key food for cardiovascular risk protection. British Journal of Nutrition 113, S19–S28.
Cui, Y.H., Jing, C.X., & Pan, H.W. (2013) Association of blood antioxidants and vitamins with risk of age-related cataract: a meta-analysis of observational studies. American Journal of Clinical Nutrition 98, 778–786.
Dai, Z., Wang, R., Ang, L.W., Low, Y.L., Yuan, J.M., & Koh, W.P. (2014) Protective effects of dietary carotenoids on risk of hip fracture in men: the Singapore Chinese Health Study. Journal of Bone and Mineral Metabolism 29, 408–417.

De Blas, J., & del Valle, G. (1996) Determination of sterols by capillary column gas chromatography: differentiation among different types of olive oil: virgin, refined and solvent extracted. Journal of the American Oil Chemists' Society 73, 1685–1689.
De La Cruz, J.P., Del Río, S., Arrebola, M.M., López-Villodres, J.A., Jebrouni, N., & González-Correa, J.A. (2010) Effect of virgin olive oil plus acetylsalicylic acid on brain slices damage after hypoxia-reoxygenation in rats with type 1-like diabetes mellitus. Neuroscience Letters 471, 89–93.
De la Puerta, R., Martinez-Dominguez, E., & Ruiz-Gutierrez, V. (2000) Effect of minor components of virgin olive oil on topical antiinflammatory assays. Zeitschrift für Naturforschung C 55, 814– 819.
Delgado-Lista J, Garcıa-Rios A, Perez-Martınez P *et al.* (2011) Olive oil and haemostasis: platelet function, thrombogenesis and fibrinolysis. Current Pharmaceutical Design 17, 778–785.
del Monaco, G., Officioso, A., D'Angelo, S., La Cara, F., Ionata, E., Marcolongo, L., Squillaci, G., Maurelli, L., & Morana, A. (2015) Characterization of extra virgin olive oils produced with typical Italian varieties by their phenolic profile. Food Chemistry 184, 220–228.
Denke, M., & Grundy, S. (1992) Comparison of effects of lauric acid and palmitic acid on plasma lipids and lipoproteins. American Journal of Clinical Nutrition 56, 895–898.
Diraman, H., Saygi, H., & Hisil, Y. (2010) Relationship between geographical origin and fatty acid composition of Turkish virgin olive oils for two harvest years. Journal of the American Oil Chemists' Society 87, 781–789.
EFSA. (2011) Panel on dietetic products, nutrition and allergies (NDA). EFSA Journal 9, 2474–2486.
Escriba, P.V., Sanchez-Dominguez, J.M., Alemany, R., Perona, J.S., & Ruiz-Gutierrez, V. (2003) Alteration of lipids, G proteins, and PKC in cell membranes of elderly hypertensives. Hypertension 41, 176–182.
Escurriol, V., Cofan, M., Serra, M., Bullo, M., Basora, J., Salas-Salvado, J., Corella, D., Zazpe, I., Martinez-Gonzalez, M.A., Ruiz-Gutierrez, V., Estruch, R., & Ros, E. (2009) Serum sterol responses to increasing plant sterol intake from natural foods in the Mediterranean diet. European Journal of Nutrition 48, 373–382.
Esti, M., Cinquanta, L., & Carrone, A. (1996) Anti-oxidative compounds and quality parameters in virgin olive oils produced in Molise. Rivista Italiana Delle Sostanze Grasse 73, 147–150.
European Union (EU) Commission Regulation. (1991) On the characteristics of olive oil and olive: residue oil and the relevant methods of analysis. Official Journal of the European Communities, No. L 248.
Faouzia, M.H., Krichène, D., Manai, H., Oueslati, I., Daoud, D., & Zarrouk, M. (2008) Analytical evaluation of six monovarietal virgin olive oils from Northern Tunisia. European Journal of Lipid Science and Technology 110, 905–913.
Farr, S.A., Price, T.O., Dominguez, L.J., Motisi, A., Saiano, F., & Niehoff, M.L. (2012) Extra virgin olive oil improves learning and memory in SAMP 8 mice. Journal of Alzheimers Disease 28, 81–92.
Fedeli, E. (1977) Lipids of olives. Progress in the Chemistry of Fats and Other Lipids 15, 57–74.
Fedeli, E., & Cortesi, N. (1993) Quality, origin and technology of virgin olive oils. Rivista Italiana Delle Sostanze Grasse 70, 419–426.
Fedeli, E., & Jacini, G. (1971) Lipid composition of vegetable oils. Advances in Lipid Research 9, 335–382.
Feeney, J., Finucane, C., Savva, G.M., Cronin, H., Beatty, S., Nolan, J.M., & Kenny, R.A. (2013) Low macular pigment optical density is associated with lower cognitive performance in a large, population-based sample of older adults. Neurobiology of Aging 34, 2449–2456.
Fernández-Cuesta, L. León, L., Velascoa, & R. (2013) Changes in squalene and sterols associated with olive maturation. Food Research International 54, 1885–1889.
Ferrara, L.A., Raimondi, A.S., d'Episcopo, L., Guida, L., Dello Russo, A., & Marotta, T. (2000) Olive oil and reduced need for antihypertensive medications. Archives of Internal Medicine 160, 837–842.
Ferrucci, L., Cherubini, A., Bandinelli, S., Bartali, B., Corsi, A., Lauretani, F., Martin, A., Andres-Lacueva, C., Senin, U., & Guralnik, J.M. (2006) Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. Journal of Clinical Endocrinology and Metabolism 91, 439–446.
Fitó, M., Estruch, R., & Salas-Salvado, J. (2014) Effect of the Mediterranean diet on heart failure biomarkers: a randomized sample from the PREDIMED trial. European Journal of Heart Failure 16, 543–550.
Fitó, M., Guxens, M., & Corella, D. (2007) Effect of a traditional Mediterranean diet on lipoprotein oxidation: a randomized controlled trial. Archives of Internal Medicine 167, 1195–203.
Fraser, T., Tayler, H., & Love, S. (2010) Fatty acid composition of frontal, temporal and parietal neocortex in the normal human brain and in Alzheimer's disease. Neurochemical Research 35, 503–513.
Fu, J., Kim, J., Oveisi, F., Astarita, G., & Piomelli, D. (2008) Targeted enhancement of oleoyl ethanolamide production in proximal small intestine induces across-meal satiety in rats. American Journal of Physiology: Regulatory, Integrative and Comparative Physiology 295, 45–50.
Gallardo-Guerrero, L., Roca, M., & Minguez-Mosquera, I. (2002) Distribution of chlorophylls and carotenoids in ripening olives and between oil and alperujo when processed using a two-phase extraction system. Journal of the American Chemical Society 79, 105–109.

Gandul-Rojas B., & Mínguez-Mosquera, M. (1996) Chlorophyll and carotenoid composition in virgin olive oils from various Spanish olive varieties. Journal of the Science of Food and Agriculture 72, 31–39.
Garcia, A., Brenes, M., Martinez, F. (2001) High-performance liquid chromatography evaluation of phenols in virgin olive oil during extraction at laboratory and industrial scale. Journal of the American Oil Chemists' Society 78, 625–629.
Garcia, B., Magalhães, J., Fregapane, G., Salvador, M.D., & Paiva-Martins, F. (2012) Effect of cultivar and ripeness stage on nutritional value of monovarietal olive oils. European Journal of Lipid Science and Technology 114(9), 1070–1082.
García-González, D.L., Tena N., & Aparicio, R. (2010) Quality characterization of the new virgin olive oil var. Sikitita by phenols and volatile compounds. Journal of Agricultural and Food Chemistry 58, 8357–8364.
Gardner, C.D., & Kraemer, H.C. (1995) Monounsaturated versus polyunsaturated dietary fat and serum lipids: a meta-analysis. Arteriosclerosis, Thrombosis, and Vascular Biology 15, 1917–1927.
Gariboldi, P., Jommi, G., & Verotta, L. (1986) Secoiridoids from *Olea europaea*. Phytochemistry 25, 865–869.
George, V. C., Kumar, D. R., Suresh, P. K., & Kumar, R. A. (2012) Apoptosis-induced cell death due to oleanolic acid in HaCaT keratinocyte cells – a proof-of-principle approach for chemopreventive drug development. Asian Pacific Journal of Cancer Prevention 13, 2015–2020.
Ghanbari, R., Anwar, F., Alkharfy, K.M., Gilani, A.H., & Saari, N. (2012) Valuable nutrients and functional bioactives in different parts of olive (*Olea europaea* L.): a review. International Journal of Molecular Sciences 13, 3291–3340.
Gomez-Alonso, S., Fregapane, G., Salvador Desamparados, M., & Gordon, M.H., (2003) Changes in phenolic composition and antioxidant activity of virgin olive oil during frying. Journal of Agricultural and Food Chemistry 57, 667–672.
Gómez-Rico, A., Fregapane, G., & Salvador, M. D. (2008) Effect of cultivar and ripening on minor components in Spanish olive fruits and their corresponding olive oils. Food Research International 41, 433–440.
González-Correa, J.A., Navas, M.D., Lopez-Villodres, J.A., Trujillo, M., Espartero, J.L., & De La Cruz, J.P. (2008) Neuroprotective effect of hydroxytyrosol and hydroxytyrosol acetate in rat brain slices subjected to hypoxia-reoxygenation. Neuroscience Letters 446, 143–146.
Gordon, M.H., Paiva-Martins, F., & Almeida, M. (2001) Antioxidant activity of hydroxytyrosol acetate compared with that of other olive oil polyphenols. Journal of Agricultural and Food Chemistry 49, 2480–2485.
Grigoriadou, D., Androulaki, A., Psomiadou, E., & Tsimidou, M.Z. (2007) Solid phase extraction in the analysis of squalene and tocopherols in olive oil. Food Chemistry 105, 675–680.
Grob, K., Lanfranchi, M., & Mariani, C. (1990) Evaluation of olive oils through the fatty alcohols, the sterols and their esters by coupled LC-GC. Journal of the American Oil Chemists' Society 67, 626–634.
Gupta, A.K., Savopoulos, C.G., Ahuja, J., & Hatzitolios, A.I. (2011) Role of phytosterols in lipid-lowering: current perspectives. QJM: An International Journal of Medicine 2011(104), 301–308.
Guth, H., & W. Grosch (1993) Quantitation of potent odorants of virgin olive oil by stable-isotope dilution assays, Journal of the American Oil Chemists' Society 70, 513–518.
Gutierrez-Rosales, F., Rios, J.J., & Gomez-Rey, M.L. (2003) Main polyphenols in the bitter taste of virgin olive oil. Structural confirmation by on-line high-performance liquid chromatography electrospray ionization mass spectrometry. Journal of Agricultural and Food Chemistry 51, 6021–6025.
Han, R.M., Zhang, J.P., & Skibsted, L.H. (2012) Reaction dynamics of flavonoids and carotenoids as antioxidants. Molecules 17, 2140–2160.
Harwood, J., & Aparicio, R. (2000) Handbook of olive oil: analysis and properties. Aspen Publishers, Gaithersburg, MD.
Hauss, T., Dante, S., Dencher, N.A., & Haines, T.H. (2002) Squalane is in the midplane of the lipid bilayer: implications for its function as a proton permeability barrier. Biochimica et Biophysica Acta 1556, 149–154.
Hegsted, D.M., Ausman, L.M., Johnson, J.A., & Dalal, G.E. (1993) Dietary fat and serum lipids: an evaluation of the experimental data. American Journal of Clinical Nutrition 57, 875–883.
Helal, O., Berrougui, H., Loued, S., & Khalil, A. (2013) Extra-virgin olive oil consumption improves the capacity of HDL to mediate cholesterol efflux and increases ABCA1 and ABCG1 expression in human macrophages. British Journal of Nutrition 109, 1844–1855.
Hernaez, A., Fernandez-Castillero, S., & Farras M. (2014) Olive oil polyphenols enhance high density lipoprotein function in humans: a randomized controlled trial. Arteriosclerosis, Thrombosis, and Vascular Biology 34, 2115–2119.
Hodson, L., & Fielding, B.A. (2013) Stearoyl-CoA desaturase: rogue or innocent bystander? Progress in Lipid Research 52, 15–42.
Impellizzeri, D., Esposito, E., Mazzon, E., Paterniti, I., Di Paola, R., & Bramanti, P. (2012) The effects of a polyphenol present in olive oil, oleuropein aglycone, in an experimental model of spinal cord injury in mice. Biochemical Pharmacology 83, 1413–1426.

Inouye, H., & Uesato, S. (1986) Biosynthesis of iridoids and secoiridoids. Progress in the Chemistry of Organic Natural Products 50, 169–236.

Jahn, U., Galano, J.M., & Durand, T. (2008) Beyond Prostaglandins–Chemistry and Biology of Cyclic Oxygenated Metabolites Formed by Free-Radical Pathways from Polyunsaturated Fatty Acids, Angew. Chem. Int. Ed. 47, 5894–5955.

Jiménez, B., Sánchez-Ortiz, A., Lorenzo, M.L., & Rivas, A. (2013) Influence of fruit ripening on agronomic parameters, quality indices, sensory attributes and phenolic compounds of Picudo olive oils. Food Research International 54, 1860–1867.

Jones, A. (2011) Can a Mediterranean-type diet prevent Parkinson's disease? Neurology Reviews 19, 1–21.

Juan, M.E., Planas, J.M., Ruíz-Gutiérrez, V., Daniel, H., & Wenzel, U. (2008a) Antiproliferative and apoptosis-inducing effects of maslinic and oleanolic acids, two pentacyclic triterpenes from olives, on HT-29 colon cancer cells. British Journal of Nutrition 100, 36–43.

Juan, M.E., Wenzel, U., Daniel, H., & Planas, J.M. (2008b) Erythrodiol, a natural triterpenoid from olives, has antiproliferative and apoptotic activity in HT-29 human adenocarcinoma cells. Molecular Nutrition & Food Research 52, 595–599.

Kanavouras, A., Kiritsakis, A., & Hernandez, R. 2005. Comparative study on volatile analysis of extra virgin olive oil by dynamic headspace and solid phase micro-extraction. Food Chemistry 90, 69–79.

Karantonis, H.C., Antonopoulou, S., & Demopoulos, C.A. (2002) Antithrombotic lipid minor constituents from vegetable oils: comparison between olive oils and others. Journal of Agricultural and Food Chemistry 50, 1150–1160.

Karppi, J., Kurl, S., Makikallio, T.H., Ronkainen, K., & Laukkanen, J.A. (2013) Low levels of plasma carotenoids are associated with an increased risk of atrial fibrillation. European Journal of Epidemiology 28, 45–53.

Keys, A., Anderson, J.T., & Grande, F. (1957) Prediction of serum-cholesterol responses of man to changes in fats in the diet. Lancet 273, 959–966.

Keys, A., Anderson, J.T., & Grande, F. (1965) Serum cholesterol response to changes in the diet. IV. Particular saturated fatty acids in the diet. Metabolism 14, 776–787.

Keys, A., Menotti, A., & Karvonen, M.J. (1986) The diet and 15-year death rate in the Seven Countries Study. American Journal of Epidemiology 124, 903–915.

Kiosseoglou, V., & Kouzounas, P. (1993) Role of diglycerides, monoglycerides and free fatty acids in olive oil minor surface-active lipid interaction with proteins at oil–water interfaces. Journal of Dispersion Science and Technology 14, 527–539.

Kiosseoglou V., Vlachopoulou, I., & Boskou, D. (1987) Esterified 4-monomethyl- and 4,4-dimethyl-sterols in some vegetable oils. Grasas Aceites 38, 102–103.

Kiralan, M., Ozkan, G., Koyluoglu, F., Ugurlu H.A., Bayrak, A., & Kiritsakis, A. (2012) Effect of cultivation area and climatic conditions on volatiles of virgin olive oil. European Journal of Lipid Science and Technology 114, 552–557.

Kiritsakis, A.K. (1991) Chemistry of olive oil. In: Olive oil, ed. A.K. Kiritsakis. AOCS Press, Champaign, IL.

Kiritsakis, A.K. (1993) Flavor chemistry of olive oil. Olivae 45, 28–33.

Kiritsakis A.K. (1998a) Olive oil: from the tree to the table, 2nd ed. Food and Nutrition Press, Trumbull, CT.

Kiritsakis, A.K. (1998b) Flavor components of olive oil: a review. Journal of the American Oil Chemists' Society 75, 673–681.

Kiritsakis A.K., & Dugan, L.R. (1985) Studies in photooxidation of olive oil. Journal of the American Oil Chemists' Society 62, 892–896.

Kiritsakis, A.K., Kanavouras A., & Kiritsakis, K.A. (2002) Chemical analysis, quality control and packaging issues of olive oil. European Journal of Lipid Science and Technology 104, 628–638.

Kiritsakis, A.K., & Markakis, P. (1987) Olive oil: a review. Advances in Food Research 31, 453–482.

Kiritsakis, A.K., & Min, D. (1989) Flavor chemistry of olive oil. In: Flavor chemistry of lipid foods, ed. D. Min & T. Smouse. AOCS Press, Champaign, IL.

Kiritsakis, A.K., & Osman, M. (1995) Effect of β-carotene and α-tocopherol on light stability of olive oil. Olivae 56, 25–29.

Konstantinidou, V., Covas, M.I., Munoz-Aguayo, D., Khymenets, O., de la Torre, R., & Saez, G. (2010) In vivo nutrigenomic effects of virgin olive oil polyphenols within the frame of the Mediterranean diet: a randomized controlled trial. FASEB Journal 24, 2546–2557.

Kotronen, A., Seppanen-Laakso, T., Westerbacka, J., Kiviluoto, T., Arola, J., Ruskeepaa, A.L., Yki-Jarvinen, H., & Oresic, M. (2010) Comparison of lipid and fatty acid composition of the liver, subcutaneous and intra-abdominal adipose tissue, and serum. Obesity 18, 937–944.

Kremmyda, L.S., Tvrzicka, E., Stankova, B., & Zak, A. (2011) Fatty acids as biocompounds: their role in human metabolism, health and disease: a review. Part 2: fatty acid physiological roles and applications. Biomedical Papers (an official journal of the Palacký University, Faculty of Medicine and Dentistry, Olomouc, Czech Republic) 155, 195–218.

Krichene, D., Taamalli, W., Daoud, D., Salvador, M. D., Fregapane, G., & Zarrouk, M. (2007) Phenolic compounds, tocopherols and other minor components in virgin olive oils of some Tunisian varieties. Journal of Nutritional Biochemistry 31, 179–194.

Lanza, B., Di Serio, M.G., Russi, F., Iannucci, E., Giansante, L., Di Loreto, G., & Di Giacinto, L. (2014) Evaluation of the nutritional value of oven-dried table olives (cv. Majatica) processed by the Ferrandina style. Rivista Italiana delle Sostanze Grasse 91, 117–127.

Lapointe, A., Couillard, C., & Lemieux, S. (2006) Effects of dietary factors on oxidation of low-density lipoprotein particles. Journal of Nutritional Biochemistry 17, 645–658.

Lauffer, L.M., Iakoubov, R., & Brubaker, PL. (2009) GPR119 is essential for oleoylethanolamide-induced glucagon-like peptide-1 secretion from the intestinal enteroendocrine L-cell. Diabetes 58, 1058–1066.

Lercker, G. (2011) La chimica dell'olio da olive. In: Oleum: Manuale dell'olio da olive, ed. A. Ricci. Edagricole, Bologna, Italy.

Li, C., Yang, Z., Zhai, C., Qiu, W., Li, D., Yi, Z., Wang, L., Tang, J., Qian, M., Luo, J., & Liu, M. (2010) Maslinic acid potentiates the anti-tumor activity of tumor necrosis factor alpha by inhibiting NF-kappaB signaling pathway. Molecular Cancer 9, 73–76.

Lichtenstein, A.H., Appel, L.J., & Brands, M. (2006) Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. Circulation 114, 82–96.

Lin, C.C., Huang, C.Y., Mong, M.C., Chan, C.Y., & Yin, M.C. (2011) Antiangiogenic potential of three triterpenic acids in human liver cancer cells. Journal of Agricultural and Food Chemistry 59, 755–762.

Liu, J., & Ma, D.W.L. (2014) The role of n-3 polyunsaturated fatty acids in the prevention and treatment of breast cancer. Nutrients 6, 5184–5223.

Liu, Y., Xu, X., Bi, D., & Wang, X. (2009) Influence of squalene feeding on plasma leptin, testosterone & blood pressure in rats. Indian Journal of Medical Research 129, 150–153.

Lo Curto, S., Dugo, G., Mondello, L., Errante, G., & Russo, M.T. (2001) Variation in tocopherol content in Italian virgin olive oils. Italian Journal of Food Science 13, 221–225.

Lúcio, K.A., da Graça Rocha, G., Monção-Ribeiro, L.C., Fernandes, J., Takiya, C.M., & Gattass, C.R. (2011) Oleanolic acid initiates apoptosis in non-small cell lung cancer cell lines and reduces metastasis of a B16F10 melanoma model in vivo. PLoS One 6, 28596.

Lukic, M., Lukic, I., Krapac, M., Sladonja, B., & Pilizota, V. (2013) Sterols and triterpene diols in olive oil as indicators of variety and degree of ripening. Food Chemistry 136, 251–258.

Lupulescu, A. (1995) Prostaglandins, their inhibitors and cancer. Prostaglandins, Leukotrienes & Essential Fatty Acids 54, 83–94.

Mailer, R.J., Ayton, J., & Conlan, D. (2007) Influence of harvest timing on olive (*Olea europaea*) oil accumulation and fruit characteristics under Australian conditions. Journal of Food, Agriculture and Environment 5, 58–63.

Manoukas, A.G., Mazomenos, B., & Patrinou, M.A. (1973) Amino acid compositions of three varieties of olive fruit. Journal of Agricultural and Food Chemistry, 21, 215–217.

Manzi P., Panfili, G., & Esti, M. (1998) Natural antioxidants in the unsaponifiable fraction of virgin olive oils from different cultivars. Journal of the Science of Food and Agriculture 77, 115–120.

Mariani, C., Fedeli E., & Grob, K. (1991) Evaluation of free and esterified minor components in fatty material. Rivista Italiana delle Sostanze Grasse 68, 233–242.

Markakis, P. (1982) Anthocyanins as food colors. Food Science and Technology: A Series of Monographs. Academic Press, New York.

Martín García, A.I., Moumen, A., Yáñez Ruiz, D.R., & Molina Alcaide, E. (2003) Chemical composition and nutrients availability for goats and sheep of two-stage olive cake and olive leaves. Animal Feed Science and Technology 107, 61–74

Martín, R., Ibeas, E., & Carvalho-Tavares, J. (2009) Natural triterpenic diols promote apoptosis in astrocytoma cells through ROS-mediated mitochondrial depolarization and JNK activation. PLoS ONE 4, 5975.

Mata, P., Alonso, R., Lopez-Farre, A., Ordovas, J.M., Lahoz, C., Garces, C., Caramelo, C., Codoceco, R., Baquez, E., & de Oya, M. (1996) Effect of dietary fat saturation on LDL oxidation and monocyte adhesion to human endothelial cells in vitro. Arteriosclerosis, Thrombosis, and Vascular Biology 16, 1347–1355.

Mateos, R., Espartero, J., & Trujillo, M. (2001) Determination of phenols, flavones and lignans in virgin olive oil by solid phase extraction and HPLC with diode array ultraviolet detection. Journal of Agricultural and Food Chemistry 49, 2185–2192.

Mattson, F.H., & Grundy, S.M. (1986) Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on plasma lipids and lipoproteins in man. Journal of Lipid Research 26, 194–202.

Mensink, R.P., & Katan, M.B. (1992) Effect of dietary fatty acids on serum lipids and lipoproteins, a meta-analysis of 27 trials. Arteriosclerosis, Thrombosis, and Vascular Biology 12, 911–919.

Mensink R.P., Zock, P.L., & Kester, A.D. (2003) Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. American Journal of Clinical Nutrition 77, 1146–1155.

Michas, G., Micha, R., & Zampelas, A. (2014) Dietary fats and cardiovascular disease: putting together the pieces of a complicated puzzle. Atherosclerosis 234, 320–328.

Miles, E., Wallace, F.A., & Calder, P.C. (2001) An olive oil-rich diet reduces scavenger receptor mRNA in murine macrophages. British Journal of Nutrition 85, 185–191.

Milne, G.L., Gao, B., Terry, E.S., Zackert, W.E., & Sanchez, S.C. (2013) Measurement of F2- isoprostanes and isofurans using gas chromatography–mass spectrometry. Free Radical Biology and Medicine 59, 36–44.

Minguez-Mosquera, I., & Gallardo-Guerrero, L. (1995) Disappearance of chlorophylls and carotenoids during the ripening of the olive. Journal of the Science of Food and Agriculture 69, 1–6.

Mohagheghi, F., Bigdeli, M.R., Rasoulian, B., Zeinanloo, A.A., & Khoshbaten, A. (2010) Dietary virgin olive oil reduces blood brain barrier permeability, brain edema, and brain injury in rats subjected to ischemia-reperfusion. Scientific World Journal 10, 1180–1191.

Montedoro, G.F., Servili. M., Baldioli, M., Selvaggini, E., & Miniati, E. (1993) Macchioni: simple and hydrolyzable compounds in virgin olive oil: spectroscopic characterization of the secoiridoids derivatives. Journal of Agricultural and Food Chemistry 41, 2228–2234.

Monti, M.C., Margarucci, L., Tosco, A., Riccio, R., & Casapullo, A. (2011) New insights on the interaction mechanism between tau protein and oleocanthal, an extra-virgin olive-oil bioactive component. Food & Function 2, 423–428.

Morales, M.T., Luna, G., & Aparicio, R. (2005) Comparative study of virgin olive oil sensory defects. Food Chemistry 91, 293–301.

Morrow, J., Awad, J., Boss, H., Blair, I., & Roberts, L. (1992) Non-cyclooxygenase-derived prostanoids (F2-isoprostanes) are formed in situ on phospholipids Proceedings of the National Academy of Sciences of the USA 89, 10721–10725.

Morrow, J.D., Harris, T.M., & Jackson Roberts, L., II. (1990) Nocycloxygenase oxidative formation of a series of novel prostaglandins: analytical ramifications for measurement of eicosanoids. Analytical Biochemistry 184, 1–10.

Mousa, Y. M., Gerasopoulos, D., Metzidakis, I. m& Kiritsakis, A. (1996) Effect of altitude on fruit and oil quality characteristics of 'Mastoides' olives. Journal of the Science of Food and Agriculture 71, 345–350.

Murkoviz, M., Lechner, S., Pietzka, A., Bratakos, M., & Katzogiannos, M. (2004) Analysis of minor constituents in olive oil. Journal of Biochemical and Biophysical Methods 61, 155–160.

Nigam, P., Bhatt, S., Misra, A., Chadha, D.S., Vaidya, M., Dasgupta, J., & Pasha, Q.M.A. (2014) Effect of a 6-month intervention with cooking oils containing a high concentration of monounsaturated fatty acids (olive and canola oils) compared with control oil in male Asian Indians with nonalcoholic fatty liver disease. Diabetes Technology and Therapeutics 16, 255–261.

Ninfali, P., Aluigi, G., Bacchiocca, M., & Magnani, M. (2001) Antioxidant capacity of extra-virgin olive oil. Journal of the American Oil Chemists' Society 78, 243–247.

Ochoa, J.J., Pamplona, R., Ramirez-Tortosa, M.C., Granados-Principal, S., Perez-Lopez, P., Naudi, A., Portero-Otin, M., Lopez-Frias, M., Battino, M., & Quiles, J.L. (2011) Age-related changes in brain mitochondrial DNA deletion and oxidative stress are differentially modulated by dietary fat type and coenzyme Q(10). Free Radical Biology and Medicine 50, 1053–1064.

Olias, J., Perez, A.G., Rios J.J., &. Sanz, L.C. (1993) Aroma of virgin olive oil: biogenesis of the "green" odor notes. Journal of Agricultural and Food Chemistry 41, 2368–2373.

Ortega, R.M., Palencia, A., & Lopez-Sobaler, A.M. (2006) Improvement of cholesterol levels and reduction of cardiovascular risk via the consumption of phytosterols. British Journal of Nutrition 96, 89–93.

Ostlund, R.E., Jr., Racette, S.B., & Stenson, W.F. (2002) Effects of trace components of dietary fat on cholesterol metabolism: phytosterols, oxysterols, and squalene. Nutrition Reviews 60, 349–359.

Oueslati, I., Anniva, C., Daoud, C., Tsimidou M., & Zarrouk, M. (2009) Virgin olive oil (VOO) production in Tunisia: the commercial potential of the major olive varieties from the arid Tataouine zone. Food Chemistry 112, 733–741.

Owen, R.W., Mier, W., Giacosa, A., Hull, W.E., Spiegelhalder, B., & Bartsch, H. (2000) Phenolic compounds and squalene in olive oils: the concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignans and squalene. Food and Chemical Toxicology 38, 647–659.

Paganuzzi V. (1999) Monoglycerides in vegetable oils. Note IV: raw oils of law unsaturation. Rivista Italiana Delle Sostanze Grasse 76, 457–471.

Paiva-Martins, F., & Gordon, M.H. (2001) Isolation and characterization of the antioxidant component 3,4-dihydroxyphenylethyl 4-formyl-3-formylmethyl- 4-hexenoate from olive (*Olea europaea*) leaves. Journal of Agriculture and Food Chemistry 49, 4214–4219.

Paiva-Martins, F., & Gordon, M.H. (2002) Effects of pH and ferric ions on the antioxidant activity of olive polyphenols in oil-in-water emulsions. Journal of the American Oil Chemists' Society 79, 571–576.

Paiva-Martins, F., & Gordon, M.H. (2005) Interactions of ferric ions with olive oil phenolic compounds. Journal of Agricultural and Food Chemistry 53, 2704–2709.

Paiva-Martins, F., Gordon, M.H., & Gameiro, P. (2003) Activity and location of olive oil phenolic antioxidants in liposomes. Chemistry and Physics of Lipids 124, 23–36.

Paiva-Martins, F., Santos, V., Mangericão, H., & Gordon, M.H. (2006) Effects of copper on the antioxidant activity of olive polyphenols in bulk oil and oil-in-water emulsions. Journal of Agricultural and Food Chemistry 54, 3738–3743.

Pardo, J.E., Cuesta, M.A., Alvarruiz, A., Granell, J.D., & Álvarez-Ortí, M. (2011) Evaluation of potential and real qualities of virgin olive oil from the designation of origin (DO) 'Aceite Montes de Alcaraz' (Albacete, Spain). Food Chemistry 124, 1684–1690.

Pavon, F.J., Serrano, A., Romero-Cuevas M., Alonso, M., & Rodrıguez de Fonseca, F. (2010) Oleoylethanolamide: a new player in peripheral control of energy metabolism: therapeutic implications. Drug Discovery Today: Disease Mechanisms 7, 175–183.

Pellegrini, N., Visioli, F., Buratti, S., & Brighenti, F. (2001) Direct analysis of total antioxidant activity of olive oil and studies on the influence of heating. Journal of Agricultural and Food Chemistry, 49, 2532–2538.

Pérez-Camino, M.C., & Cert, A. (1999) Quantitative determination of hydroxypentacyclic triterpene acids in vegetable oils. Journal of Agricultural and Food Chemistry, 47, 1558–1562.

Perez-Galvez, A., Pacheco, Y.M., Bermudez, B., Lopez, S., Abia, R., Muriana, F.J., Villar, J., & Garrido-Fernández, J. (2005) Postprandial evolution of the carotenoid content in the triacylglycerol-rich lipoprotein fraction after a single ingestion of virgin olive oil in humans. Food Research International 38, 1097–1102.

Perk, J., De Backer, G., & Gohlke, H. (2012) European guidelines on cardiovascular disease prevention in clinical practice. The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). European Heart Journal 33, 1635–1701.

Pitozzi, V., Jacomelli, M., Zaid, M., Luceri, C., Bigagli, E., & Lodovici, M. (2010) Effects of dietary extra-virgin olive oil on behavior and brain biochemical parameters in ageing rats. British Journal of Nutrition 103, 1674–1683.

Psomiadou E., & Tsimidou, M. (1998) Simultaneous HPLC determination of tocopherols, carotenoids and chlorophylls for monitoring their effect on virgin olive oil oxidation. Journal of Agricultural and Food Chemistry 46, 5132–5138.

Psomiadou E., & Tsimidou, M. (2001) Pigments in Greek virgin olive oils: occurrence and levels. Journal of the Science of Food and Agriculture 41, 640–647.

Psomiadou E., & Tsimidou, M. (2002) Stability of virgin olive oil. 2. Photo-oxidation studies. Journal of Agricultural and Food Chemistry 50, 722–727.

Ramsden, C.E., Hibbeln, J.R., Majchrzak, S.F., & Davis, J.M. (2010) n-6 Fatty acid-specific and mixed polyunsaturate dietary interventions have different effects on CHD risk: a meta-analysis of randomised controlled trials. British Journal of Nutrition 104, 1586–1600.

Ranalli, A., & Angerosa, F. (1996) Integral centrifuges for olive oil extraction: the qualitative characteristics of products. Journal of the American Oil Chemists' Society 73, 417–422.

Reaven, P., Parthasarathy, S., & Grasse, B.J. (1993) Effects of oleate-rich and linoleate-rich diets on the susceptibility of low density lipoprotein to oxidative modification in mildly hypercholesterolemic subjects. Journal of Clinical Investigation 91, 668–676.

Reddy, L.H., & Couvreur, P. (2009) Squalene: a natural triterpene for use in disease management and therapy. Advanced Drug Delivery Reviews 61, 1412–1426.

Reiter, B., & Lorbeer, E. (2001) Analysis of the wax ester fraction of olive oil and sunflower oil by gas chromatography and gas chromatography-mass spectrometry. Journal of the American Oil Chemists' Society 78, 881–888.

Ridgway, N., & McLeod, R. (2016) Biochemistry of lipids, lipoproteins and membranes, 6th ed. Elsevier, Amsterdam.

Ristagno, G., Fumagalli, F., Porretta-Serapiglia, C., Orrù, A., Cassina, C., & Pesaresi, M. (2012) Hydroxytyrosol attenuates peripheral neuropathy in streptozotocin-induced diabetes in rats. Journal of Agricultural and Food Chemistry 60, 5859–5865.

Rodríguez, G., Lama, A., Rodríguez, R., Jiménez, A., Guillén, R., & Fernández-Bolaños, J. (2007) Olive stone an attractive source of bioactive and valuable compounds. Bioresource Technology 99, 5261–5269.

Rodriguez-Rodriguez, R.A., Tabernero, A., Velasco, A., Lavado, E.M., & Medina, J.M. (2004) The neurotrophic effect of oleic acid includes dendritic differentiation and the expression of the neuronal basic helix-loop-helix transcription factor NeuroD2. Journal of Neurochemistry 88, 1041–1051.

Romani, A., Lapucci, C., Cantini, C., Ieri, F., Mulinaci, N., & Visioli, F. (2007) Evolution of minor polar compounds and antioxidant capacity during storage of bottled extra virgin olive oil. Journal of Agricultural and Food Chemistry 55, 1315–1320.

Romero, C., García, A., Medina, E., Ruíz-Méndez, V., de Castro, A., & Brenes, M. (2010) Triterpenic acids in table olives. Food Chemistry 118, 670–674.

Rondanini, D.P., Castro, D.N., Searles, P.S., & Rousseaux, M.C. (2011) Fatty acid profiles of varietal virgin olive oils (*Olea europaea* L.) from mature orchards in warm arid valleys of Northwestern Argentina (La Rioja). Grasas y Aceites 62, 399–409.

Rose, D. P. (1997) Effects of dietary fatty acids on breast and prostate cancers: evidence from in vitro experiments and animal studies. American Journal of Clinical Nutrition 66, 1513S–1522S.

Roy, J., Oger, C., Thireau, J., Roussel, J., Mercier-Touzet, O., Faure, D., Pinot, E., Farah, C., Taber, D.F., Cristol, J.P., Lee, J.C.Y., Lacampagne, A., Galano, J.M., Durand, T., & Le Guennec, J.Y. (2015) Nonenzymatic lipid mediators, neuroprostanes, exert the antiarrhythmic properties of docosahexaenoic acid. Free Radical Biology and Medicine 86, 269–278.

Rufino-Palomares, E.E., Reyes-Zurita, F.J., Garcia-Salguero, L., Mokhtari, K., Medina, P.P., Lupianez, J.A., & Peragon, J. (2013) Maslinic acid, triterpenic anti-tumoural agent, interferes with cytoskeleton protein expression in HT29 human colon-cancer cells. Journal of Proteomics 83, 15–25.

Ryan, D., Antolovich, M., Prenzler, P.D., Robards, K., & Lavee, S. (2002) Biotransformations of phenolic compounds in *Olea europaea* L. Scientia Horticulturae 92, 147–176.

Salvador, M.D., Aranda, F., Gómez-Alonso, S., & Fregapane, G. (2003) Influence of extraction system, production year and area on Cornicabra virgin olive oil: a study of five crop seasons. Food Chemistry 80, 359–366.

Schaffer, S., Podstawa, M., Visioli, F., Bogani, P., Müller, W.E., & Eckert, G.P. (2007) Hydroxytyrosol-rich olive mill wastewater extract protects brain cells in vitro and ex vivo. Journal of Agricultural and Food Chemistry 55, 5043–5049.

Schwingshackl, L., Strasser, B., & Hoffmann, G. (2011) Effects of monounsaturated fatty acids on cardiovascular risk factors: a systematic review and meta-analysis. Annals of Nutrition and Metabolism 59, 176–86.

Scoditti, E., Nestola, A., Massaro, M., Calabriso, N., Storelli, C., & De Caterina, R. (2014) Hydroxytyrosol suppresses MMP-9 and COX-2 activity and expression in activated human monocytes via PKCalpha and PKCbeta1 inhibition. Atherosclerosis 232, 17–24.

Scolastici, C., Ong, T.P., & Moreno, F.S. (2004) Squalene does not exhibit a chemopreventive activity and increases plasma cholesterol in a Wistar rat hepatocarcinogenesis model. Nutrition and Cancer 50, 101–109.

Servili, M., Baldioli, M., Selvaggini, R., Macchioni, A., & Montedoro, G.F. (1999a) Phenolic compounds of olive fruit: one- and two-dimensional nuclear magnetic resonance characterization of nüzhenide and its distribution in the constitutive parts of fruit. Journal of Agricultural and Food Chemistry 47, 12–18.

Servili, M., Baldioli, M., Selvaggini, R., Miniati, E., Macchioni, A., & Montedoro, G.F. (1999b) HPLC evaluation of phenols in olive fruit, virgin olive oil, vegetation waters and pomace and 1D and 2D-NMR characterization. Journal of the American Oil Chemists' Society 76, 873–882.

Servili, M., Sordini, B., Esposto, S., Urbani, S., Veneziani, G., Di Maio, I., Selvaggini, R., & Taticchi, A. (2014) Biological activities of phenolic compounds of extra virgin olive oil. Antioxidants 3, 1–23; doi:10.3390/antiox3010001.

Shahidi, F., & Ambigaipalan, P. (2015) Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects – a review. Journal of Functional Foods 18, 820–897.

Shahidi, F., Wanasundara, U., & Brunet. N. (1994) Oxidative stability of oil from blubber of harp seal (*Phoca groenlandica*) as assessed by NMR and standard procedures. Food Research International 27, 555–562.

Shahidi, F., & Zhong, Y. (2007) Measurement of antioxidant activity in food and biological systems. In: Antioxidant measurement and applications, ed. F. Shahidi & C.T. Ho. ACS Symposium series 956. American Chemical Society, Washington, DC.

Shan, J.Z., Xuan, Y.Y., Ruan, S.Q., & Sun, M. (2011) Proliferation inhibiting and apoptosis-inducing effects of ursolic acid and oleanolic acid on multi-drug resistance cancer cells in vitro. Chinese Journal of Integrative Medicine 17, 607–611.

Shi, C., Wu, F., & Xu, J. (2013) Incorporation of β-sitosterol into mitochondrial membrane enhances mitochondrial function by promoting inner mitochondrial membrane fluidity. Journal of Bioenergetics and Biomembranes 45, 301–305.

Silva, L., Carrola, J., Pinto, J., & Paiva-Martins, F. (2010a) Oxidative stability of olive oil after food processing and comparison with other vegetable oils. Food Chemistry 121, 1177–1187.

Silva, L., Garcia, B., & Paiva-Martins, F. (2010b) Oxidative stability of olive oil and its polyphenolic compounds after food processing. LWT – Food Science and Technology 43, 1336–1344.

Simopoulos, A.P. (2003) Importance of the ratio of omega-6/omega-3 essential fatty acids: evolutionary aspects. World Review of Nutrition and Dietetics 92, 1–22.

Simopoulos, A.P. (2008) The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Experimental Biology and Medicine (Maywood), 233, 674–688.

Solà, R., La Ville, A.E., & Richard, J.L. (1997) Oleic acid rich diet protects against the oxidative modification of high density lipoprotein. Free Radical Biology and Medicine 22, 1037–1045.

Solinas, M., Angerosa, F., & Camera, L. (1998) Oxidation progress in vegetable oils during frying: determination of volatile components by HRGC and HPLC. Rivista Italiana Delle Sostanze Grasse 65, 567–574.

Spyros, A., Philippidis, A., & Dais, P. (2004) Kinetics of diglyceride formation and isomerization in virgin olive oils by employing 31P NMR spectroscopy: formulation of a quantitative measure to assess olive oil storage history. Journal of Agricultural and Food Chemistry, 52, 157–164.

Steinberg. D., Parthasarathy, S., & Carew, T.E. (1989) Beyond cholesterol: modifications of low density lipoprotein that increases its atherogenicity. New England Journal of Medicine 320, 915–924.

Stone, W.L., Krishnan, K., Campbell, S.E., & Palau, V.E. (2014) The role of antioxidants and pro-oxidants in colon cancer. World Journal of Gastrointestinal Oncology 6, 55–66.

Sujak, A., Okulski, W., & Gruszecki, W.I. (2000) Organisation of xanthophyll pigments lutein and zeaxanthin in lipid membranes formed with dipalmitoylphosphatidylcholine. Biochimica et Biophysica Acta 1509, 255–263.

Taamalli, A., Gómez-Caravaca, A.M., Zarrouk, M., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2010) Determination of a polar and minor polar compounds and other chemical parameters for the discrimination of six different varieties of Tunisian extra-virgin olive oil cultivated in their traditional growing area. European Food Research and Technology 231, 965–975.

Taber, D.F., & Roberts, L.J. (2005) Nomenclature systems for the neuroprostanes and for the neurofurans, Prostaglandins Other Lipid Mediat 78, 14–18.

Tasset, I., Pontes, A.J., Hinojosa, A.J., de la Torre, R., & Túnez, I. (2011) Olive oil reduces oxidative damage in a 3-nitropropionic acid-induced Huntington's disease-like rat model. Nutritional Neuroscience 14, 106–111.

Tegenge, M.A., & Mitkus, R.J. (2013) A physiologically-based pharmacokinetic (PBPK) model of squalene-containing adjuvant in human vaccines. Journal of Pharmacokinetics and Pharmacodynamics 40, 545–556.

Teres, S., Barcelo-Coblijn, G., Benet, M., Alvarez, R., Bressani, R., Halver, J.E., & Escriba, P.V. (2008) Oleic acid content is responsible for the reduction in blood pressure induced by olive oil. Proceedings of the National Academy of Sciences of the USA 105, 13811–13816.

Tsimidou, M., Macrae, M., & Wilson, I. (1987) Authentification of virgin olive oils using principal component analysis of triglyceride and fatty acid profiles. Part I. Classification of Greek oils. Food Chemistry 25, 227–239.

Tsimikas, S., Philis-Tsimikas, A., Alexopoulos, S., Sigari, F., Lee, F., & Reaven, P.D. (1999) LDL isolated from Greek subjects on a typical diet or from American subjects on an oleate-supplemented diet induces less monocyte chemotaxis and adhesion when exposed to oxidative stress. Arteriosclerosis, Thrombosis, and Vascular Biology 19, 122–130.

Tunstall-Pedoe, H., Kuulasmaa, K., & Mahonen, M. (1999) Contribution of trends in survival and coronary-event rates to changes in coronary heart disease mortality: 10-year results from 37 WHO MONICA project populations: monitoring trends and determinants in cardiovascular disease. Lancet 353, 1547–1557.

Tura, D., Failla, O., Bassi, D., Pedo, S., & Serraiocco, A. (2009) Environmental and seasonal influence on virgin olive (*Olea europaea* L.) oil volatiles in northern Italy. Scientia Horticulturae 122, 385–392.

Valls, R.M., Farràs, M., Suárez, M., Fernández-Castillejo, S., Fitó, M., Konstantinidou, V., Fuentes, F., López-Miranda, J., Giralt, M., Covas, M.I., Motilva, M.J., & Solà, R. (2015) Effects of functional olive oil enriched with its own phenolic compounds on endothelial function in hypertensive patients: a randomised controlled trial. Food Chemistry 167, 30–35.

van Dijk, S.J., Feskens, E.J., Bos, M.B., de Groot, L.C., de Vries, J.H., Müller, M., & Afman, L.A. (2012) Consumption of a high monounsaturated fat diet reduces oxidative phosphorylation gene expression in peripheral blood mononuclear cells of abdominally overweight men and women. Journal of Nutrition 142, 1219–1225.

Viola, P., & Viola, M. (2009) Virgin olive oil as a fundamental nutritional component and skin protector. Clinical Dermatology 27, 159–165.

Vlahov, G., & Solinas, M. (1993) Anthocyanin polymerization in black table olives. Agricoltura Mediterranea 123, 7–11.

Vogler, O., López-Bellan, A., Alemany, R., Tofé, S., González, M., & Quevedo, J. (2008) Structure–effect relation of C18 long-chain fatty acids in the reduction of body weight in rats. International Journal of Obesity 32, 464–473.

Wang, X., Bai, H., Zhang, X., Liu, J., Cao, P., Liao, N., Zhang, W., Wang, Z., & Hai, C. (2013) Inhibitory effect of oleanolic acid on hepatocellular carcinoma via ERK-p53-mediated cell cycle arrest and mitochondrial dependent apoptosis. Carcinogenesis 34, 1323–1330.

Weijers, R.N. (2012) Lipid composition of cell membranes and its relevance in type 2 diabetes mellitus. Current Diabetes Reviews 8, 390–400.

Weinstock-Guttman, B., Baier, M., Park, Y., Feichter, J., Lee-Kwen, P., & Gallagher, E. (2005) Low fat dietary intervention with omega-3 fatty acid supplementation in multiple sclerosis patients. Prostaglandins, Leukotrienes & Essential Fatty Acids 73, 397–404.

Wisniewska-Becker, A., Nawrocki, G., Duda, M., & Subczynski, W.K. (2012) Structural aspects of the antioxidant activity of lutein in a model of photoreceptor membranes. Acta Biochimica Polonica 59, 119–124.

Wolak, T., & Paran, E. (2013) Can carotenoids attenuate vascular aging? Vascular Pharmacology 59, 63–66.

Wolosik, K., Knas, M., Zalewska, A., Niczyporuk, M., & Przystupa, A.W. (2013) The importance and perspective of plant-based squalene in cosmetology. Journal of Cosmetic Science 64, 59–66.

Woollard, D.C., & Indyk, H.E. (2003) Tocopherols. In: Encyclopedia of food science and nutrition. Academic Press, London, 5789–5796.

Yang, X., Askarova, S., & Lee, J.C. (2010) Membrane biophysics and mechanics in Alzheimer's disease. Molecular Neurobiology 41, 138–148.

Yonova-Doing, E., Hysi, P.G., Venturini, C., Williams, K.M., Nag, A., Beatty, S., Liew, S.H., Gilbert, C.E., & Hammond, C.J. (2013) candidate gene study of macular response to supplemental lutein and zeaxanthin. Experimental Eye Research 115C, 172–177.

Yu, S., Derr., J., & Etherton, T.D. (1995) Plasma cholesterol-predictive equations demonstrate that stearic acid is neutral and monounsaturated fatty acids are hypocholesterolemic. American Journal of Clinical Nutrition 61, 1129–1139.

Zrelli, H., Matsuoka, M., Kitazaki, S., Zarrouk, M., & Miyazaki, H. (2011) Hydroxytyrosol reduces intracellular reactive oxygen species levels in vascular endothelial cells by upregulating catalase expression through the AMPK-FOXO3a pathway. European Journal of Pharmacology 660, 275–282.

6 Mechanical harvesting of olives

Sergio Castro-Garcia and Louise Ferguson

6.1 Introduction

The need to reduce reliance on manual olive tree planting and harvesting promoted progressive changes in the mechanical systems used for this purpose. In addition, new requirements for environmental sustainability and for enhanced quality of both olive oil and table olives have also stimulated this change. An increasing demand for technology and capital investment has been associated with these new systems. The rate of development of mechanical harvesting for olives has been continuous and progressive since the 1960s. The following three main approaches have been developed since that time: (a) fruit removal from the tree; (b) collection, cleaning, and transport of fallen fruits; and (c) integration of all of these harvest operations into a single operation using continuous harvesters.

6.2 Fruit removal from the tree

Most of the labor demand for traditional manual harvesting was focused on the removal of fruits from the trees, either by hand or with poles (Figure 6.1a). Humanes-Guillen (1976) measured the time required for the different single operations included in the whole process of olive harvesting. Whereas the total demand for harvesting represented 80% of the total manual labor for annual olive orchard management, 40–60% of this demand corresponded to fruit removal by poling or by hand (Klonsky, 2009). Two different complementary approaches that were designed to achieve fruit removal are (a) fruit-loosening products and (b) mechanical devices.

6.2.1 Fruit-loosening products

Spraying with different chemicals to reduce the fruit attachment force was explored for table olives. The first experiments were applied to table olives (Hartmann *et al.*, 1968; Lavee *et al.*, 1973) and oil olives (Collinna & Zucconi, 1966) to facilitate harvesting. At that time, plant growth regulators were considered potential agents for modulating fruit ripening and for controlling other associated processes such as fruit abscission. Many different products were tested, but ethrel was the most promising (Hartmann *et al.*, 1970; Ben-Tal & Lavee, 1976), and large-scale experiments were performed in most olive-producing countries to determine the appropriate concentrations and environmental conditions for its commercial use (Lavee, 2010). However, leaf drop was also associated with ethylene-releasing compounds at concentrations that were effective for fruit abscission, which therefore presented a major drawback for the commercial use of such compounds. Different additives (Shulman *et al.*, 1982; Ben-Tal 1987) were applied to restrict the effect of ethephon on the abscission zone of fruits and leaves and overcome or reduce the induction of leaf drop. Some promising initial responses to new formulations (Vitagliano, 1975; Birger *et al.*, 2008) and other additives such as monopotasium phosphate (Barranco *et al.*, 2000) and 1-methylcyclopropene (Burns *et al.*, 2008) did not provide consistent results in extensive commercial applications. Consequently, no reliable fruit abscission

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Figure 6.1 Hand olive harvesting and tools to facilitate fruit removal process: (a) hand harvest. *Source*: Courtesy of d'Abrunhosa family. (b) Handheld combing. (c) Trunk shaker mounted in tractor. (d) Self-propelled trunk shaker.

agents with commercial potential for both oil and table olives have been identified (Ferguson *et al.*, 2010). In a recent review, Lavee (2010) concluded that using fruit attachment-loosening agents is currently very limited in the olive industry. The problems of leaf drop and variability of response across different years and locations have not yet been solved.

Ethephon applications have been reported to have significant effects on oil quality. In a study of the effects of preharvest ethephon treatments on 'Manzanilla' and 'Frantoio' olives. Alowaiesh (2015) reported that 1000–2000 mg L^{-1} ethephon treatments two to four weeks prior to harvest produced significant increases in peroxide value and most of the fatty acids but significant decreases in the oleic acid, monounsaturated fatty acids (MUFAs), and ratio of MUFAs to polyunsaturated fatty acids (PUFAs). Concentrations of the polyphenols hydroxytyrosol, tyrosol, oleuropein, and total polyphenols, as well as the sensory attributes of fruitiness, bitterness, and pungency decreased significantly with increasing ethephon application rates. Specifically, when trees were sprayed four weeks prior to harvest, the free fatty acids were 0.42%, the

peroxide value was 11.02 meqO_2 kg^{-1}, palmitic acid was 3.19%, stearic acid was 4.19%, linoleic acid was 11.12%, and PUFAs were 11.60%. The significantly reduced phenolic compounds were hydroxytyrosol, 3.91 mg kg^{-1}; tyrosol, 6.05 mg kg^{-1}; and oleuropein at 59.54 mg kg^{-1}. The reduced scores for fruitiness, bitterness, and pungency were 1.74, 1.51, and 1.72, respectively.

6.2.2 Mechanical harvest aids

Many different devices have been designed to facilitate fruit removal. Ferguson *et al.* (2010) indicated that different handheld harvesting units are used in small, hilly orchards in California but cannot be considered to represent mechanical harvesting because the speed and efficiency of the unit are determined by the operator and there is no collection mechanism. Different units (such as pneumatic, handheld combing, and clamping shaking) have been described (Gil-Ribes *et al.*, 2008; Ferguson *et al.*, 2010; Lavee, 2010) (Figure 6.1b). Branch shakers and combing units are still widely used in small olive orchards in many countries because of their low cost and high rate of fruit removal (>90%) (Gil-Ribes *et al.*, 2008; Lavee, 2010) (Figure 6.1b). These devices increase the fruit collection to 350–400 kg per worker per day (Ferguson *et al.*, 2010).

6.2.3 Inertia trunk shaker

Scaffold shakers were initially developed for traditional vase-trained trees with low or nondefined trunks (Lavee, 2010). Inertia head trunk shakers were designed by the University of California for table olive harvesting by the end of the 1960s (Fridley *et al.*, 1971). Since that time and for many years, trunk and scaffold shakers have been considered the most efficient method for fruit removal. Shakers are composed of a head and a clamp that attaches the shaker to the trunk (Figure 6.1c). Three types of trunk shaker heads have been designed to date: linear, multidirectional, and orbital. Three types of clamps (i.e., three-point, scissors, and pincers) are used. Three point clamps are well suited for cylindrical trunks, whereas scissors and pincers are better adapted to the trunks of old trees with irregular shaped cross-sections. The efficiency of the clamp that attaches the shaker to the trunk is a critical point for all shaker heads, as any movement between the clamp and the trunk during shaking can tear the barks and cause severe trunk wounding (Affeldt *et al.*, 1988; Castro-Garcia *et al.*, 2007; Lavee, 2010). Shaker heads of different sizes are mounted on heavier or lighter tractors as front, lateral, or back modules. Self-propelling shakers are also widely used (Figure 6.1d). A review of shakers used for olives has recently been published (Gil-Ribes *et al.*, 2008).

Many factors influence the efficiency and side effects of shakers, and these factors determine the choice of shaker and its operation (Visco *et al.*, 2008a, 2008b). Internal factors refer to the shakers themselves. Shakers transform mechanical energy from a diesel engine to hydraulic energy that is transmitted to the shaker head to be transformed again into a mechanical force vibration that is applied to the tree branch or trunk.

This vibration transmitted by the tree frame detaches the fruits. The energy of the trunk vibration used to detach olive fruits can damage the bark, shoot, and branches and even the machine. Blanco-Roldan *et al.* (2001) reported that the energy leak was higher than 33% during energy transformation from a 59 to 73.6 kW tractor to a multidirectional shaker under regular harvesting conditions, indicating that improvements in the design of the shaker to increase its energy efficiency is needed.

The frequency, acceleration, stroke, vibration time, and desired degree of fruit removal are major parameters that affect the vibration transmitted from the shaker to the tree. Most commercial orbital trunk shakers operate at frequencies between 20 and 40 Hz (1200–2400 cycles min^{-1}) (Gil-Ribes *et al.*, 2007). Intensively planted olive trees have an interesting vibration response when they are excited between 28 and 30 Hz (1680–1800 cycles min^{-1}). In this frequency range, the force vibration excites the first and second tree modes (20.2 and 37.7 Hz, respectively), which reduces vibration damping and prevents large resonance phenomena in the tree (Castro-Garcia *et al.*, 2008). Tsatsarelis (1987) found that amplitude values between 25 and 10 mm in the frequency range of 11.7 to 28.3 Hz (700–1700 cycles min^{-1}) were more effective to detach olives with a vibration duration of 2 to 6 sec without violent motion that could cause fruit damage.

The time of vibration is also a critical parameter for efficient shaker operation. Studies on cumulative olive detachment using artificial vision techniques indicate that more than 50% of the olives drop in the first 3 sec of vibration and that shaking the olive tree beyond 5 sec causes progressive damage to both the tree and the shaker (Gil-Ribes *et al.*, 2008). Further studies indicate that two vibrations of 10 sec were more

effective for fruit removal at the beginning and mid-ripening but not at later harvesting dates and that these vibrations caused negligible damage to the trees (Blanco-Roldan *et al.*, 2009). These findings have resulted in modifications to the usual time of vibration, which was 20 sec. In short, the mode and time of vibration are critical factors for safe fruit removal and to maximize the lifespan of the shaker and tractor. Therefore, the design, choice, and operation of the shaker should take into consideration these critical factors.

6.2.4 Agronomical factors

The introduction of shakers stimulated many studies to define the influence of agronomic factors on the efficiency of shakers for fruit detachment and the accompanying side effects. Tree size and architecture are related to the transmission of vibration to the canopy. Fridley *et al.* (1971) stated that vibration was transmitted well throughout an upright scaffold and that the vibration was damped in low-hanging branches, thus requiring complementary fruit removal by poling. The training of trees as well as their distances became recognized as a major problem for efficient fruit removal in traditional olive plantings in Spain (Humanes-Guillén, 1976). A one-trunk medium-sized vase configuration with 3–4 main limbs (20–40 m^3 volume) has been found to be an adequate training system that is suited to mechanical harvesting (Humanes-Guillén, 1976; Gucci & Cantini, 2000; Pastor & Humanes, 2000; Tombesi, 2006; Visco *et al.*, 2008a, 2008b). In Italy, orchards trained in monocone (Gucci & Cantini, 2000) have also been harvested using shakers, although the fruit removal efficiency was less than that in young vase-trained trees.

Currently, tree density in orchards to be harvested with trunk shakers ranges between 150 and 400 trees per ha, and corresponds to planting distances of 9 × 7 to 7 × 6 m in rain-fed plantations and 8 × 6 to 6 × 4 m in irrigated plantations (Table 6.1). In a preliminary study on vibration, D'Agostino *et al.* (2008) determined that shakers should maximize the vibration effects and that shakers should only be applied to small trees that have a rigid structure. Ferguson (2006) further determined that the lifespan of the orchard should be less than 20 years to avoid excessive tree size and poorly structured trees that are not suited to mechanical harvesting.

Fruit removal force (FRF), fruit fresh weight (FW), oil content, and variation throughout ripening are critical parameters for efficient mechanical harvesting when using shakers. In all cases, the FRF decreases, and the natural fruit drop increases with the progress of ripening, whereas the fruit oil content and fruit weight remain constant or decrease slightly once the color of the fruit begins to change progressively from green to black. These changes determine the optimum period for mechanical harvesting using shakers (Humanes-Guillén, 1976). Extensive variability has been reported among traditional cultivars (Humanes-Guillén, 1976; Lavee *et al.*, 1982; Kouraba *et al.*, 2004; Visco *et al.*, 2008a, 2008b, 2008c; Farinelli *et al.*, 2012) and among genotypes from breeding progenies that further increase the variability inherited from the progenitors (De la Rosa *et al.*, 2008). These data indicate the advantages of establishing several cultivars that have fruits that detach readily by shaking and that ripen at different overlapping times to extend the period of harvesting and the annual use of the shaker, thus reducing the cost of harvesting. The possibility of obtaining new suitable cultivars by breeding also remains a challenge.

Differences in oil composition and quality throughout ripening have been extensively reported. A recent review (El Riachy *et al.*, 2011) indicated that the type of cultivar and the progress of ripening represent the main sources of variability in virgin olive oil composition and sensorial profiles. In general, harvesting at the beginning of fruit ripening is related to a high level of antioxidants, bitterness, pungency, and fruity flavor. Late harvesting is associated with the occurrence of natural drop of the fruit, the severity of which varies with the cultivar (Beltrán *et al.*, 2008). High acidity and deterioration of sensory qualities of olive oil have been found when dropped fruits collected late from the ground are processed (Uceda *et al.*, 2008). Early mechanical harvesting prior to the occurrence of natural fruit drop is therefore recommended as a good husbandry practice.

6.3 Collection, cleaning, and transport of fallen fruits

Traditional olive harvest includes several successive single operations. First, naturally dropped fruits are hand-collected from the ground, cleaned, bagged, and transported to the oil mill. Then, fruits from the trees are collected manually or detached by poling. For table olives, fruits are manually collected, whereas fruits

Table 6.1 Types of specialized olive plantations (orchards): planting systems, yields and methods of harvest.

	Typology of plantings						
	Discontinuous canopy				Continuous canopy (hedgerow)		
	Traditional orchards (T)		Intensive orchards (I)		Wide (WH)	Narrow (NH)	
Variable	**Rainfed (RT)**	**Irrigated (IT)**	**Rainfed (RI)**	**Irrigated (II)**	**Irrigated (IWH)**	**Rainfed (RNH)**	**Irrigated (INH)**
Cultivars	Diverse and local		Selected by high yield, early bearing, and adapted to mechanical harvest				
Density (trees ha^{-1})	17–300[a]	70–120	150–250	200–400	450–800	800–1,000	>1,500
Volume (10^3 m^3 ha^{-1})	6.3–9.8	11.0–13.0	8.0–10.5	12.0–15.0	14.0–18.0	No data	6.0–7.0
Tree training	Diverse, vase		Open vase & central leader		Wide wall	Narrow wall	Narrow wall
Trunks per tree	1–4	1–4	1	1	1	variable	variable
Production							
Years to full bearing	10–15	8–10	6–8	5–7	4–6	4–5	3–4
Yield at full bearing (t ha^{-1})	1–5	5–10	4–6	8–10	>10	4–6	>10
Years of bearing	>100	>100	>40	>40	>20	>15	>15
Oil quality & harvest method							
Oil quality	Irregular and PDO[b]		High standard quality		High standard quality		
Harvest method	Manual, branch shakers and poling		Shakers and catching frames		Diverse	Straddle harvesters	

[a] Related to rainfall
[b] PDO (Protected Denomination of Origin)

Figure 6.2 Olive harvesters: (a) trunk shaker with an inverted umbrella catch frame, (b) side-by-side trunk shaker, (c) modified vineyard straddle harvester, and (d) canopy shaker straddle harvester.

for oil are usually detached by poling and subsequently collected using shade cloths (Figure 6.2b). These fruits are then cleaned and loaded into trailers to be transported to oil mills or table olive processing factories. Fruit removal by shakers represents only the first step toward mechanical harvesting of olives. The olives fall from the shaken trees into nets or shade cloths, or on the ground, and then the same steps used for fruits removed by poling are followed for cleaning, loading, and transport.

The time required for different harvest operations has been extensively recorded in many oil olive plantations since the first field trials of shaker mechanical harvesting. Humanes-Guillén (1976) summarized data from this extensive field experimentation. The time required for fruit removal by shakers is constant without consideration of the tree crop, whereas the time required for other operations is related to tree crop load. The working time for fruit removal by shakers ranges between 1.2 and 3.8% of the total time required for the whole harvest operation, according to the tree crop load. This substantial reduction should be complemented by mechanization of other operations. Different approaches to mechanize the collection of fruits from the ground, extension and moving of nets, and cleaning and loading of fruits into bins or the simultaneous integration of fruit falling and catching have been developed to complement the mechanical removal of fruits by shakers (Gil-Ribes *et al.*, 2008; Lavee, 2010).

The collection of fruits from the ground requires previous preparation of the soil by rollers to facilitate subsequent operations. Picking these dispersed fruits from the ground involves grouping them into rows and gathering them up, followed by cleaning and loading into containers for transport to the mill. Mechanical and pneumatic sweepers and sweeper gatherers are currently used for these operations (Gil-Ribes *et al.*, 2008). The main drawback of collecting fruits from the ground is the damage incurred to the fruits, which can severely reduce the oil quality (Uceda *et al.*, 2008).

Lavee's (2010) review describes methods developed to facilitate fruit collection. A catching frame in the form of an inverted umbrella and a conveyor carrying the harvested fruits to a tank mounted in the same rig with the shaker was the initial approach and represents the first step toward a continuous harvester. These systems have been used largely in widely spaced, traditional, and intensively planted commercial orchards and are highly efficient for plant densities of <250 trees/ha. In more industrial orchards with higher densities, the higher number of trees per hectare increases the cost of harvest. The insufficient space between trees also impedes the use of the inverted umbrella connected to the shaker (Figure 6.2a). A winding catching canvas deployed from a drum has been the most common catching frame and is still used. Both systems require a large amount of manual labor and two individual mechanical devices. A step forward to integrated mechanical harvest was therefore needed for orchards with densities greater than 250 trees/ha.

6.4 Continuous harvesters

The tendency to increase the plant density above 250 trees/ha drove the search for alternative mechanical harvest systems. Several systems have been developed in the past 15 years, and some of them are based on the association of trunk shakers and contact head machines with slanted canvas catch frames working in tandem. The integration of both machine and catching frames in a single unit (side-by-side systems) (Figure 6.2b) or straddle harvesters for wide and narrow hedgerows that integrate all operations (i.e., removing, cleaning, and loading the fruits into bins), from which the fruits are poured into a tractor trailer or truck to be transported to the oil mill (Figure 6.2c and 6.2d), are now typically used (Gil-Ribes *et al.*, 2008; Ferguson *et al.*, 2010; Lavee, 2010; Ravetti & Robb, 2010; Tous *et al.*, 2010; Vieri & Sarri, 2010; Tous, 2011). These continuous harvesters have been designed to address the requirements of high-density orchard typologies (Table 6.1), and each one is best suited for different circumstances. Trunk shakers and inverted umbrellas are very well adapted to medium-sized orchards with fewer than 250 trees/ha and tree trunk heights of approximately 0.8–1.0 m. Fruit removal efficiency is influenced by fruit and tree sizes, the position of fruits within the canopy, and the ripening process. The adaptation of cultivars, tree training, and pruning are therefore critical factors for this system that have required relevant experimental study in most countries.

Narrow hedgerow orchards have been designed to be harvested with the modified grape straddle harvester (Rius & Lacarte, 2010). This machine is well suited for harvesting in many farms, as it may work efficiently in diverse slopes and soils and may move easily from farm to farm. The size of the farm is not currently a problem, as many companies offer harvesting services to farmers. The harvest efficiency is very high and does not depend on the size of the fruit, the position of the fruit within the canopy, or the ripening index (Tous, 2011). The most common self-propelled machines have variable sizes, depending on the model, and are between 2.5 and 4 m tall and 0.5 to 1.5 m wide. The speed of the modified vineyard straddle-harvesting machine ranges between 1 and 2 km per hour, and the area covered is approximately 3–4 ha per day (Lavee, 2010; Tous *et al.*, 2010). Maintaining the same size of tree becomes a critical point for this system, and only a few early-bearing traditional and new cultivars are well adapted to this harvester. However, this harvester has been efficiently used to date in the oldest narrow hedges planted in 1994, by adjusting the height and width of the hedge through deficit irrigation and through pruning the trees to suit the shape and size of the harvester.

Side-by-side shakers, lateral-contact hedge harvesters, and large overhead straddle harvesters are currently being tested or are already used in farms in California (Ferguson, 2006; Vossen, 2007; Ferguson *et al.*, 2010; Ferguson & Castro Garcia, 2014), Italy (Vieri & Sarri, 2010), Argentina and Australia (Ravetti & Robb 2010), as well as Spain and Portugal (Gil-Ribes *et al.*, 2008; Tous *et al.*, 2010; Tous, 2011) for intensive and large hedgerow plantations (high density) or orchards with a density greater than 250 trees/ha, particularly those in the range of 450–800 trees/ha. Ravetti and Robb (2010) compared the performance of a large straddle harvester with side-by-side shakers in full-canopy trees with a total volume of 16,000 m^3/ha in an orchard planted at 7 × 4 m and trained in a free palm form at Boort, Australia, where large farms on flat lands are common. They evaluated the speed of operation, efficiency of fruit removal, canopy damage, trunk damage, and net harvesting time and cost of operation, and concluded that both types of machine are competitive and safe alternatives. Whereas shakers are viable options for small-scale operations in light-cropping or late-harvest situations, straddle harvesters are more competitive for large-scale modern intensive plantations with high levels of production. The maneuverability of the large straddle harvester is a main limiting factor

when it is used on small farms within sloping terrains. These machines offer the possibility to harvest a wider range of cultivars with different levels of vigor when compared with the modified grape harvester.

In summary, olive mechanical harvesting began with harvest shakers for removal of fruits off the trees in the early 1960s. The association of diverse devices with shakers to provide complementary operations for the whole process of harvesting as well as the integration of all operations into a single machine since the early 1990s have opened many possibilities and have led to the design and use of integrated continuous harvesters. These changes in mechanical harvesting have been associated with contemporary modifications of olive planting systems.

6.5 Effects on oil and fruit quality

The final objective of all harvesting is to produce high-quality marketable olive oil, whether hand or mechanically harvested. As a result, preharvest, harvest, and postharvest practices have been evaluated for their ability to affect olive oil quality. The major preharvest production factor that affects olive oil quality is irrigation. The practice of decreasing irrigation prior to harvest to facilitate soil conditions for tractor tires and to decrease trunk barking has not been investigated. However, the effects of decreasing irrigation prior to harvest on both table olive and oil olive quality are well documented. Decreasing irrigation prior to harvest will affect size, and possibly induce shrivel of table olives (Goldhamer, 1999.) The huge volume of olive irrigation literature generally concludes that, dependent upon the cultivar, water quality, and amount, excessive irrigation prior to olive oil harvest will decrease the percentage of oil content and quality, while deficit irrigation prior to olive oil harvest will decrease the olive oil content by percentage but may increase quality (Rallo *et al.*, 2013). The use of abscission compounds has not proven reliable (Burns *et al.*, 2008) enough to be incorporated into mechanical harvesting and is not considered a factor affecting table fruit or olive oil quality. Reported experimental effects of the fruit-loosening compound ethephon on oil quality was discussed in Section 6.2.1, "Fruit-Loosening Compounds."

Generally, unless harvesting damages the fruit, or the fruit is harvested overripe and more susceptible to damage, the harvest method has little direct effect on olive fruit or oil quality (Ferguson, 2006; Ferguson *et al.*, 2010; Saglam *et al.*, 2013; Ferguson & Castro-Garcia, 2014). The effects that harvesting, or postharvest handling, have on table fruit or olive oil quality are processes precipitated by fruit damage during harvest, transport, or storage. It is interesting to note that Yousefi *et al.* (2012) reported that mechanical harvesting caused internal fruit damage inducing rapid softening and decay. The oil produced had similar fatty acid composition but lower tocopherol content, phenolic content, and oxidative stability than oil from manually harvested olives. The fruit deteriorated quickly during fruit storage. Cold storage (3 °C) delayed the deterioration, producing a commercial level of quality in the oil from mechanically harvested olives for 10 days. This suggests cold storage of mechanically harvested fruit that cannot be processed within 24 hours as a way to ensure quality olive oil production when mechanized harvesting produces higher volumes of olive fruit than mills can accommodate.

6.6 Conclusion

Harvest, postharvest transport, and storage methods can have an effect on both table and olive oil quality if they produce damage that accelerates fruit deterioration and causes elimination of the functional compounds of these two products.

References

Affeldt, H.A., Marshall, D.E., & Brown, G.K. (1988) Relative dynamic displacements within a trunk shaker clamp. Transactions of the ASAE 31, 323–330.

Alowaiesh, B.F. (2015) Mechanical harvesting, fruit and oil quality in olives influenced by harvest time and exogenous application of ethyene. PhD dissertation, Curtin University, Australia.

Barranco, D., Cimato, A., Fiorino, P., Rallo, L., Touzani, A., Castañeda, C., Serafini, F., & Trujillo, I. (2000) World catalogue of olive cultivars. International Olive Oil Council, Madrid, Spain.
Beltrán, G., Uceda, M., Hermoso, M., & Frías, L. (2008) Maduración. In: El cultivo de olivo, ed. D. Barranco, R. Fernández-Escobar, & L. Rallo. Junta de Andalucía Ediciones Mundi-Prensa, Madrid, Spain, 163–187.
Ben-Tal, Y. (1987) Improving ethephon's effect on olive fruit abscission by glycerin. HortScience 22, 869–871.
Ben-Tal, Y., & Lavee, S. (1976) Ethylene influence on leaf and fruit detachment in 'Manzanillo' olive trees. Scientia Horticulturae 4, 337–344.
Birger, R., Abd-Elhadi, F., Ronen, A., Cohen, E., Ankorion, Y., Najjar, A., & Moreno, J. (2008) Olive HarvestVant, a new harvest-aid formulation for improving fruit abscission and mechanical harvesting. Acta Horticulturae 791, 257–263.
Blanco-Roldán, G.L, Gil-Ribes, J.A., & Agüera-Vega, J. (2001) Optimization of the design and use of shaker machines for mechanical harvesting of the olive trees in Spain. ASAE Paper 01-1096. ASAE, Sacramento, CA.
Blanco-Roldán, G.L., Gil-Ribes, J.A., Kouraba, K., & Castro-García, S. (2009) Effects of trunk shaker duration and repetitions on removal efficacy for the harvesting of oil olives. Applied Engineering in Agriculture 25, 329–334.
Burns, J.K., Ferguson, L., Glozer, K., Krueger, W.H., & Rosecrance, R.C. (2008) Screening fruit loosening agents for black ripe processed table olives. HortScience 43, 1449–1453.
Castro-García, S., Blanco-Roldán, G.L., Gil-Ribes, J.A., & Agüera-Vega, J. (2008) Dynamic analysis of olive trees in intensive orchards under forced vibration. Trees Structure Function 22, 795–802.
Castro-García, S., Gil-Ribes, J.A., Blanco-Roldán, G.L., & Agüera-Vega, J. (2007) Mode shape evaluation of trunk shakers used in oil olive harvesting. Transactions of the ASAE 50, 727–732.
Collinna, F., & Zucconi, F. (1966) Richere sull'impiego di produtti chemiciatt a facillitare la recolta del'olive. III ricera comparative sulla efficacia de prodotti diversi. Atti Reale Accademia Geogofili 142 (Suppl. XIII), 155–164.
De la Rosa, R., Leon, L., Moreno, I., Barranco, D., & Rallo, L. (2008) Ripening time and fruit characteristics of advanced olive selections for oil production. Australian Journal of Agricultural Research 59, 46–51.
El Riachy, M., Priego-Capote, F., León, L., Rallo, L., & Luque de Castro, M.D. (2011) Hydrophilic antioxidants of virgin olive oil (a review). Part II: biosynthesis and biotransformation of phenolic compounds in virgin olive oil as affected by agronomic and processing factors. European Journal of Lipid Science and Technology 113, 692–707.
Farinelli, D., Ruffolo, M., Bocco, M., & Tombesi, A. (2012) Yield efficiency and mechanical harvesting with trunk shaker of some international olive cultivars. Acta Horticulturae 949, 379–384.
Ferguson, L. (2006) Trends in olive harvesting. Grasas y Aceites 57, 9–15.
Ferguson, L., & Castro Garcia, S. (2014) Transformation of an ancient crop: preparing California 'Manzanillo' table olives for mechanical harvesting. HortTechnology 24, 274–280.
Ferguson, L., Rosa, U.A., Castro-García, S., Lee, S.M., Guinard, J.X., Burns, J., & Glozer, K. (2010) Mechanical harvesting of California table and oil olives. Advances in Horticultural Science 24, 53–63.
Fridley, R.B., Hartmann, H.T., Melschau, J.J., Chen, P., & Whisler, J. (1971) Olive harvest mechanization in California. California Agricultural Experiment Station Bulletin 855. University of California, Davis, CA.
Gil-Ribes, J., Castro Garcia, S., Blanco-Roldán, S., Kouraba, K., & Agüera, J. (2007) Estudio de las propiedades dinámicas de los olivos en una plantación intensiva. IV Congreso Nacional de Agroingenieria, Albacete, Spain.
Gil-Ribes, J., López-Giménez, F.J., Blanco-Roldán, G.L., & Castro-García, S. (2008) Mecanización. In: El cultivo de olivo, ed. D. Barranco, R. Fernández-Escobar, & L. Rallo. Junta de Andalucía Ediciones Mundi-Prensa, Madrid, Spain, 435–507.
Gucci, R., & Cantini, C. (2000) Pruning and training systems for modern olive growing. CSIRO, Collingwood, Australia.
Hartmann, H.T., Heslop, D.J., & Whisler, J. 1968. Fruit abscission in olives. California Agriculture 22, 14–16.
Hartmann, H.T., Tombesi, A., & Whisler, J. (1970) Promotion of ethylene evolution and fruit abscission in the olive by 2-chloroethanephosphonic acid and cyclohexamide. Journal of the American Society for Horticultural Science 95, 635–640.
Klonsky, K.M. (2009) What yield, harvest cost and price are profitable? Department of Agriculture & Resource Economics, University of California, Davis. Available from http://ucanr.edu/sites/mecholive/files/48443.pdf
Kouraba, K., Gil-Ribes, J., Blanco-Roldán, G.L., De Jaime, M.A., & Barranco, D. (2004). Suitability of olive varieties for mechanical harvesting. Olivae 101, 38–43.
Lavee, S. (2010) Integrated mechanical, chemical and horticultural methodologies for harvesting of oil olives and the potential interaction with different growing systems: a general review. Advances in Horticultural Science 24, 5–15.
Lavee, S., Avidan, B., & Ben-Tal, Y. (1982) Effect of fruit size and yield on fruit-removal force within and between olive cultivars. Scientia Horticulturae 17, 27–32.

Lavee, S., Barshi, G., & Haskal, A. (1973) Natural fruit drop and induced abscission to facilitate chemical harvesting of 'Manzanillo' and 'Souri' olives. Scientia Horticulturae 1, 63–75.
Pastor, M., & Humanes, J. (2000) Poda del Olivar: moderna olivacultura. Editoria Agricola Espanola, Madrid, Spain.
Rallo, L., Barranco, D., Castro Garcia, S., Conner, D., Gomez del Campo, M., & Rallo, L. (2013) High density olive plantations, ed. J. Janick. Horticultural Reviews vol. 41. Chichester, John Wiley & Sons Ltd, 303–383.
Ravetti, L., & Robb, S. (2010) Continuous mechanical harvesting in modern Australian olive growing systems. Advances in Horticultural Science 24, 71–77.
Rius, X., & Lacante, J.M. (2010) La revolución del olivar: el cultivo en seto. Agromillora. Bayer Crop Science, New Holland, Olinto, Naszo. Teodolito, Barcelona.
Saglam, C., Tuna, Y.T., Gucgel, U., & Atar, E.S. (2013) Effects of olive harvesting methods on olive oil quality. APCBEE Procedia 8, 334–342.
Shulman, Y., Avidan, B., Ben-Tal, Y., & Lavee, S. (1982) Sodium bicarbonate, a useful agent for pH adjustment of ethephon controlling grapevine shoot growth and loosening olive fruits. Riv. Ortoflorofrutt. Italia 66, 181–187.
Tombesi, A. (2006) Planting systems, canopy management and mechanical harvesting. In: Recent advances in olive industry: Second International Seminar Olivebioteq, Special Seminars and Invited Lectures, 5–10 November, Marsala–Mazara del Vallo (TP), Italy, ed. T. Caruso, S. Motisi, & L. Sebastiani, 307–316.
Tous, J. 2011. Olive production systems and mechanization. Acta Horticulturae 924, 169–184.
Tous, J., Romero, A., & Hermoso, J.F. (2010) New trends in olive orchard design for continuous mechanical harvesting. Advances in Horticultural Science 24, 43–52.
Tsatsarelis, C.A. (1987) Vibratory olive harvesting: the response of the fruit-stem system to fruit removing actions. Journal of Agricultural Engineering Research 38, 77–90.
Uceda, M., Hermoso, M., & Aguilera, M. P. (2008) La calidad del aceite de oliva. In: El cultivo de olivo, ed. D. Barranco, R. Fernández-Escobar, & L. Rallo. Junta de Andalucía Ediciones Mundi-Prensa, Madrid, Spain, 699–727.
Vieri, M., & Sarri, D. (2010) Criteria for introducing mechanical harvesting of oil olives: results of a five-year project in central Italy. Advances in Horticultural Science 24, 78–90.
Visco, T., Molfese, M., Cipolletti, M., Corradetti, R., & Tombesi. A. (2008a) The influence of training system, variety and fruit ripening on the efficiency of mechanical harvesting of young olive trees in Abruzzo, Italy. Acta Horticulturae 791, 387–392.
Visco, T., Molfese, M., Cipolletti, M., Corradetti, R., & Tombesi. A. (2008b) The influence of variety and harvesting period on the efficiency of mechanical harvesting of adult olive trees in Abruzzo, Italy. Acta Horticulturae 791, 431–434.
Visco, T., Molfese, M., Cipolletti, M., Corradetti, R., & Tombesi. A. (2008c) The influence of vibration applied to the trunk and to the branches of different sized olive trees on the efficiency of mechanical harvesting. Acta Horticulturae 791, 435–438.
Vitagliano, C. (1975) Ethylene-releasing compounds to loosen olive fruits for mechanical harvesting. 2nd Sem. Oleic. Intl., Cordoba, Spain.
Vossen, P. (2007) Olive oil: history, production, and characteristics of the world's classic oils. HortScience 42, 1093–1100.
Yousefi, K., Welland, C.M., & Garcia, J.M. (2012) Effect of harvesting system and fruit cold storage on virgin olive oil chemical composition and quality of superintensive cultivated 'Arbequina' olives. Journal of Agricultural and Food Chemistry 60, 4743–4750.

7 Olive fruit harvest and processing and their effects on oil functional compounds

Apostolos Kiritsakis and Nick Sakellaropoulos

7.1 Introduction

The harvest of olive fruits and their processing have an impact on the contents of functional compounds in the fruit and the resultant oil. These effects would in turn exert their influence on the characteristic and hence the associated health implications. These factors are discussed in the subsequent sections of this chapter.

7.2 Harvest time

Olives should be harvested after obtaining the required characteristics for their subsequent use. Olive fruit destined for oil is harvested close to the maximal oil accumulation, but prior to quality deterioration. Table olives could be harvested at the green, cherry, or black stage, depending on the de-bittering and fermentation process used (Blekas *et al.*, 2002; Charoenprasert & Mitchell, 2012; Tsantili *et al.*, 2012).

The period of fruit growth and development for olives is 6 to 7 months, and it is extremely long in comparison with other stone fruits. There are considerable differences between cultivars, growing conditions, level of fruit yield per tree, and so on, but the average cycle takes approximately 200 days to be completed (RIRDC, 2008). The olive development follows, like in other stone fruits, a double sigmoid curve with a latent period at the beginning of the process and another one at the end. After pollination, the cell division process is relatively fast. During this first phase of fast-growing conditions, almost all the cell division processes are completed. During the second phase of slow-growing conditions, the pit-hardening process takes place and both pit and embryo reach their final size that will not vary in the following months. The final phase of fast-growing conditions determines the real size of the fruit due to the enlargement of the cells in the flesh and the processes of oil and moisture accumulation (RIRDC, 2008).

During the green stage, olive fruit reach their final shape and size, and their color is yellow green. When the skin pigment (chlorophyll) is replaced by anthocyanin, the fruit enters the black stage, with purple-black color. The phenolic compounds change qualitatively and quantitatively during the maturity period, reaching their highest content at the stage between the yellow-green and purple color. At the late maturity stage, the total amount of phenolic compounds, especially oleuropein, is degraded by the enzyme esterase and decreases significantly (Esti *et al.*, 1998). In the late ripening stage, the "higher molecular weight" components are hydrolyzed to form tyrosol and hydroxytyrosol (Cimato, 1990, cited by Gharbi *et al.*, 2015).

Differences in phenolic profile and levels of compounds in relation to maturity stages have been observed in 29 Portuguese cultivars (Vinha *et al.*, 2005), 'Arauco' from Argentina (Bodoira *et al.*, 2014), 'Chetoui' from Tunisia (Damak *et al.*, 2008), and the Greek cultivar 'Koroneiki' (Anastasopoulos *et al.*, 2010). Gimeno

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et al. (2002) denoted that phenols and generally the antioxidant content of the oil are higher in green olives than in ripe olives.

Compositional changes in olives, however, are much affected by other factors that might have an impact on the determination of harvest time. Farshid *et al.* (2014), for instance, found that the higher ratio of oleic/linoleic acids in 'Koroneiki' and 'Mari' than in 'Arbequina' and 'Shengeh' was attributed to higher gene expression levels of the enzyme stearoyl desaturase (OeSAD). This suggests that 'Arbequina' and 'Shengeh' have to be harvested earlier than 'Koroneiki' and 'Mari'. The levels of each phenolic compound also depend on the geographical origin of the cultivar (Vinha *et al.*, 2005; Dag *et al.*, 2010), while crop year effects also have been frequently reported in the literature (Anastasopoulos *et al.*, 2010). The particular climate conditions during the fruit growth affect the rate of maturation and quality. This unpredictable factor indicates that harvest time cannot be determined in advance. Moreover, cultivation practices influence the olive characteristics and ripening rate. Indicatively, studies concerning the optimal harvesting stage in order to obtain products of high quality in high-density (HD) and super-high-density (SHD) orchards were (Metzidakis *et al.*, 2008) and still are in progress (Benito *et al.*, 2014).

Volatile compounds that develop during extraction become less dominant during oil storage with the emergence of volatile compounds from chemical oxidation. Thus, the presence or absence of particular volatile compounds partially explains quality differences in olive oils. Volatile compounds are not produced in significant amounts during fruit growth, but arise during the climacteric stage of ripening (Kalua *et al.*, 2005). During the climacteric period, fruits produce ethylene, inducing biochemical, physical, and chemical changes and an increase in some protein and enzyme activities. Enzymatic oxidation of olive oils, especially through the lipoxygenase pathway, is considered responsible for the aroma of the oil (Kiritsakis, 1998; Angerosa *et al.*, 2001). One pathway is the enzymatic splitting of linoleic and linolenic acid into C6 and C9 aldehydes and C9 and C12 oxo acids.

The most appropriate stage for harvesting olives destined for oil production is at optimum maturity. At that stage, oil content and oil quality are at their highest level. Too early or too late harvests have a negative effect on both quality and quantity (Kiritsakis & Tsipeli, 1992; Ranalli *et al.*, 2003b). During senescence, the triacylglycerols commence to hydrolyze, and acidity increases. Kiritsakis and Tsipeli (1992) observed that during the time that olive fruit remain on the tree, olive oil triacylglycerols undergo a gradual hydrolysis, and peroxide values as well as the ultraviolet (UV) absorbance are increased.

Figure 7.1 shows the effects of preharvest factors of olive fruit on the acidity of olive oil (Bustan *et al.*, 2014). It is obvious that infested fruit results in increase of olive oil acidity. Delayed harvest increases oil acidity (Kiritsakis, 1998), but this is not true when the fruit size is small (RIRDC, 2008).

The maturity index (MI) was developed to help olive farmers classify the maturity level of their cultivars, under their own specific growing conditions, over a number of years. This is a simple, but not perfect, tool

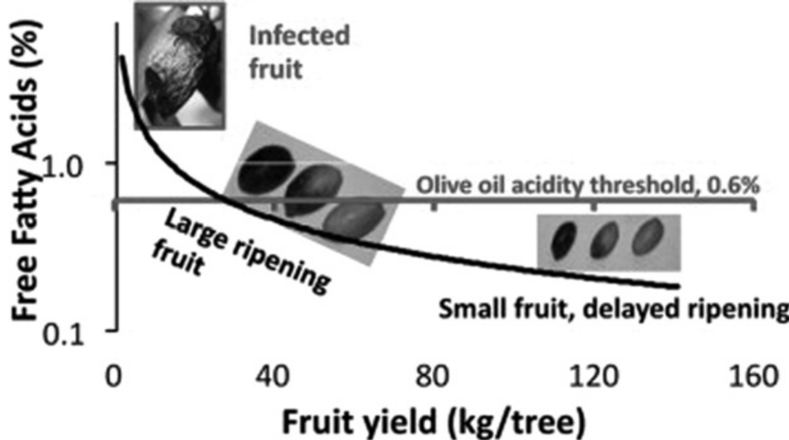

Figure 7.1 Preharvest circumstances leading to elevated oil acidity in Barnea olives. *Source*: RIRDC (2008). Reproduced with permission of Elsevier.

to estimate the proper harvest time for optimum product quality per olive cultivar (Vossen, 2002, 2005). The color chart and the mathematical equation to determine the MI are given below:

0 = Skin color deep green – fruit hard
1 = Skin color yellow-green – fruit starting to soften
2 = Skin with < half the fruit surface turning red, purple, or black
3 = Skin color with > half the surface turning red, purple, or black
4 = Skin color all purple or black with all white or green flesh
5 = Skin color all purple or black with < half the flesh turning purple
6 = Skin color all purple or black with > half the flesh turning purple
7 = Skin color all purple or black with all the flesh purple to the pit

$$MI = \frac{A \times 0 + B \times 1 + C \times 2 + D \times 3 + E \times 4 + F \times 5 + G \times 6 + H \times 7}{100}$$

where the letters A–H = number of fruit in each category.

At harvest, MI usually ranges between 1 and 4.5 (Bodoira *et al.*, 2014). Dag *et al.* (2014) found that in 'Barnea', 'Coratina', and 'Picual', the maximal oil yield was obtained at different MI and suggested the oil yield as a possible index for harvest, indicating the difference between oil content (%) and oil yield (Kg/tree). Cultivar habits, such as rapid oil accumulation or fruit shedding, as well as cultivation under hot climate, should be taken into consideration for early harvest (Dag *et al.*, 2014).

Solinas *et al.* (1987) demonstrated that fruit pigmentation is not always related with the fraction of volatile components in the oil, particularly 2-hexenal, hexanal, heptanol, and some volatile alcohols and unsaturated conjugated compounds.

Traditionally, olives are harvested at the green-yellow or black-purple stage. Since all the fruit do not mature simultaneously even on the same tree, harvesting should take place when the majority of the fruit are at optimum maturity. This is not always possible because other factors may also affect harvest time, such as *availability of farm labor*, *availability of olive oil mills*, *weather conditions*, and so on.

7.3 Harvest techniques

The methods of harvesting olives have remained unchanged for many centuries. New techniques used to facilitate the collection of olives have been introduced only recently.

7.3.1 Harvest after natural fall

In the past, olives were harvested by hand after falling on the ground. This method has been largely replaced in Greece and other countries by plastic nets that are spread under the trees and the fruit are allowed to fall on them naturally. In order to minimize deterioration of the oil, olives should not be left on the nets for more than 15 days (Kiritsakis & Markakis, 1984; Metzidakis *et al.*, 1995; Kiritsakis, 1998). When olives are left on the nets for longer periods, this results in oil of inferior quality. The time between fruit drop and harvest is the most crucial factor in increasing the acidity of the olive oil, especially in fruit infested by the olive fly dacus (*Bactocera oleae*) with insect exit holes (Economopoulos *et al.*, 1982; Kapatos & Fletcher, 1983; Michelakis, 1986).

7.3.2 Harvest from the tree by hand

Olives to be processed as table olives are almost always picked by hand. Hand picking from the tree undoubtedly yields fruit of the best quality for both table olives and olive oil production. The handpicked fruit is relatively free from foreign matter (soil, branches, leaves, etc.) (RIRDC, 2008). At the same time, hand picking results in minimum injury of the tree, but it is very expensive.

7.3.3 Harvest from the tree by beating the branches

Olives are collected by hand beating the branches of the trees with long poles, wooden or plastic, after the olive skin has darkened and the oil content has increased substantially. At times plastic combs are also used to detach the olives. This method sometimes injures fruiting branches and spreads the disease caused by *Bacterium savastanoi* (Penyalver *et al.*, 2000). Great efforts have been made to invent cheap, efficient methods of harvesting olives such as the Greek-made flogging machines. These harvesting machines increase the harvesting rate of each worker by two or three times. Flogging machines have spread very rapidly in the olive-growing areas.

7.3.4 Harvest with shakers

Tree shakers designed for use with deciduous tree fruits have been used for olive harvesting. Since the attachment of olive fruit to trees, as measured by the ratio of removal force to fruit weight (F/W), is very strong (Lamouria & Brewer 1965), abscission chemicals that help the release of the fruit from the tree have been introduced (Hartmann *et al.*, 1970, 1972). However, according to Ferguson *et al.* (2010), no successful abscission compounds able to decrease fruit removal force have been identified.

One of the most important research lines in olive harvesting is to maximize the percentage of fruit removed. The harvesting efficiency in many cases is dictated by the operational parameters of the machines used and the tree adaptation to mechanical harvesting. It has been determined that both moderate and severe pruning, leading to a lower canopy density, improve harvesting with a trunk shaker, when the soil area permits. Metzidakis (1999) and Gerasopoulos *et al.* (1999) provide results from field studies for mechanical harvesting of the Greek cultivars 'Koroneiki' and 'Mastoides' by using chemicals, while Ferguson *et al.* (2010) describe in detail factors affecting mechanical harvest for oil and table olives.

7.4 Olive storage and transportation to the olive oil mill

Ideally, the processing of oil olives should follow harvest without delay. As this is often impractical, they are stored for a period of time before processing. During storage, several chemical and biochemical changes may occur, leading to deterioration of the oil.

The most serious damage is caused by fermentation of the olives. Fermentation comprises the reactions caused by both the enzymes that are endogenous in the olive tissue and those produced by bacteria, yeasts, and molds. As the olive tissue respires, heat is produced that, if not effectively dissipated, will accelerate enzyme actions. Lipolysis, lipid oxidation, and other undesirable reactions occur, causing an increase in acidity and elimination of functional compounds of the oil. To avoid olive deterioration, storage in cool buildings is recommended with the olives piled in layers not exceeding 25 cm in height. It has also been suggested to store olives under water in tanks. Adding mild preservatives such as NaCl (3%), or citric acid (0.03%), or metabisulfite (2%), to the water would result in better storage (Suarez, 1975). Partial drying of the olives with infrared radiation before storage has been tried, although such a treatment appears too costly. Olive fruit preservation in closed tanks containing inert or antiseptic gases, such as nitrogen or ammonia, has given satisfactory results (Suarez, 1975). Olive paste has also been preserved in lined tanks. Research involving storage of olive fruit under controlled atmospheres is carried out at various research institutions (Kiritsakis *et al.*, 1998). According to Gutierrez *et al.* (1992), storage of olive fruit in controlled atmospheres at 5 °C with 3% CO_2 and/or 5% O_2 did not show any clear positive effect on the quality characteristics of the oil. Olive oil from fruit that has been stored at 0 °C or 5 °C for 30 days had acceptable acidity, peroxide value, and absorption coefficients, but showed low resistance to oxidation, which was attributed to low chlorophyll and phenol content, as shown in Figure 7.2 (Kiritsakis *et al.*, 1998).

An effective and relatively inexpensive way of storing olive fruit is to keep the fruit in cool rooms near the olive oil mill. Cooling of the fruit protects the physical, chemical, and sensory characteristics of the oil, even for a period of 60 days (Gutierrez *et al.*, 1992). Vichi *et al.* (2009) evaluated the production of volatile phenols during 12 days of storage of olive fruit from three cultivars ('Arbequina', 'Arbosana', and 'Leccino') in closed plastic bags. A different resistance of each cultivar to the microbiological attack was observed, and

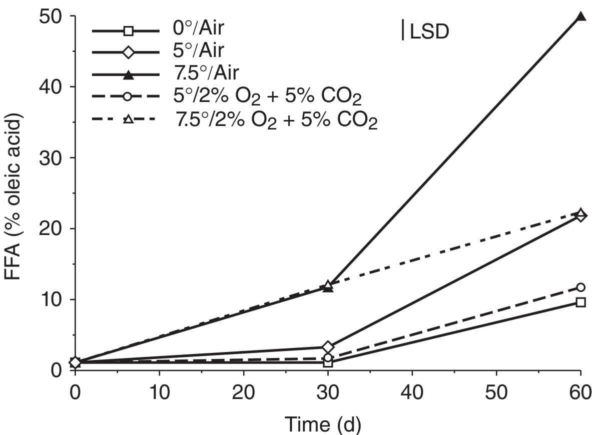

Figure 7.2 Changes in acidity of oil during storage of olives at various conditions. *Source*: Kiritsakis (1998). Reproduced with permission of Springer.

it was reflected by the evolution of guaiacol, 4-ethylphenol, and 4-ethylguaiacol, and related to free acidity values.

Kader *et al.* (1990) recommended ideal preprocessing storage for fresh, green Manzanillo table olives at 5 to 7.5 °C and 95% relative humidity. Under these conditions, the olives can be stored for 6 to 8 weeks in air, or 9 to 12 weeks in a 2% oxygen atmosphere with no significant loss in the quality of the olives. More details on the effect of storage conditions on the quality of olives, and olive oil quality, are reported by Garcia *et al.* (1996) and Agar *et al.* (1998).

According to Sanchez *et al.* (2013), olives were also immersed in several chemical solutions (0.5% ascorbic acid, 0.3% NaOH, 0.05% SO_2, and 1% glycerol) before, and in nitrogen after, the postharvest storage with the aim of reducing oxygen diffusion into the fruit. Only the use of glycerol in the form of a coating layer before applying nitrogen atmosphere showed good results. Moreover, it has been demonstrated that the browning reactions in bruised olives were developed due to the oxidation of oleuropein without the requirement of any previous hydrolysis reactions of this polyphenol. The decrease in oleuropein and increase in darkening of the bruised areas, of olives preserved in nitrogen and re-exposed to air, were also caused by enzymatic reactions.

A common but wrong practice for the transportation of the olive fruit is to place them in sacks made of plastic or other material. The use of plastic sacks should be discouraged, since the conditions created inside them favor microbial growth and spoilage of the fruit. The best way to transport the olives is in open-mesh, plastic or wooden crates that allow air to circulate and prevent heating caused by catabolic activity of the fruit.

Upon arrival at the oil mill, the olive fruit should be classified in terms of cultivar, method of harvesting (picked from the tree or from the ground), and condition (sound or attacked by insects) (IOOC, 1984). Each class of fruit must be processed separately. Good-quality oil, as it has already been said, is obtained when the olives are processed without any delay, right after harvest.

7.5 Processing steps

Olive oil exists in the form of minute drops in the vacuoles of mesocarp cells in the olive fruit (Herrera, 1975). It is also scattered in the colloidal system of the cell's cytoplasm and, to a smaller degree, in the epicarp and endosperm (IOOC, 1984). While it is possible for all of the oil in the vacuoles to be released during processing, it is hard to obtain the dispersed in the cytoplasm oil. This oil generally remains in the olive pomace, as a by-product of olive fruit processing.

The main processing steps in the olive oil mill in order to obtain olive oil include: *feeding*, *washing*, *crushing*, *malaxation* (*mixing*), *separation of the olive oil from olive paste*, and *final centrifugation of the oil in a vertical centrifugal*.

The presence of hydrophilic phenols in olive oil depends on the different endogenous enzymes of the olive fruit and on malaxation conditions during olive fruit processing. The modifications occurring include hydrolysis of glycerides by lipases, hydrolysis of glycosides and oligosaccharides by β-glucosidases, oxidation of phenolic compounds by phenoloxidases, and polymerization of free phenols (Ryan *et al.*, 1999).

7.5.1 Feeding

The fruit is put initially into a big feeding hopper attached to a moving belt. Removal of the leaves follows, although part of the leaves should remain since they contain important functional constituents. Transfer of chlorophyll from the leaves to the oil promotes olive oil deterioration in the presence of light due to photooxidation, but this pigment works as an antioxidant during storage of oil in the dark (Kiritsakis, 1998).

7.5.2 Washing

Washing the fruit eliminates the presence of foreign material. A washing machine is a basic accessory of an olive oil mill.

7.5.3 Crushing (milling)

After washing, the olive fruit is transferred to the crushing unit. Crushing or milling is the first main step in olive fruit processing. The purpose of crushing is to tear the flesh cells and facilitate the release of the oil from the vacuoles. Thus, during crushing, microscopic drops coalesce to form larger drops of oil, which can be separated from the other phases (Solinas, 1992; Kiritsakis, 1998). In the past and even today in some olive oil producing countries, the crushing was (is) done by conical or cylindrical oil stones. In recent years, however, metal olive-crushing equipment has been introduced for the new processing systems (centrifugal). The metal crushers may be hammer, toothed, disc, cylindrical, or roller. They rotate at high speeds. The oil stones have some advantages compared to metal crushers, because they mix the paste properly and amalgamate the oil droplets dispersed in the vegetable water. Oil stones revolve slowly and achieve both crushing and partial malaxation at the same time. The reasons for replacing the old crushing mills (oil stones) with new ones are: large size, low efficiency, and high labor cost. Metal crushers, however, have some disadvantages as well: the paste is not always properly prepared, because crushing is accomplished quickly; the sensory characteristics of the oil may be affected; and the metal parts of the crushers, which rotate at high speeds, suffer from wear and tear.

Ranalli (1988) found that the oil yield was increased with the degree of comminution of the olive kernel (pit). When the olive paste is pounded more finely, higher yields are obtained. The main types of oil stones used for olive crushing are described by Mendoza (1975).

7.5.4 Mixing (malaxation)

After the olive fruit has been crushed, the resulting paste undergoes the mixing stage (malaxation). Malaxation entails stirring the olive mash slowly and constantly for about 30 min. For olive fruit that have reached maturity, 20–25 minutes of malaxation is sufficient. The purpose of this operation is to increase the percentage of "free" oil. It also aids in coalescing small oil drops into larger ones, thereby facilitating separation of the oil and water phases. It also aids in breaking up the oil/water emulsion drops (Suarez, 1975; Clodoveo, 2012). Figure 7.3 (Kiritsakis, 1998) shows oil drops formed during malaxation.

Malaxators differ in shape, size, and arrangement between olive oil plants. They are mostly cylindrical vats with rotating blades and double walls. A rotating helix with several wings mixes the paste. Rotation is usually slow (19–20 rpm) and depends on the kind of paste. For greater efficiency, malaxators have double walls for circulation of warm water. Temperature increase lowers the viscosity of the oil and results in a greater olive oil yield (Mendoza, 1975). However, the temperature of the water should not be high (no more than 27 °C) to prevent destruction of volatile constituents, which results in the reduction of functional value of the oil. To avoid the above effect, malaxators must be equipped with an automatic thermostat.

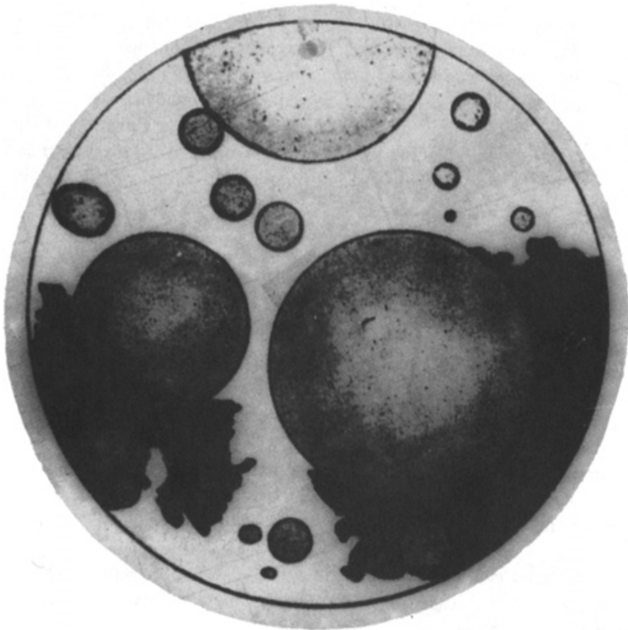

Figure 7.3 Shows oil drops formed during malaxation.

During milling and malaxation, lipoprotein membranes may be formed on the surface of the original drops due to their contact with water. If the malaxation process is slow, the formation of larger oil drops is possible, while at the same time emulsion formation is retarded and separation of the oil phase is easier. Prolonged malaxation favors the formation of emulsions that retard oil separation (Mendoza, 1975).

In the olive oil extraction process, 10–20% of the oil remains inside the unsheltered cells or is left in the colloidal system of the olive paste (microgels), and some is bound in an emulsion with the vegetable water. In the "bound" oil, the droplets of dispersed or emulsified oil are surrounded by a lipoprotein membrane (phospholipids and proteins) that keeps them in that state (Kiritsakis, 1998). This phenomenon is more pronounced in some olive cultivars and in cases when fruit are harvested early or frozen. The paste obtained from such conditions is called "difficult paste" and usually the use of a coadjuvant, which is added at the malaxation step, is needed to break down emulsions, allowing most of the oil to be released.

Spain was the first country to regulate coadjuvant, and natural talc (hydrated magnesium silicate) was the first product allowed to be used for olive oil extraction. Moya *et al.* (2010) found that the use of coadjuvants improves the oil extraction yield. The extraction efficacy, defined as the percentage of initial oil that is extracted, increased by 2% when carbonate was used as coadjuvant. For the same particle size, calcium carbonate was found to extract a greater oil amount than talc. The best results were obtained when using 0.3% calcium carbonate. The coadjuvant list was modified to include the enzyme carbohydrase from *Aspergillus aculeatus*.

Malaxation in recent years has been recognized as one of the most critical points in the mechanical extraction process for virgin olive oil (VOO), and the design of the malaxation equipment has undergone great changes. According to Clodoveo (2012), who summarizes the results of more than 80 articles, malaxation conditions such as time, temperature, and the composition of the atmosphere in contact with the olive paste can influence the activity of the enzymes that are responsible for the healthy and organoleptic properties of the product. In order to achieve the best quality from the olive fruit, the malaxation machine should be hermetically closed during the extraction process to strategically control the oxygen concentration, also employing inert gases. The use of inert gas during malaxation reduces the oxidative degradation of phenolic compounds, extending malaxation time without damaging the produced oil. Moreover, the increasing malaxation time permits an increment in the oil extraction yield, in the case of "difficult" pastes. A low malaxation temperature (<27 °C) and a malaxation time between 30 and 45 min are recommended to obtain

good olive oil quality without compromising the yields. The use of the CO_2 naturally produced by the olive pastes during malaxation could be used as a method of obtaining a natural inertization of the headspace in the malaxation equipment, allowing a large reduction in the costs, derived by the use of inert gases. The addition of lukewarm water to the paste during malaxation can improve oil extractability, but at the same time phenols of a hydrophilic nature decrease as a function of the amount of water added. Reboredo-Rodriguez (2014) tested the influence of olive paste preparation conditions on the standard quality parameters, as well as volatile profiles of extra virgin olive oils (EVOOs) from the 'Morisca' and 'Manzanilla de Sevilla' cultivars, produced in an olive-growing area in north-western Spain. The volatile profile of the oils underwent a substantial change in terms of odorant series when different malaxation parameters were applied. According to Salas and Sanchez (1999), the malaxation temperature generally causes a decrease of levels of volatile compounds from lipoxygenase (LOX) pathways, as a consequence of proved inactivation of hydroperoxide lyases.

Malaxation time, and especially temperature, negatively affect the intensity of sensory attributes, and the content of secoiridoid compounds modifies the composition of metabolites arising from LOX pathways, reducing volatile compounds displaying pleasant sensations and increasing those giving less attractive perceptions; it also elevates the production of 2-methyl butanal and 3-methyl butanal through amino acid conversion. Malaxation conditions affect the flavor of the resulting oil, involving changes of concentration of both polyphenols (Servili *et al.*, 2011) and volatile compounds (Kiritsakis, 1998; Angerosa *et al.* 2001; Stefanoudaki *et al.*, 2011).

According to Angerosa *et al.* (2001), the total amount of the secoiridoid fraction was negatively affected by malaxation time. Servili *et al.* (2011) attributed the reduction of phenolic concentration to the activation of endogenous oxidoreductase enzymes. The greater losses appear when temperature is raised from 25 °C to 35 °C (Servili *et al.*, 2011). The malaxation temperature causes a marked decrease in concentration, in particular of derivatives containing hydroxytyrosol (3,4-dihydroxyphenylethanol). The volatiles obtained by using different malaxation conditions are mainly formed of C6 and C5 compounds, produced from LnA and LA through the LOX pathways.

Rannalli *et al.* (2005) studied the effects of two new enzyme processing aids, Bioliva and Rapidase adex D, as well as the olive paste malaxation temperature factor on the composition of natural colorings (chlorophylls, xanthophylls, and carotenes) and the chromatic parameters (chroma, brightness, and hue) in VOOs. The two enzyme complexes affected the values of green and yellow pigments and color index, as well as the profile of other functional fractions. This resulted in an enhanced release of lipochromes and other bioactive non-glyceridic compounds and in a greater accumulation of them into the oil phase. Increasing malaxation temperatures in the range of 20–30 °C resulted in an increase of the concentrations of lipochromes and other functional unsaponifiable components (excluding volatiles) in the oils (Ranalli *et al.*, 2005). Generally, the temperature in the malaxation step is mainly responsible for: (a) the sensory characteristics of oils; (b) considerable losses of secoiridoid compounds; (c) the marked decrease of concentration of C6 esters, which are very important contributors of delicate green perceptions, and of cis-3-hexen-1-ol, which gives pleasant real green sensations; (d) the increase of hexan-1-ol and trans-2-hexen-1-ol, which are considered elicitors of less attractive perceptions (Angerosa *et al.*, 2001); and (e) the production of very high contents of 2-methyl butanal and 3-methyl butanal through the activation of the amino acid conversion pathway. The time of paste malaxation ranging between 30 and 45 min, according to rheology of the olive pastes, seems to give satisfactory results regarding the quality and the quantity of the oil obtained. Compounds related to attractive perceptions, such as esters, are still present at high levels, and concentrations of those giving unpleasant sensations such as trans-2-hexen-1-ol and hexan-1-ol are rather low. In addition, the amount of secoiridoid compounds is great enough to assure a suitable shelf life of the product, and the content of branched aldehydes is in a range typical of olive oils of high quality (Angerosa *et al.*, 2001).

Malaxation has been recognized as one of the most critical points in the mechanical extraction process for VOO. As a matter of fact, the malaxation step is the only discontinuous phase in a continuous extraction process. In the near future, the essential challenge of the VOO industrial plant manufacturing sector is to design and build advanced machines in order to transform the discontinuous malaxation step in a continuous phase and improve the working capacity of the industrial plants. In order to reduce the malaxation time and to improve the quality of the oil, two ultrasound treatments were tested in contrast with the traditional method (Clodoveo *et al.*, 2013). The sonication treatment was applied to olives submerged in a water bath (before the

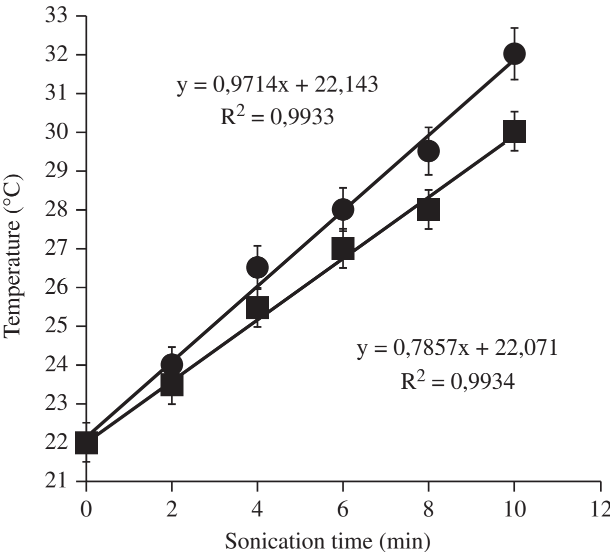

Figure 7.4 Positive correlation between the olive paste temperature and the duration of sonication treatment applied to the whole (d) and crushed olives (j). Error bars represent standard deviations from mean values of three experiments.

crushing) and to olive paste (after the crushing). Better extractibility and higher minor compounds contents were obtained by sonicating the olives submerged in a water bath than the olive paste.

Figure 7.4 shows a positive correlation between the olive paste temperature and the sonication time when the treatment is applied to the whole and to crushed olives for 2, 4, 6, 8, and 10 min, respectively. In either case, the temperature of olive paste linearly increased when ultrasound treatment time was extended. Thus, further research is needed for temperature control.

A processing line equipped with the prototype of the innovative malaxation machine is shown in Figure 7.5. The malaxator is equipped with a set of sensors useful in measuring the processing conditions (oxygen, temperature, and viscosity). All the sensors are connected to a personal computer to acquire the data (Clodoveo *et al.*, 2013).

The VOO industry can take advantage of ultrasound systems in the extraction process. As a matter of fact, ultrasounds reduce the duration of malaxation, enhancing the quality of the product. Moreover, the ultrasound technology provides a quicker heating of olive paste, improvements in the extractability process, and a high minor compounds content in the resultant oils.

Reboredo-Rodriguez *et al.* (2014) observed that the volatile profile of olive oil undergoes a substantial change in terms of odorant series when different malaxation parameters are applied and differ from cultivar to cultivar. In one of the two tested cultivars, C6 aldehydes decreased as the time of malaxation of olive paste increased and were statistically significant ($p < 0.05$). In another cultivar, the trend differed completely, a fact attributed to the behavior of enzymes, which differs from cultivar to cultivar.

Taticchi *et al.* (2013) studied the effect of the malaxation temperature on the composition of the phenolic compounds in VOOs produced from olives processed at two atmospheric conditions. In both cases, the results show a positive relationship between temperature and the concentration of the derivatives of the secoiridoid aglycones. The effect of the temperature on the oxidoreductases (polyphenoloxidase and peroxidase) was investigated; and it was observed that while olive peroxidase (POD) showed the highest activity at 37 °C, polyphenoloxidase (PPO) exhibited the optimum activity at approximately 50 °C. There was a large variation according to the olive cultivar tested and to the temperatures applied during the malaxation process. We may have an increase in the phenol concentration in VOOs obtained following higher temperatures of malaxation. However, we may take into consideration the opinion that a malaxation temperature higher than 27 °C reduces the VOO quality (European Union [EU], 2002). Evolution of the volatile compounds

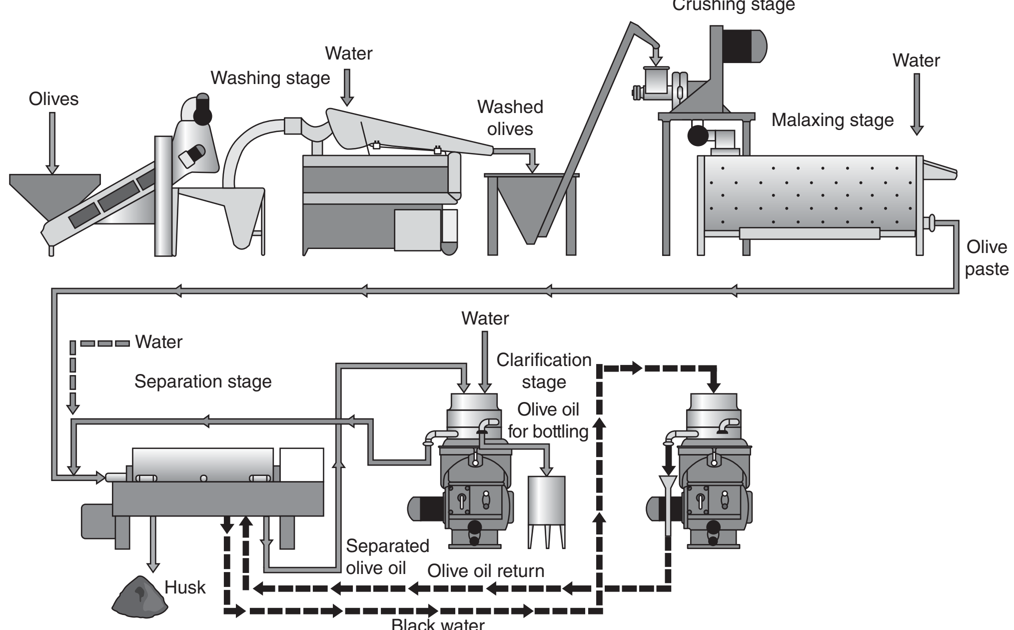

Figure 7.5 Scheme of the olive oil processing line equipped with the prototype of the innovative malaxation machine. The malaxer is equipped with a set of sensors useful to measure the oxygen concentration in the headspace of the tank (A) and inside the olive paste (B), the temperature of olive paste (C), the viscosity of the olive paste (D), the increase of CO_2 in the headspace of the malaxer (E) and also the water temperature in the heating jacket (F). All the sensors are connected to a personal computer (PC) to acquire the data. *Source*: Clodoveo (2012). Reproduced with permission of Elsevier.

produced by the lipoxygenase pathway must also be considered as a quality marker having the same importance as the phenolic concentration in VOO. Thus, temperature not higher than 27 °C is the right choice (Kiritsakis, 1998).

Generally, the phenolic compounds of VOO, once released or formed during processing of olives, are distributed between the water (approximately 53% of the available pool of antioxidants in the olive fruit) and oil phases (1–2%). Another part of the phenolics (approximately 45%) is trapped in the solid phase (pomace) (Rodis *et al.*, 2002). The distribution of the released amount of the phenolic compounds between water and oil is dependent on their solubility in each of these two phases. Consequently, only a fraction of the phenolics enters the oil phase.

7.5.5 Separation of olive oil from the olive paste

The main constituents of olive paste are: *olive oil, small pieces of kernel (pit), water*, and *cellular debris of the crushed olives. Pressure, centrifugation, or a selective filtration process* may be applied for the separation of oil from the other constituents.

7.6 Pressure process

In this process, pressure is applied to separate the oil from the olive paste. It is the oldest and the most widespread method for processing olive fruit in order to obtain olive oil. It has been used since the beginning of olive tree cultivation. However, the pressure process has changed drastically over the years. In very old

oil mills, the oil separation was achieved with applying pressure by either human or animal force. The invention of the hydraulic press was a revolution for the operation of old olive oil mills. Hydraulic presses are still used in improved traditional olive oil plants. In the pressing system, the olive paste obtained after crushing and malaxation is placed in oil diaphragms. Olive paste of 2–3 cm thickness is placed uniformly on each diaphragm. The oil diaphragms are put in a moving unit with a pierced cylinder-guide (trolley fitted with a central shaft) to provide even arrangement of the diaphragms and support them in order to avoid any movement when the system is subjected to hydraulic pressure. A metal tray and a diaphragm, not containing olive paste, are placed after every 3 or 4 full diaphragms to obtain uniform application and a more stable load. The moving unit, with its load, is placed under a hydraulic pressure unit. When applying pressure, the liquid phases (oil and water) run out from the olive paste. Thus, the diaphragms are working as filtering means. The kinetics of pressure, as a filtering process, is based on Carman's equation. This is expressed by Di Giovacchino (1994) as follows:

$$\frac{dV}{dt} = \frac{PA}{\eta\beta}\left(1 - \frac{\alpha\omega}{\beta \mathrm{A}}V\right),$$

where V = volume of liquid separated, t = time, P = pressure applied, A = filtering area (of diaphragms), η = viscosity of the liquid, β = resistance of the solid matter deposited in the oil diaphragms, α = resistance of the filtering material, and ω = quantity of solid matter deposited per unit of liquid filtered.

The pressure applied depends on the kind of the paste. A better quality of oil is obtained by applying low pressure (100–150 kg/cm^2). Petruccioli (1975) and Moreno (1975) reported that several factors affect the pressure process. Di Giovacchino (1994) and Kiritsakis (1991, 1998) summarized the advantages and the disadvantages of the pressure process.

7.7 Centrifugation process

Centrifugation compared to the pressure process is a relatively new process used for the separation of oil from the olive paste. It is based on the differences in density of the olive paste constituents (olive oil, water, and insoluble solids). The speed at which two immiscible liquids are dissociated, when they are subjected to centrifugal force, is governed by Stoke's law (Di Giovacchino, 1994). It is expressed in its modified version as follows:

$$V = \frac{D^2}{18} \times \frac{(d_2 - d_1)\omega^2 r}{\eta},$$

where V = speed of separation, D = diameter of the drops of liquid with the higher density, d_2 = density of the heavier liquid, d_1 = density of the lighter liquid, ω = angular speed, r = distance from the rotating arms, and η = viscosity of the liquid with the lower density.

The separation of the oil is accomplished through a horizontal centrifugal (decanter). The efficiency of a centrifugal separator (decanter) is determined in terms of the relationship between the centrifugal acceleration (ω^2r) and the gravitation acceleration (g). Centrifugal action can reach values as high as 2500–3000 G, with $G = \frac{\omega^2 r}{g}$.

After crushing and malaxation, the olive oil in the olive paste is either completely free or in the form of small droplets inside microgels or emulsified in the aqueous phase. The separation of the oil by centrifugation is much easier when most of the oil is free (Mendoza, 1975).

Horizontal centrifuges (decanters) consist of a cylindrical-conical bowl. Inside them is a hollow, similarly shaped component with helical blades. A slight difference between the speed at which the bowl rotates and that at which the inner screw gyrates results in the movement of the olive pomace, wastewater, and oily must (oil with some water).

The optimum amount of water needed to dilute the paste is chiefly determined by the characteristics of the olive paste and the centrifuge. The temperature of the added water must be low to avoid overheating of the olive paste.

Rheological characteristics of the olive paste, which change in relation to water content, fruit cultivar, maturity level, and seasonal temperature variations, greatly affect the efficiency of centrifugal extraction. Altieri *et al.* (2013) tested a suitable flow mass sensor in order to set up an automatic system to control the olive paste mass flow rate that is fed to a decanter centrifuge during olive oil extraction.

7.8 Selective filtration (Sinolea) process

Besides pressure and centrifugation, selective filtration (Sinolea) combined with centrifugation is used for the separation of olive oil from the olive paste. Selective filtration is based on the different interfacial tensions of oil and water coming in contact with a steel plate. The steel plate is coated with oil when plunged into olive paste since the interfacial tension of the oil is less than that of the water.

The most common selective filtration system is the Sinolea unit. It is made up of one or more units that can handle about 350–370 kg of olive paste, each of which has a stainless-steel grating unit at the bottom. Five to seven thousand moving sheets are slotted through the slits in the grating unit and slowly penetrate the paste in reciprocating motion. The best rotation speed for the mechanical arm that stirs the paste is 7.5 rpm. When the steel plate is moved into the paste, it will preferably be coated with oil that then drips off, creating a flow of oily must consisting almost solely of oil. When this process is repeated several times, most of the oil is recovered from the paste.

The oil obtained by the selective filtration process is called "Sinolea oil," and it can be considered as "prime" oil. It retains the natural composition and sensory characteristics of the fruit, and it has a high functional value. To obtain the remaining oil, the olive paste is further moistened, undergoes new malaxation, and is subjected to centrifugation. The oil obtained by this process is called "decanter oil." The combination of the two processes (selective filtration and centrifugation) results in a high yield. Combined selective filtration and centrifugation is a good processing system, producing oil of good quality, but unfortunately it has not been accepted widely. Table 7.1 gives the advantages and disadvantages of the press, selective filtration, and centrifugation processes.

7.8.1 Final centrifugation of olive oil

A final centrifugation of the oil is needed regardless of the process applied, namely, pressure, centrifugation, or selective filtration (Sinolea). Thus, oil containing some water passes through a vertical centrifugal that revolves at 6000 to 7000 rpm for the final separation and clarification of the oil. The centrifugal consists of a stable and a mobile part, which turns very quickly. A large number of cone-shaped disks are attached to

Table 7.1 Advantages and disadvantages of the press, selective filtration and centrifugation process.

System	Advantages	Disadvantages
Press	Less energy consumption, Less moisture in the pomace, Less construction cost	More labor required, Difficult to maintain clean in the olive diaphragm, More exposition of olive paste and oil to atmospheric oxygen
3-phase Decanter	Relatively drier pomace, easier extraction process	More water consumption, High energy cost to heat the extra water, Lots of waste water, Losses of polyphenols
2-phase Decanter	Saves water and heat sice less water is added to the olive paste, Oil has higher polyphenol content, continuous flow	Produces pomace with high moisture, More difficult extraction
Selective Filtration (Sinolea)	In conjuction with decanter or press system more oil is extracted, Produces oil of unique sinolea quality	Difficult to clean the Sinolea unit, More overall cost, Greater maintenace

Source: Kiritsakis (1998). Reproduced with permission of Springer.

the centrifugal unit. The liquid phase is distributed on the total surface in thin layers, and the centrifugation in the vertical centrifuge results in a final separation of the oil from the water and other substances.

7.9 Processing systems

Generally, the processing systems used today can be divided into those that result in olive pomace of low moisture content (25–30%) and those that produce olive pomace with high moisture content (more than 45%). The pressure (traditional) olive oil mills are of the first type, while the second type includes those based on centrifugation (two-phase and three-phase centrifugal olive oil mills), or based on selective filtration (Sinolea) and centrifugation (combination-type olive oil mills) (Kiritsakis *et al.*, 1985).

Regardless of the processing system, process automation is needed to obtain a high-quality product and optimal process yields at low costs (Altieri *et al.*, 2015). The use of sensors and detectors to continuously measure important chemical properties could have significant technical benefits. Near-infrared (NIR) transmittance spectroscopy could be applied to on-line quality control and characterization of VOOs. Regarding the VOO aroma, electronic noses could be used for analyzing olive pastes (Clodoveo, 2012). On-line monitoring of the evolution of volatile compounds (VCs) during VOO processing could be very useful for defining the operative conditions of malaxation (i.e., time, temperature, and atmosphere) in order to improve the VOO sensory quality according to product type (i.e., cultivar, ripening stage, sanitary aspects, etc.). Electronic tongues are also promising techniques in olive oil extraction process control (Apetrei *et al.*, 2010).

7.9.1 Centrifugal-type olive oil mills: three-phase and two-phase decanters

The problem created by the wastewater of three-phase centrifugal-type olive oil mills, where water is added to the decanter to facilitate the separation of olive oil from the olive paste, consists of the difficulties in recycling it for use in agriculture, together with the demand for better oil quality. These were the cause for the modification of the three-phase decanter (Kiritsakis *et al.*, 1985). New centrifuges (decanters) that separate the oily paste into two phases (oil and oily pomace), without the addition of water, have been developed (Ranalli & Angerosa, 1996). Figure 7.6 shows the structure of a two-phase decanter and a three-phase decanter. In the two-phase decanter, we have two exits (for olive oil and solids); and in the three-phase one, we have three exits, including the exit for wastewater.

The two-phase decanters are called ecological for commercial purposes. They appear to possess many advantages, with the most outstanding being the elimination of the pollutant effluent fraction (Di Giovacchino *et al.*, 1994) and the higher phenol content in the olive oil (Kiritsakis, 1998). Such an oil has

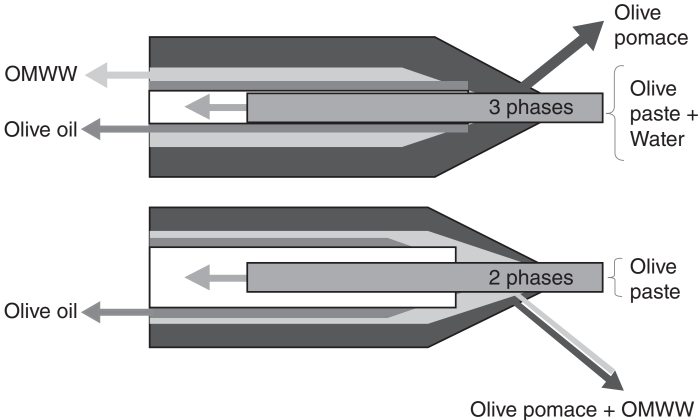

Figure 7.6 Two - phase and three - phase decanter structures.

great functional and nutraceutical value. However, olive pomace produced by the two-phase decanter has higher moisture content than that obtained by conventional centrifuges (three-phase decanters).

Kalogeropoulos *et al.* (2014) reported that the significantly higher phenolic content in oil produced by a two- versus three-phase centrifuge was attributed to higher hydroxytyrosol, as well as to higher tyrosol, vanillin, and homovanillic alcohol levels. Evaluation of the effect of the centrifuge system on oils' functional properties showed a clear superiority of two-phase decanter oil. It exerted higher ferric reducing capacity, elongation of serum lipid lag time, and decrease in cytokine response of stimulated human mononuclear cells, compared to the oil obtained from the three-phase decanter (Kalogeropoulos *et al.*, 2014).

Generally, the type of olive oil mill and the conditions applied may significantly affect the quality and functional characteristics of the oil.

7.10 Olive fruit processing by-products and their significance

Olive pomace, wastewater, and olive leaves are the main by-products of the oil fruit processing containing very important constituents that can be isolated by applying different procedures (Codounis *et al.*, 1983; Vigo *et al.*, 1983; Petruccioli *et al.*, 1988; Guzman *et al.*, 2002; Vlyssides *et al.*, 2004; Roig *et al.*, 2006; Guillermo *et al.* 2009; Kiritsakis *et al.*, 2010; Rincón *et al.*, 2013; Casanovas *et al.*, 2015; Gonzalez and Cuadros, 2015; Goula and Lazarides, 2015; Proietti *et al.*, 2015). Generally, olive oil by-products are considered to be good sources of phenolic compounds (Servili *et al.*, 2011). Olive leaves are considered as a by-product with significant functional value.

Details for olive leaves and wastewater by-products are given in specific chapters of this book. Olive pomace and the production of olive pomace oil are described in this section.

Olive pomace is the pulpy material remaining after removing most of the oil from the olive paste. The commercial value of the olive pomace depends mainly on its oil and water content, which in turn depends on the process applied and on the operating conditions.

Olive pomace contains fragments of skin, pulp, pieces of kernels, and some oil. The main constituents are cellulose, proteins, and water (Carola, 1975; Ranalli & Angerosa, 1996; Kiritsakis, 2007). Olive pomace also contains some polyphenols, which give a significant functional value to the product. A significant amount of phenolic acids is retained in the olive pomace, such as gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, and ferulic acid.

Phenolic constituents can be extracted from olive pomace using acetone or ethanol (Sheabar & Neeman, 1988; Hurtado-Fernandez *et al.*, 2010). Phenolic constituents present in olive pomace could be utilized as antioxidants for other oils or fats, increasing their functional and nutritional value at the same time.

An innovative approach to alternate uses of olive pomace is suggested by Dashti *et al.* (2015), who explain how the bacteria present in the pomace can be used for hydrocarbon biodegradation, nitrogen fixation, and mercury resistance.

The oil present in the olive pomace undergoes rapid deterioration due to the moisture content, the presence of enzyme lipase, and the development of microorganisms (*Gliomastix chartarum*, *Cephalosporium* sp., Aspergillus *glaucus*, etc.), which favors triacylglycerol hydrolysis. The acidity of olive pomace oil may increase from 5% to 60% in a short time (Carola, 1975). Besides the increase in the acidity, oxidation products (aldehydes and ketones) are also formed during storage of the olive pomace. These products significantly affect the quality of olive pomace oil and eliminate the functional value. In order to minimize the deterioration of the olive pomace oil, olive pomace should be processed as soon as possible.

Olive pomace is further processed to extract olive pomace oil and other important constituents. Paini *et al.* (2015) proposed the isolation of phenolic compounds from olive pomace using spray drying. Polyphenols from olive pomace were extracted with a high-pressure, high-temperature agitated reactor and for the first time encapsulated by spray drying, using maltodextrin as coating agent at different concentrations. In addition, effects of inlet temperature (130 °C and 160 °C) and feed flow (5 mL/min and 10 mL/min) were studied. Physicochemical, antioxidant properties and stability of microparticles were evaluated. High inlet temperature implied lower moisture and bulk density without affecting antioxidant properties. Increasing maltodextrin concentration caused lower bulk density and higher microparticles sizes, while higher feed flow led to increased moisture content. Spray drying at inlet temperature of 130 °C, maltodextrin

concentration of 100 g/L, and feed flow of 10 mL/min resulted in high microencapsulation yield (94%) and encapsulation efficiency (76%) with high polyphenols content (39.5 mg CAE/g DP) and antiradical power (33.8 mmol DPPH/L extract). High-performance liquid chromatography and thermogravimetric analysis revealed the thermal protection effect of maltodextrin for phenolic compounds. Microcapsules were stable at 5 °C in dark condition for 70 days, and only 21% were degraded when increasing storage temperature up to 25 °C. UV light exposure resulted in a 66% loss in polyphenols after 48 h of exposure.

Small kernel particles can be used for the production of plastics and other materials. Kernel wood can also be used after the removal of the stones as livestock feed. It is converted into small granules to facilitate transportation and use. The ash of kernel wood is a good fertilizer due to its P, K, and Ca content (Frezzotti & Manni, 1956). Karapinara and Worgan (1983) studied the growth of Aspergillus oryzae*,* Aspergillus niger*, and* Sporotrichum sp. in olive pomace after extraction of oil and their effect on protein formation. Sporotrichum sp. increased the protein content of the culture by 76% and reduced the cellulose content by 52%.

Olive pomace oil can be consumed only after refining. This includes neutralization, deodorization, and decolorization. The products obtained from refining pomace oil are: *refined olive pomace oil, FFA, olive soap paste, and exhausted olive pomace.*

Refined olive pomace oil is an acceptable edible oil. Its fatty acid composition does not differ from that of refined olive oil. High-acidity olive pomace oils were mostly used for the production of household soaps, before detergents appeared on the market.

7.11 The effect of enzymes in olive fruit processing and oil composition

Efforts have been made to increase the yield of oil from olives by adding enzymes during the olive fruit processing (Montedoro & Petruccioli 1974; Petruccioli *et al.*, 1988; Najafian *et al.*, 2009). Enzymes as biocatalysts facilitate the breakdown of cellulose and pectin, thus helping to release the droplets of oil by reducing the stability and resistance of the cell walls. Enzymes may be added either during the crushing-milling step or during the malaxation of the olive paste. Addition of enzymes during crushing gives better results, but it is more common to add the enzymes during the malaxation step (Montedoro & Petruccioli, 1974). Montedoro and Petruccioli (1974) and Petruccioli *et al.* (1988) reported that the enzymes pectin depolymerase, papain, cellulase, hemicellulose, and acid protease increased oil yield and decreased processing time. Servili *et al.* (2011) studied the effect of endo-polygalacturonase obtained from the treatment of vegetable water and observed an increase in oil yield and an improvement in oil quality (more aroma and better resistance to oxidation). The enzyme polygalacturonase (yeast pectinase) increased the oil yield up to 8% and improved oil quality. Olive oil obtained using this particular enzyme had lower turbidity, a lower degree of oxidation, and more aroma compounds than oil obtained without using this enzyme. The enzyme Olivex (the trademark of a Novo Nordisk ferment product) is recommended for increasing oil yield. Olivex is a natural enzyme preparation produced by the fungus *Aspergillus aculeatus.* When Olivex is added to the olive paste, it breaks down the cell walls, liberating additional amounts of oil. Oil obtained by the use of enzymes shows an increase in polyphenol and tocopherol content, and therefore better oxidative stability and higher functional value.

Enzyme preparations were shown to increase oil extraction yields in the range of 10.2–13.5 kg oil per tonne of olives, regardless of the olive cultivar processed (Ranalli *et al.*, 2003a). Other reports showed that the enzyme treatments resulted in higher overall oil yields with an increase ranging from 0.9% to 2.4% on a wet basis, compared to untreated pastes (Najafian *et al.*, 2009).

7.12 Effect of processing systems on olive oil quality and functional properties

Ben-Hassine *et al.* (2013) studied the effect of processing systems on olive oil quality and found that it affects acidity, PV, and sensorial characteristics (mainly the musty attribute).

The processing system affects the values of some chemical compounds of olive oil, such as tetracosanol and hexacosanol, Δ-5-avenasterol, and others (Lanzon *et al.*, 1986; Aparicio *et al.*, 1991; Nergiz & Unal, 1991; Capriotti *et al.*, 2014). Nergiz and Unal (1991) also reported that oil obtained by pressure contained higher amounts of polyphenols than that obtained by centrifugation and therefore had a higher functional value.

The processing system and the conditions applied may destroy the chlorophylls and carotenoids of the oil and reduce its functional activity (Kiritsakis, 1991). Vekiari *et al.* (2007) observed that oil samples extracted using the centrifugal-type machines and kept in glass containers in the dark had higher peroxide values than those extracted by the classic method. Di Giovacchino *et al.* (1994), however, noted that olive oils obtained from good-quality olive fruit did not differ in their characteristics regardless of the processing system used. The values of these parameters mainly depend on the quality of olives and on the enzyme action on the olives before processing (Di Giovacchino, 1994). A strong linear correlation between the polyphenol content and the induction period of the oil has been observed. The induction time is significantly lower in olive oils obtained by centrifugation, because it is well correlated with total polyphenol content. The water used in centrifugal olive oil mills removes the polyphenol hydroxytyrosol to a great extent. Olive oil obtained by the two-phase decanter process contains more phenolic compounds than oil from the three-phase process (Ranalli & Angerosa, 1996). Olive oil from two-phase decanting contained higher amounts of polyphenols, ortho-diphenols, hydroxytyrosol, tocopherols, trans-2-hexenal (the most important aroma component, with a grassy aroma), and total aroma volatile compounds than oil obtained by the three-phase decanter. This indicates higher functional and nutritional value of the oil.

7.13 Conclusion

Harvest and processing techniques, as well as conditions applied, affect significantly the olive oil quality and the presence of phenols and other functional compounds in the final product.

Efforts have been made to control the production of olive oil from olive fruit and especially temperature and time during olive paste malaxation, leading to a product with high phenol content, aromatic compounds, and furthermore increased functional value and characteristics.

References

Agar, I.T., Hess-Pierce, B., Sourour, M.M., & Kader, AA. (1998) Quality of fruit and oil of black-ripe olives is influenced by cultivar and storage period. Journal of Agricultural and Food Chemistry 46, 3415–3421.

Altieri, G., Di Renzo, G.C., & Genovese, F. (2013) Horizontal centrifuge with screw conveyor (decanter): optimization of oil/water levels and differential speed during olive oil extraction. Journal of Food Engineering 119, 561–572.

Altieri, G., Genovese, F., Tauriello, A., & Di Renzo, G.C. (2015) Innovative plant for the separation of high quality virgin olive oil (VOO) at industrial scale. Journal of Food Engineering 166, 325–334.

Anastasopoulos, E., Kalogeropoulos, N., Kaliora, A.C., Kountouri, A., & Andrikopoulos, N.K. (2010) The influence of ripening and crop year on quality indices, polyphenols, terpenic acids, squalene, fatty acid profile, and sterols in virgin olive oil (Koroneiki cv.) produced by organic versus non-organic cultivation method. International Journal of Food Science & Technology 46, 170–178.

Angerosa, F., Mostallino, R., Basti, C., & Vito, R. (2001) Influence of malaxation temperature and time on the quality of virgin olive oils. Food Chemistry 72, 19–28.

Aparicio, R., Sanchez Navarro, M., & Ferreiro, M.S. (1991) Definite influence of the extraction methods on the chemical composition of virgin olive oil. Grasas y Aceites 42, 356–362.

Apetrei, C., Apetrei, I.M., Villanueva, S., de Saja, J.A., Gutierrez- Rosales, F., & Rodriguez-Mendez, M.L. (2010) Combination of an e-nose, an e-tongue and an e-eye for the characterisation of olive oils with different degree of bitterness. Analytica Chimica Acta 663(1), 91–97. Erratum in: Analytica Chimica Acta 673, 212–218.

Ben-Hassine, K., Taamalli, A., Ferchichi, S., Mlaouah, A., Benincasa, C., Romano, E., Flamini, G., Lazzez, A., Grati-kamoun, N., Perri, E., Malouche, D., & Hammami, M. (2013) Physicochemical and sensory characteristics of virgin olive oils in relation to cultivar, extraction system and storage conditions. Food Research International 54, 1915–1925.

Benito, M., Lasa, J.M., Gracia, P., Oria, R., Abenoza, M., Varona, L., & Sánchez-Gimeno, A.C. (2014) Olive oil quality and ripening in super-high-density Arbequina orchard. Journal of the Science of Food and Agriculture 93, 2207–2220.

Blekas, G., Vassilakis, C., Harizanis, C., Tsimidou, M., & Boskou, D.G. (2002) Biophenols in table olives. Journal of Agricultural and Food Chemistry 50, 3688–3692.

Bodoira, R., Torres M., Pierantozzi, P., Taticchi, A., Servili, M., & Maestri, D. (2014) Oil biogenesis and antioxidant compounds from 'Arauco' olive (*Olea europaea* L.) cultivar during fruit development and ripening. European Journal of Lipid Science and Technology 117, 377–388.

Capriotti, A.L., Cavaliere, C., Crescenzi, C., Foglia, P., Nescatelli, R., Samperi, R., & Laganà, A. (2014) Comparison of extraction methods for the identification and quantification of polyphenols in virgin olive oil by ultra-HPLC-QToF mass spectrometry. Food Chemistry 158, 392–400.

Carola, C. (1975) By products. In: Olive oil technology, ed. J.M. Moreno Martinez. FAO, Rome, 77–87.

Casanovas, A., Galvis, A., & Llorca, J. (2015) Catalytic steam reforming of olive mill wastewater for hydrogen production. International Journal of Hydrogen Energy 40, 7539–7545.

Charoenprasert, S., & Mitchell A. (2012) Factors influencing phenolic compounds in table olives. Journal of Agricultural and Food Chemistry 60, 7081–7095.

Cimato, A. (1990) La qualité de l'huile d'olive et les facteurs agronomiques. Olivae 31, 20–31.

Clodoveo, M.L. (2012) Malaxation: influence on virgin olive oil quality. Past, present and future: an overview. Trends in Food Science & Technology 25, 13–23.

Clodoveo, M.L., Durante, V., & La Notte, D. (2013) Working towards the development of innovative ultrasound equipment for the extraction of virgin olive oil. Ultrasonics Sonochemistry 20, 1261–1270.

Codounis, M., Katsaboxakis, K., & Papanicolaou, D. (1983) Progress in the extraction and purification of anthocyanin pigments from the effluents of olive oil extracting plants. European Federation of Chemical Engineering: Food Working Party; Food Eng. Symp., Athens, Greece, 567–572.

Dag, A., Bustan, A., Avni, A., Tzipori, I., Lavee, S., & Riov, J. (2010) Timing of fruit removal affects concurrent vegetative growth and subsequent return bloom and yield in olive (*Olea europaea* L.). Scientia Horticulturae 123, 469–472.

Dag, A., Harlev, G., Lavee, S., Tzipori, I., & Kerem, Z. (2014) Optimizing olive harvest time under hot climatic conditions of Jordan valley, Israel. European Journal of Lipid Science and Technology 116, 169–176.

Damak, N., Bouaziz, M., Ayadi, M., Sayadi, S., & Damak, M. (2008) Effect of the maturation process on the phenolic fractions, fatty acids, and antioxidant activity of the Chétoui olive fruit cultivar. Journal of Agricultural and Food Chemistry 56, 1560–1566.

Dashti, N., Nedaa, A., Khanafer, M., Al-Awadhi, H., Sorkhoh, N., & Radwan, S. (2015) Olive-pomace harbors bacteria with the potential for hydrocarbon-biodegradation, nitrogen-fixation and mercury-resistance: promising material for waste-oil-bioremediation. Journal of Environmental Management 155, 15, 49–57.

Di Giovacchino, L., Solinas, M., & Miccoli, M. (1994) Effect of extraction systems on the quality of virgin olive oil. Journal of the American Oil Chemists' Society 71, 1189–1194.

Economopoulos, A.P, Haniotakis, G.E., Michelakis, S., Tsiropoulos, G.J., Zervas, G.A., Tsitsipis, J.A., Manoukas, A.G., Kiritsakis, A., & Kinigakis, P. (1982) Zeitschrift für Angewandte Entomologie 93, 463–476.

Esti, M., Messia, M.C., Bertocchi, P., Sinesio, F., Moneta, E., Nicotra, A., Fantechid, P., & Palleschi, P. (1998) Chemical compounds and sensory assessment of kiwifruit (*Actinidia chinensis* (*Planch.*) var. *chinensis*): electrochemical and multivariate analyses. Food Chemistry 61, 293–300.

European Union (EU). (2002) Regulation No. 1019/2002 of 13 June 2002 on marketing standards for olive oil. Official Journal of the European Union L155, 27–31.

Farshid, P., Zeinanloo, A.A., Ebrahimie, E., Tahmasebi-Enferadi, S., & Hosseini-Mazinani, M. (2014) Differential expression of fatty acid desaturases in Mari and Shengeh olive cultivars during fruit development and ripening. European Journal of Lipid Science and Technology 117, 523–531.

Ferguson, L., Rosa, U.A., Castro-Garcia, S., Lee, S.M., Guinard, J.X., Burns, J., Krueger, W.H., O'Connell, N.V., & Glozer, K. (2010) Mechanical harvesting of California table and oil olives. Advances in Horticultural Science 24, 53–63.

Fleming, H.P., & Etchells, J.C. (1967) Occurrence of an inhibitor of lactic acid bacteria in green olives. Applied Microbiology 15, 1178–1184.

Frezzotti, G., & Manni, M. (1956) Olive oil processing in rural mills. Agricultural development paper no. 58. FAO, Rome.

García, J.M., Gutiérrez, F., Castellano, J.M., Perdiguero S., Morilla, A., & Albi, M.A. (1996) Influence of storage temperature on fruit ripening and olive oil quality. Journal of Agricultural and Food Chemistry 44, 264–267.

Gerasopoulos, D., Metzidakis, I., & Naoufel, E. (1999) Ethephon sprays affect harvest parameters of 'Mastoides' olives. Acta Horticulturae 474, 223–225.

Gharbi, I., Issaoui, M., Mehri, S., Cheraief, I., Sifi, S., & Hammami, M. (2015) Agronomic and technological factors affecting Tunisian olive oil quality. Agricultural Sciences 6, 513–526.

Gimeno, E., Castellote, A.I., Lamuela-Raventos, R.M., De la Torre, M.C., & Lopez-Sabater, M.C. (2002) The effects of harvest and extraction methods on the antioxidant content (phenolics, a-tocopherol, and b-carotene) in virgin olive oil. Food Chemistry 78, 207–211.

González-González, A., & Cuadros, F. (2015) Effect of aerobic pretreatment on anaerobic digestion of olive mill wastewater (OMWW): an ecoefficient treatment. Food and Bioproducts Processing 95, 339–345.

Goula, A.M., & Lazarides, H.N. (2015) Integrated processes can turn industrial food waste into valuable food by-products and/or ingredients: the cases of olive mill and pomegranate wastes. Journal of Food Engineering 167, 45–50.

Guillermo, R., Lama, A., Trujillo, M., Espartero, J.L., & Fernandez-Bolanos, F. (2009) Isolation of a powerful antioxidant from *Olea europaea* fruit-mill waste: 3,4-dihydroxyphenylglycol. LWT – Food Science and Technology 42, 483–490.

Gutierrez, F., Perdiguero, S., Garcia, J.M., & Castellano, J.M. 1992. Quality of oils from olives stored under controlled atmosphere. Journal of the American Oil Chemists' Society 69, 1215–1218.

Guzman, J.F.B. (2002) Method for obtaining purified hydroxytyrosol from products and by-products derived from the olive tree. US Patent 6849770 B2, PCT/ES02/00058.

Hartmann, H.T., El-Hamady, M., & Whisler, J. (1972) Abscission induction in olive by cycloheximide. Journal of the American Society for Horticultural Science 97, 781–785.

Hartmann, H.T., Tombesi, A., & Whisler, J. (1970) Promotion of ethylene evolution and fruit abscission in the olive by 2-chloroethanephosphonic acid and cycloheximide. Journal of the American Society for Horticultural Science 95, 635–640.

Herrera Gomez, C. (1975) Mechanical properties of ground olive pastes. In: Olive oil technology, ed. J.M. Moreno Martinez. FAO, Rome, 17–30.

Hurtado-Fernández, E., Gómez-Romero, M., Carrasco Pancorbo, A., & Fernández-Gutiérrez, A. (2010) Application and potential of capillary electroseparation methods to determine antioxidant phenolic compounds from plant food material. Journal of Pharmaceutical and Biomedical Analysis 53, 1130–1160.

International Olive Oil Council (IOOC). (1984) Olive oil quality improvement. Technical Handbook. IOOC, Madrid, Spain, 25–45.

Kader, A.A., Nanos, G.D., & Kerbel, E.L. (1990) Storage potential of fresh 'Manzanillo' olives. California Agriculture 40, 23–24.

Kalogeropoulos, N., Kaliora, A.C., Artemiou, A., & Giogios, I. (2014) Composition, volatile profiles and functional properties of virgin olive oils produced by two-phase vs three-phase centrifugal decanters. LWT – Food Science and Technology 58, 272–279.

Kalua, C.M., Allen, M.S., Bedgood, D.R., Jr., Bishop, A.G., Prenzler, P.D., & Robards, K. (2007) Olive oil volatile compounds, flavour development and quality: a critical review. Food Chemistry 100, 273–286.

Kapatos, E.T., & Fletcher, B.S. (1983) Seasonal changes in the efficiency of McPhail traps and a model for estimating olive fly densities from trap catches using temperature data. Entomologia Experimentalis et Applicata 33, 20–26.

Karapinara, M., & Worgan, J.T. (1983) Bioprotein production from the waste products of olive oil extraction. Journal of Chemical Technology & Biotechnology 33, 185–188.

Kiritsakis, A. (1991) Extraction of olive oil. In: Olive oil, ed. A. Kiritsakis. American Oil Chemistry Society, Champaign, IL, 61–79.

Kiritsakis, A.K. (1998) Olive oil: from the tree to the table, 2nd ed. Food & Nutrition Press, Trumbull, CT.

Kiritsakis, A. (2007) Olive oil: conventional and organic, tables olives and olive paste, 4th ed. Copy City, Thessaloniki, Greece, 219–244. (in Greek)

Kiritsakis, A., Koutsaftakis, A., & Mikros, L. (1985) The effect of processing systems "Pieralisi, Hiller and Rapanelli (Sinolea – Decanter)" on olive oil quality. Grasas y Acietes 35, 165–170.

Kiritsakis, A., & Markakis, P. (1984) Effect of olive collection regime on olive oil quality. Journal of the Science of Food and Agriculture 35, 677–678.

Kiritsakis, A., Nanos, G.D., Polymenopoulos Z., Thomai, T., & Sfakiotakis, E.M. (1998) Effect of fruit storage conditions on olive oil quality. Journal of the American Oil Chemists' Society 75, 721–724.

Kiritsakis, A., & Tsipeli, A. (1992) Relationship of the acidity of olive oil to its resistance to oxidation. Rivista Italiana Delle Sostanze Grasse 69, 513–515.

Kiritsakis, K., Kontominas, M.G., Kontogiorgis, C., Hadjipavlou-Litina, D., Moustakas, A., & Kiritsakis, A. (2010) Composition and antioxidant activity of olive leaf extracts from Greek olive cultivars. Journal of the American Oil Chemists' Society 87, 369–376.

Lamouria, L.H., & Brewer, H.L. (1965) Determining selected bio-engineering properties of olives. Transactions of American Society of Agricultural Engineers 8, 271–274.

Lanzon, A., Albi, T., & Gracian, J. (1986) Influence of the extraction system on some components of olive oil: preliminary assays. Grasas y Aceites 37, 254–258.

Mendoza Alba, J. (1975) Milling – malaxation. In: Olive oil technology, ed. J.M. Moreno Martinez. FAO, Rome, 23–30.

Metzidakis, I. (1999) Field studies for mechanical harvesting by using chemicals for the loosening of olive pedicel on cv. Koroneiki. Acta Horticulturae 474, 197–201.

Metzidakis, I., Gerasopoulos, D., & Kiritsakis, A. (1995) Length of time that olive fruit remains on collection nets: effect quality characteristics of olive oil. Rivista Italiana Delle Sostanze Grasse 72, 253–257.

Metzidakis, I., Martinez-Vilela, A., Castro Nieto, G., & Basso, B. (2008) Intensive olive orchards on sloping land: good water and pest management are essential. Journal of Environmental Management 89, 120–128.

Michelakis, S. (1986) Bio-ecological data of olive fly *Dacus oleae* (Gmel) in Crete-Greece. Proceedings of the Second International Symposium on Fruit Flies, Crete, 397–406.

Montedoro, G., & Petruccioli, G. (1974) Treatment with enzymatic preparation and tannin absorbents in extracting olive oil by single pressure and percolation processes. Rivista Italiana Delle Sostanze Grasse 51, 378–385.

Moreno Martinez, J.M. (1975) Other systems of extraction. In: Olive oil technology, ed. J.M. Moreno Martinez. FAO, Rome, 42–46.

Moya, M., Espinola, F., Fernandez, D.G., De Torres, A., Marcos, J., Vilar, J., Josue, J., Sanchez, T., & Castro, E. (2010) Industrial trials on coadjuvants for olive oil extraction. Journal of Food Engineering 97, 57–63.

Najafian, L., Ghodsvali, A., Haddad Khodaparast, M.H., & Diosady, L.L. (2009) Aqueous extraction of virgin olive oil using industrial enzymes. Food Research International 42, 171–175.

Nergiz, C., & Unal, K. (1991) Effect of method of extraction on the total polyphenol, 1,2-diphenol content and stability of virgin olive oil. Journal of the Science of Food and Agriculture 56, 79–84.

Paini, M., Aliakbarian, B., Casazza, A.A., Perego, P., Ruggiero, C., & Pastorino, L. (2015) Chitosan/dextran multilayer microcapsules for polyphenol co-delivery. Material Science and Engineering: C 46, 374–380.

Penyalver, R., García, A., Ferrer, A., Bertolini, E., & López, M.M. (2000) Detection of *Pseudomonas savastanoi* pv. *savastanoi* in olive plants by enrichment and PCR. Applied and Environmental Microbiology 66, 2673–2677.

Petruccioli, G. (1975) Oil extraction. In: Olive oil technology, ed. J.M. Moreno Martinez. FAO, Rome, 31–41.

Petruccioli, M., Servili, M., Montedoro, F., & Federici, F. (1988) Development of recycle procedure for the utilization of vegetation waters in the olive oil extraction process. Biotechnology Letters 10, 55–60.

Proietti, P., Federici, E., Fidati, L., Scargetta, S., Massaccesi, L., Nasini, L., Regni, L., Ricci, A., Cenci, G., & Gigliotti, G. (2015) Effects of amendment with oil mill waste and its derived-compost on soil chemical and microbiological characteristics and olive (*Olea europaea* L.) productivity. Agriculture, Ecosystems and Environment 207, 51–60.

Ranalli, A. (1991) The effluent from olive mills: proposals for re-use and purification with reference to Italian legislation. Part I. Olivae 37, 30–35.

Ranalli, A. (1998) Influence of paste stone fragment size on the output of olive oil extraction: centrifugation system. Rivista Italiana Delle Sostanze Grasse 65, 581–587.

Ranalli, A., & Angerosa, F. (1996) Integral centrifuges for olive oil extraction: the qualitative characteristics of products. Journal of the American Oil Chemists' Society 73, 417–422.

Ranalli, A., Gomes, T., Delcuratolo, D., Contento, S., & Lucera, L. (2003b) Improving virgin olive oil quality by means of innovative extracting biotechnologies. Journal of Agricultural and Food Chemistry 51, 2597–2602.

Ranalli, A., Malfatti, A., Lucera, L., Contento, S., & Sotiriou, E. (2005) Effects of processing techniques on the natural colourings and the other functional constituents in virgin olive oil. Food Research International 38, 873–878.

Ranalli, A., Pollastri, L., Contento, S., Lannucci, E., & Lucera, L. (2003a) Effect of olive paste kneading process time on the overall quality of virgin olive oil. European Journal of Lipid Science and Technology 105, 57–67.

Reboredo-Rodriguez, P., Gonzalez-Barreiro, C., Cancho-Grande, B., & Simal-Gandara, J. (2014) Improvements in the malaxation process to enhance the aroma quality of extra virgin olive oils. Food Chemistry 158, 534–545.

Rincón, B., Bujalance, L., Fermoso, F.G., Martín, A., & Borja, R. (2013) Biochemical methane potential of two-phase olive mill solid waste: influence of thermal pretreatment on the process kinetics. Bioresource Technology 140, 249–255.

Rodis, P.S., Karathanos, V.T., & Mantzavinou, A. (2002) Partitioning of olive oil antioxidants between oil and water phases. Journal of Agricultural and Food Chemistry 50, 596–601.

Roig, A., Cayuela, M.L., & Sanchez-Monedero, M.A. (2006) An overview on olive mill wastes and their valorisation methods. Waste Management 26, 960–969.

Rural Industries Research and Development Corporation (RIRDC). (2008) New ideas for rural Australia. Publication No. 08/157. RIRDC, Wagga Wagga, Australia.

Ryan, D., Robards, K., & Lavee, S. (1999) Determination of phenolic compounds in olives by reversed-phase chromatography and mass spectrometry. Journal of Chromatography A 832, 87–96.

Salas, J.J., & Sanchez, J. (1999) The decrease of virgin olive oil flavor produced by high malaxation temperature is due to inactivation of hydroperoxides. Journal of Agricultural and Food Chemistry 47, 809–812.

Sanchez, H.A., Romero, C., Ramirez, E., & Brenes, M. (2013) Storage of mechanically harvested Manzanilla olives under controlled atmospheres. Postharvest Biology and Technology 81, 60–65.

Servil, M., Esposto, S., Veneziani, G., Urbani, S., Taticchi, A., Di Maio, I., Selvaggini, R., Sordini, B., & Montedoro, G.F. (2011) Improvement of bioactive phenol content in virgin olive oil with an olive-vegetation water concentrate produced by membrane treatment. Food Chemistry 124, 1308–1315.

Sheabar, F.Z., & Neeman, I. (1988) Separation and concentration of natural antioxidants from the rape olives. Journal of the American Oil Chemists' Society 65, 990–993.

Solinas, M. (1992) Principles of olive oil extraction. Olivae 42, 31–35.

Solinas, M., Marsilio, V., & Angerosa, F. (1987) Behaviour of some components of virgin olive oil flavour in connection with the ripening of olives. Rivista Italiana Delle Sostanze Grasse 64, 475–480.

Stefanoudaki, E., Koutsaftakis, A., & Harwood, J.L. (2011) Influence of malaxation conditions on characteristic qualities of olive oil. Food Chemistry 127, 1481–1486.

Suarez, M.J.M. (1975) Preliminary operations. In: Olive oil technology, ed. J.M. Moreno Martinez. FAO, Rome.

Taticchi, A., Esposto, S., Veneziani, G., Urbani, S., Selvaggini, R., & Servili, M. (2013) The influence of the malaxation temperature on the activity of polyphenoloxidase and peroxidase and on the phenolic composition of virgin olive oil. Food Chemistry 136, 975–983.

Tsantili, E., Kafkaletou, M., Roussos, P.A., & Christopoulos, M.V. (2012) Phenolic compounds, maturation and quality in fresh green olives for table use during exposure at 20 °C after preharvest ReTain treatment. Scientia Horticulturae 140, 26–32.

Vekiari, S.A., Papadopoulou, P., & Kiritsakis, A. (2007) Effects of processing methods and commercial storage conditions on the extra virgin olive oil quality indexes. Grasas y Aceites 58, 237–242.

Vichi, S., Romero, A., Gallardo-Chacon, J., Tous, J., Lopez-Tamames, E., & Buxaderas, S. (2009) Volatile phenols in virgin olive oils: influence of olive variety on their formation during fruits storage. Food Chemistry 116, 651–656.

Vigo, F., Avalle, L., & Paz, M.D. (1983) Elimination of vegetation water from olive oil plants: the electrochemical process. Rivista Italiana Delle Sostanze Grasse 60, 125–132.

Vinha, A.F., Ferreres, F., Silva, B.M., Valentao, P., Goncalves, A., Pereira, J.A., Beatriz Oliveira, M., Seabra, R.M., & Andrade, P.B. (2005) Phenolic profiles of Portuguese olive fruits (*Olea europaea* L.): influences of cultivar and geographical origin. Food Chemistry 89, 561–568.

Vlyssides, A.G., Loizides, M., & Karlis, P.K. (2004) Integrated strategic approach for reusing olive oil extraction by-products. Journal of Cleaner Production 12, 603–611.

Vossen, P. (2002) Quality table olive and olive oil production: olive oil processing technology influences on quality. Australian Olive Association: National Olive Industry Conference, Adelaide, Australia.

Vossen, P. (2005) Variety and maturity: the two largest influences on olive oil quality. Available from http://cesonoma.ucdavis.edu/files/27177.pdf

8 Application of HACCP and traceability in olive oil mills and packaging units and their effect on quality and functionality

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8.1 Introduction

In recent years, consumer demands for high-quality products, as well as the need to align business with standards, such as the European standards, and secure compliance with legislation in the production of quality products, have led organizations involved in the olive oil and olives industry to take measures in order to ensure the quality of their products. The certification of quality assurance and product safety is the companies' compliance to the requirements of some *standards* (ISO and HACCP) and the implementation of a *traceability* system.

HACCP (Hazard Analysis and Critical Control Points) is a quality control system, which specifies the rules to be followed by olive oil mills and packaging units concerning hygiene, occupational safety, environmental protection, hazard identification, and evaluation of critical control points (CCPs), among others. The proper implementation and application of these rules aim at achieving overall quality assurance and product safety and thus satisfy consumers' growing demand for virgin olive oil with exceptional quality and functionality.

Quality and safety are both linked to traceability, although safety is implicated by traceability more often. They are two very important elements of people's conceptions of food and associated decision making (i.e., food choice). Traceability is primarily viewed as a tool for determining the food safety by providing a means for recall as well as proof of the authenticity of food, but it is also related to food quality (Aung & Chang, 2014). Since both quality and safety were shown to be related to confidence, traceability may indeed boost consumer confidence through quality and safety assessments (Rijswijk & Frewer, 2006).

To foster continuous improvement in the quality of products and processes, firms use the Total Quality Management (TQM) system. Ho (1994) stated that ISO 9000 can be seen as a route to implementing TQM. Figure 8.1 shows the relationship of food safety, quality, and traceability systems from the management point of view.

8.2 The basic HACCP benefits and rules

The application of the HACCP program in an olive oil plant helps (Kiritsakis, 2007) several aspects:

- Trace and identify all possible risks (physical, chemical, and microbiological) in all processing steps.
- Analyze and estimate the size and severity of every risk and the possibility of this risk to appear.
- Control and solve the problems caused by each risk.
- Apply systems and procedures in order to prevent these risks.

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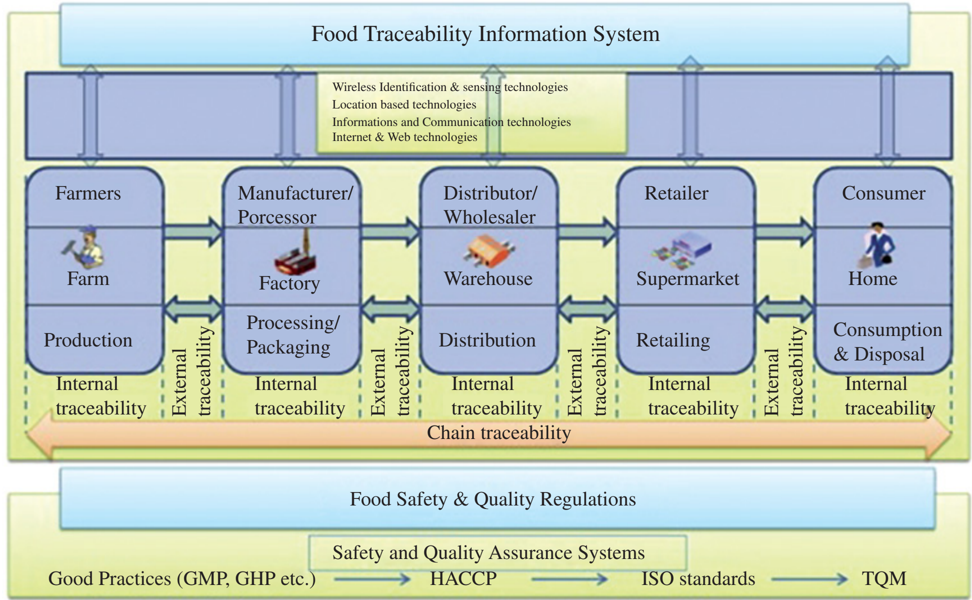

Figure 8.1 Food safety, quality, and traceability. *Source*: Aung and Chang (2014). Reproduced with permission of Elsevier.

Furthermore, the application of a HACCP system in an olive oil plant confirms the following:

- The specifications of the produced olive oil conform to the current legislation.
- Consumers' health is protected.
- Benefits of the plant are ensured against any legal disputes.
- Olive oil promotion is favored.
- Competition in international market is favored.
- Export of the olive oil is increased, while the danger of order cancellation is eliminated.
- Value of olive oil is increased.

The program can be satisfactorily applied only when the oil plant has employed the required hygiene level. Thus, for the proper application of a HACCP system, the owner of the olive oil plant has to ensure these aspects:

- Appropriate training and uniforms for the staff.
- Efficiency in sanitary-ware.
- Appropriate facilities (hygienically designed areas, drainage web, clean toilets, separate storage, garbage area, etc.).

Once the program has been applied, the company can be certified from a certification service. On 2005, the International Management Standard of food safety ISO 22000 was released. ISO 22000 was created in order to form a united and homogeneous demand platform, acceptable from all the states worldwide (Escanciano & Santos-Vijande, 2014; Fernández-Segovia *et al.*, 2014).

In general, for the application of the HACCP program in olive oil plants, it is necessary to follow several criteria (Arvanitoyiannis, 2009):

- The rules of Good Manufacturing Practice (GMP) and Good Hygiene Practice (GHP) will be followed.
- The staff will be trained sufficiently in hygiene safety issues, along with updates on developments.
- There will be prompt adaptation to any new data that arise.

For the installation of a HACCP program in an olive oil plant, the following requirements must be followed:

- Designating a person in charge of the HACCP program and informing the staff to ensure active participation in the program.
- Recording the possible risks (physical, chemical, and microbiological) linked to the olive fruit process, suggesting preventive measures, and identifying the CCPs in every process step.
- Specifying critical limits and control measures for every CCP (the "critical limit" is the borderline of a CCP beyond which there might be consequences in olive oil quality and consumers' health).
- Designating correction actions for every CCP.
- Verification and validation.
- Validation that the HACCP system works by keeping and updating the necessary data.

8.3 Description and analysis of the HACCP program in the olive oil mill

The HACCP program allocates and controls the hazards (physical, chemical, and microbial) that affect negatively the food safety. Olive oil may carry physical and chemical hazards. Microbiological hazards can only occur on the olive fruit when it is infected by fungi during storage and before processing, especially if it is kept in plastic bags. However, in the olive oil mill, microbiological hazards are eliminated because fungi are totally removed with the wastewater during the olive fruit processing. Table 8.1 shows some examples of physicochemical hazards that may appear during olive fruit processing (Pardo *et al.*, 2002, 2003).

Figure 8.2 shows the flow diagram in an olive oil plant. The type of olive oil mill chosen to apply the HACCP program here was one using a horizontal centrifugal (decanter) in order to extract the oil, as it is the most abundant among the olive oil mills, as well as the most preferable in terms of preserving the olive oil's phenolic content and sensory attributes (Di Giovacchino *et al.*, 2001, 2002; Kalogeropoulos *et al.*, 2014). The preservation of these compounds in the final product is of great importance, as they are the critical compounds that give olive oil its functional properties (Servili *et al.*, 2011).

Before olive fruit reaches the olive oil mill, there are three very important steps. These are fruit harvesting, transportation of fruits to the olive oil mill, as well as preservation of the fruit before processing. Furthermore, after the final extraction of olive oil and before it reaches the packaging unit, there is the intermediate step of olive oil storage. An analysis of the main hazards and a detection of the CCPs, in each step of the olive oil plant, are provided in Table 8.2.

Step 1. Olive fruit harvesting
Hazards:

- Olive fruit infection by insects (*Bactrocera oleae* or *Dacus oleae*) and diseases (*Colletotrichum acutatum* and *C. gloeosporioides*) (Moral *et al.*, 2009; Ordano *et al.*, 2015).

Table 8.1 Examples of physicochemical hazards in olive oil production processing.

Physical hazards	Chemical hazards
Small branches and pieces of wood attached to the olive fruit during harvest	Pesticide residues
Metals	Packaging material
Stones	Cleaning residues
Plastics	Polycyclic hydrocarbons
Leaves	Halogenated solvents
	Heavy metals

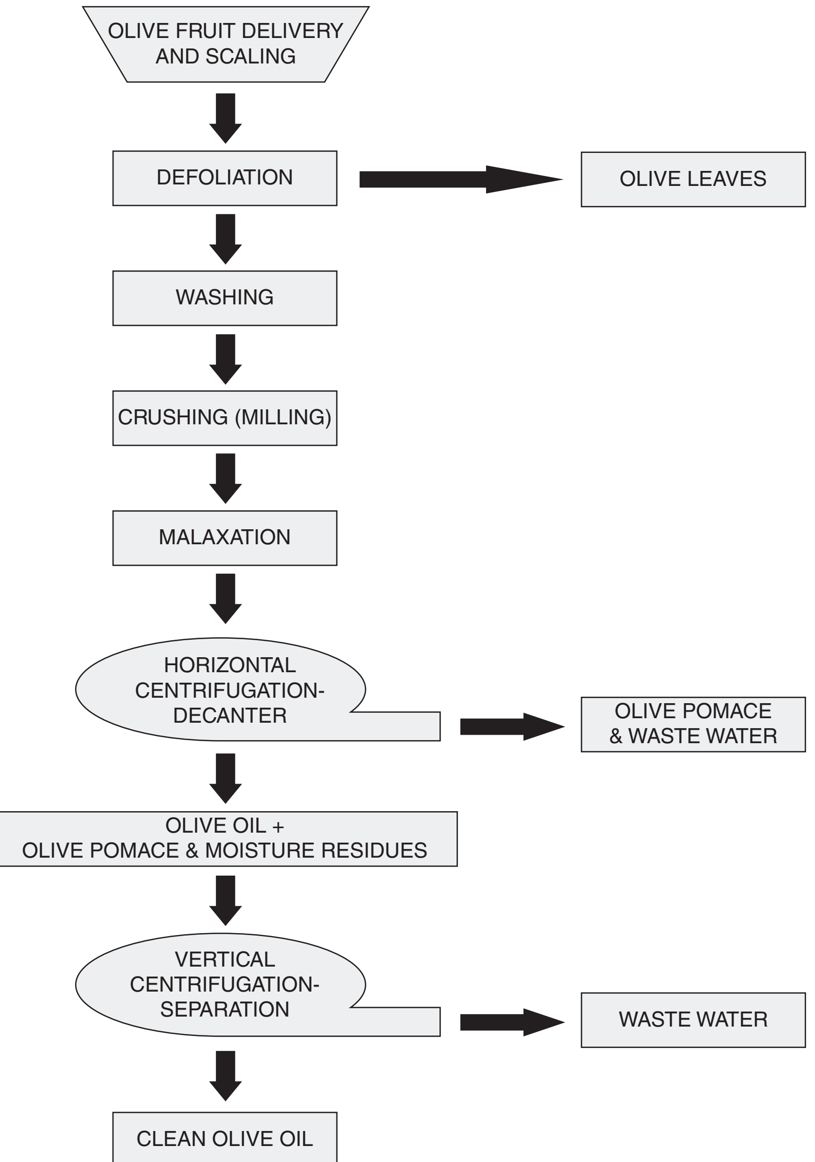

Figure 8.2 Olive oil plant flow diagram.

- Olive fruit contamination by extensive use of pesticides.
- Deterioration of the quality characteristics of the olive oil due to delayed harvesting of the olive fruit.
- Damage of the olive fruit during harvesting.

Prevention:

- Treatment of the insects and diseases by organic means or wise use of pesticides.
- Harvest of the olive fruit at the proper maturity step.
- Harvest of the olive fruit by means and ways that reduce injury.

Table 8.2 HACCP control chart of olive oil extraction by a centrifugal system.

CCP	Hazard	Control measures	Critical limits	Monitoring action	Corrective action	Verification procedures and documentation
Receiving of raw materials (olive fruits and water)	• Microbial infection • Insect infection • Mechanical damage • Pesticides residues • Trace elements • Enzymatic reactions • Foreign materials	• Separation of ground of flight and health olives • Separate ground of flight and health olives • High-quality drinking water • Good transportation handling	• Suitability of olive • Meeting the specifications of drinking water • Adequate sanitary conditions • Good transportation	• Visual inspection • Microbiological-physicochemical analysis of water • Microbiological-chemical analysis of samples	• Reject unsuitable items • Poor-quality fruits processed separately • Switch point water supply • Conduct training for operators	• Develop particular specifications for the raw materials • Corrective measures • Recording raw materials
Cleaning (leaves removal, washing)	• Water quality • Foreign materials • Washing time • Reuse of excessive wash water • Pesticides • Heavy metals	• Microbiological analysis of samples • Effectiveness program of cleaning and disinfection • Adequate renewal of the washing water	• Adequate renewal of the washing water • Increase wash time • Good hygiene • Good equipment performance	• Visual inspection of washing water • Correct application of preventive programs of maintenance, cleaning, and disinfection of equipment • Physiological inspection of washing water (e.g., for color and odor)	• Increase the rate of replacing the washing water • Correct preventive programs of maintenance, cleaning, and disinfection of equipment	• Frequency of water changes • Maintenance, cleaning, and disinfection of equipment • Corrective measures

(*continued*)

Table 8.2 (*Continued*)

CCP	Hazard	Control measures	Critical limits	Monitoring action	Corrective action	Verification procedures and documentation
Malaxation process	• Microbiological infection • Enzymatic reactions • Emulsions formation	• Equipment (state, cleaning) • Malaxation temperature and time (<35 °C, 60 min) • Oil loss	• Good equipment performance • Suitable malaxation conditions (<35 °C, 60 min)	• Malaxation temperature and time	• Visual inspection of malaxation temperature and time • Correct preventive programs of maintenance, cleaning, and disinfection of equipment	• Cleaning and disinfection programs • Visual inspection of malaxation temperature and time
Extraction of olive oil by centrifugation	• Enzymatic reactions • Emulsions formation • Water quality • Impurities • Oil degradation	• Operations (time, efficiency, and oil loss) • Equipment (state and cleaning) • Cleaning program and adequate disinfections • Oil loss • FFA, PV, TBA, K_{232}, K_{270}, and polyphenols	• Processing time • Minimum oil loss • FFA, PV, TBA, K_{232}, K_{270}, and polyphenols • Olive oil specification • Good equipment performance	• Visual inspection of processing time, water quality • Visual inspection of FFA, PV, TBA, K_{232}, K_{270}, and polyphenols • Good hygiene practices • Periodic visual inspection of equipment	• Correct preventive programs of maintenance, cleaning, and disinfection of equipment • Training program	• Preventive programs for cleaning and disinfection of equipment • Visual inspection of FFA, PV, TBA, K_{232}, K_{270}, and polyphenols

Source: El-Sayed et al., (2015).

Inspection:

- Optical inspection of the olive fruit in order to determine the appropriate harvest time so that it will coincide with the physiological ripening.

Step 2. Olive fruit transportation to the olive oil mill
Hazards:

- Contaminated olive fruit by insects or fungi (*Bactrocera oleae, Colletotrichum gloeosporioides*, etc.).
- Contaminated olive fruit by pesticide residues.
- Damage of olive fruit during loading on the tractor or other transportation means.
- Mixing healthy olive fruits with those contaminated by insects or fungi.

Prevention:

- Selection of olive farmers who produce olive fruit of good quality.
- Use of appropriate containers for the transport of olive fruit to the olive oil plant, such as open plastic crates and under no circumstances plastic bags.
- Use of appropriate cleaning materials for the transportation means and for the machineries of the olive oil plant, accompanied by certificates from the supplier.
- Separation of the healthy olive fruits from the contaminated ones or from those of bad quality.
- Separate processing for the olive fruits sprayed for insect infestation.
- Separation of the olive fruit that are picked up from the ground from the ones harvested from the tree.
- Rejection of all the olive fruit batches that do not meet the specifications established by the olive oil plant.
- Chemical analysis of the raw materials.
- Training the staff in cleaning and disinfection matters.

Correction:

- Rejection of the olive farmers who supply olive fruits of quality that does not meet the standards set by the olive oil plant.

Inspection:

- Optical inspection of the supplied olive fruits, in order to avoid mixing of olive fruits collected from the ground with these harvested from the tree.
- Optical inspection in order to avoid mixing healthy olive fruits with the contaminated ones and moreover to check for the presence of pesticide residues or contaminating substances on the olive fruits.

Record:

- All olive fruit batches processed, and the controls made.
- All rejections of inappropriate batches.
- Corrective and precaution actions.

Step 3. Preservation of olive fruit until processing
Hazards:

- Deterioration of olive fruit quality and consequently of olive oil (undesirable taste, acidity increase) as a result of the fermentation from fungal infection.
- Increase in oxidative rancidity of olive oil due to temperature increase.
- Microbial contamination of healthy olives due to preservation together with infected ones for a long period of time.
- Contamination of olives by foreign substances, organic wastes, and accumulated dirt residues.

- Contamination by animals (birds, mice, dogs, cats, etc.) that may come in contact with the olives.
- Contamination by oils and other substances derived from the tractors and other transportation means.

Prevention:

- Immediate processing of olive fruit, right after its arrival, on to the olive oil plant.
- Appropriate cleaning and disinfection of the olive fruit.
- Proper ventilation and temperature conditions.
- Removal of olive fruits of bad quality.
- Prevention of animal presence in the storing areas.
- Prevention of vehicles from entering the plant.
- Appropriate staff training to insure proper operating conditions.

Inspection:

- Proper cleaning and disinfection of the storing areas.
- Implementation of good handling practices from the workers.
- No presence of animals (dogs etc.) in the area.
- Vehicle entrance.

Record:

- Cleaning and disinfecting program.
- Any incidents noted (animal entry, oil stains in the storage place, etc.) and the corrections applied.

Step 4. Olive fruit scaling

In most olive oil plants, olive fruit are weighed with the use of a scale connected to a computer, which automatically issues certificates.

Hazards:

- Contamination of the olive fruit from a previous batch that was soiled with organic residues, dirt, oxides, or paint attached to the scale.

Prevention:

- Appropriate program for cleaning the scale.
- Checking the construction material of the scale for any undesirable substances that could get attached to the olive fruit.

Critical limit:

- Absence of oxides and other foreign substances from the surface of the scale.

Inspection:

- Optical inspection in order to confirm the good condition of the scale.

Record:

- Cleaning and disinfecting programs.
- Any problems spotted on the scale.
- Actions applied.

Step 5. Defoliation
Hazards:

- Entrance of an extremely high amount of leaves with the olive fruit (physical hazard).
- Decayed substances on the defoliator that may contaminate the olive fruit (microbial hazard).

Prevention:

- Preventive maintenance of the defoliator.
- Proper cleaning and disinfecting programs.

Inspection:

- Correct application of preventive maintenance programs.
- Periodical optical inspection of the defoliator.

Record:

- Preventive maintenance program of the defoliator.
- Cleaning and disinfection programs.
- Any correction applied.

Step 6. Washing of the olive fruit

The olive fruit must be well washed in order to prevent any problems associated with bad olive oil taste (moldy, earthy, etc.) and damage of the machinery.

Hazards:

- Presence of dirt and organic residues on the olive fruit that are attached on the conveyor belts and on the washing machine.
- Use of contaminated water for washing of the olive fruit.

Prevention:

- Regular renewal of the washing water.
- Increase of washing time if the olive fruits are very dirty or their quality is very bad.
- Visual inspection for cleanliness of the washing machine.
- Washing machine maintenance.
- Controlling the cleaning and disinfecting programs.
- Checking the water suitability.

Corrections:

- Change of the water supply resource if it is considered inappropriate or if chemical substances are used for its cleaning.

Critical limit:

- The critical limit must be detected in the proper functioning of the machinery and the maintenance of good hygiene conditions.

Inspection:

- ♦ Optical periodical inspection of the washing machine.
- ♦ Periodical physicochemical and microbiological tests of the water and the olive fruits.
- ♦ Correct implementation of the prevention maintenance programs and machinery cleaning.

Record:

- ♦ Washing water changes.
- ♦ Programs of preventive machinery maintenance.
- ♦ Cleaning and disinfection, along with corrective measures applied.
- ♦ Any physicochemical and microbiological water tests.
- ♦ Any corrective and preventive actions.

Step 7. Crushing of the olive fruit
Hazards:

- ♦ Contamination of the olive paste from dirty olive fruit (physical hazard).
- ♦ Entrance of metal substances or stone pieces into the olive paste (physical hazard).
- ♦ Initiation of oxidation due to the contact of the crushed olive fruit with the air (chemical hazard).
- ♦ Increase in olive paste temperature, leading to deterioration of olive oil quality (chemical hazard).

Prevention:

- ♦ Preventive maintenance of the olive oil plant and metal crusher with appropriate cleaning and disinfecting program.
- ♦ Placement of magnets next to the crusher, which can hold any metal bits found in the olive paste.
- ♦ Avoidance of contact between olive paste and air.
- ♦ Control of the temperature below 27 °C.

Critical limit:

- ♦ Absence of metal bits or stone pieces.
- ♦ Good function of crusher and oil plant and correct application of hygiene conditions.

Inspection:

- ♦ Correct maintenance, cleaning, and disinfection of the metal crusher.
- ♦ Checking the magnets for any metal bits present (the presence of bits indicates bad condition of the metal crusher).

Record trace:

- ♦ Cleaning and disinfection programs of the metal crusher.
- ♦ Corrections applied.
- ♦ Temperature data.

Step 8. Olive paste malaxation
Hazards:

- ♦ Contamination of the olive paste from color residues, mineral oils, and organic residues (physical hazard).
- ♦ Usage of water that does not meet the requirements of the legislation (chemical hazard).
- ♦ Loss of oil aroma and flavor and initiation of oxidation when malaxation temperature exceeds 27 °C and/or olive paste comes in contact with atmospheric air (chemical hazard).
- ♦ Presence of insects and/or rodents.

Prevention:

- ♦ Regular maintenance of the malaxator (oiling with cooking oil).
- ♦ Appropriate cleaning program.
- ♦ Water purity control.
- ♦ Water temperature control.
- ♦ Check for insects and/or rodents.

Critical limit:

- ♦ Maximum temperature limit of the olive paste at 27 °C.
- ♦ Zero contamination limits from mineral oils or other substances.

Inspection:

- ♦ Correct application of the maintenance, cleaning, and disinfection programs.

Record:

- ♦ Preventive maintenance, cleaning, and disinfection programs.
- ♦ Any applied correction.
- ♦ Temperature of the olive paste.

Step 9. Horizontal centrifugation (decanting)
Hazards:

- ♦ Entrance of mineral oil in the olive paste (physical hazard).
- ♦ Entrance of foreign substances in the olive paste, coming from the centrifugal (decanter) (physical hazard).
- ♦ Decayed substances on the decanter that may contaminate the olive paste (microbial hazard).
- ♦ Usage of inappropriate water or water with high temperature (chemical hazard).

Prevention:

- ♦ Preventive maintenance of the centrifugal.
- ♦ Proper cleaning and disinfecting programs.
- ♦ Pipes coverage to avoid the entrance of foreign bodies.
- ♦ Usage of cooking fat for oiling the machinery.
- ♦ Appropriate staff training.

Critical limit:

- ♦ Zero contamination from mineral oils, metals, dirt, organic residues, or foreign bodies.
- ♦ Usage of water with drinking specifications and temperature not higher than 27 °C.

Inspection:

- ♦ Correct application of preventive maintenance programs.
- ♦ Periodical optical inspection of the piping and machinery.

Record:

- ♦ Preventive maintenance program of the piping and machinery.
- ♦ Cleaning and disinfection programs.

- Any correction applied.
- Water temperature.

Step 10. Vertical centrifugation (oil separation)
Hazards:

- Entrance of grease, foreign objects, insects, or organic residues that are attached on the oil separator or are transferred from the staff to the olive oil (physical and chemical hazard).

Prevention:

- Maintenance of the pipes that transfer the olive oil to the separator.
- Appropriate cleaning and disinfecting program of the oil separator.
- Appropriate piping insulation.
- Usage of cooking fat for oiling the oil separator.
- Limited access to people where the oil separator is located.

Critical limit:

- Zero contamination from mineral oils, dirt, organic residues, or foreign substances.
- Water temperature not higher than 27 °C.

Inspection:

- Correct application of each preventive maintenance and disinfecting program.
- Correct operation of each machine in the oil plant.

Record:

- Maintenance, cleaning, and disinfecting programs.
- Any correction applied.
- Water temperature.

Step 11. Olive Oil Storage
Hazards:

- Entrance of foreign materials in the olive oil, if the storage tanks do not close properly (physical hazard).
- Entrance of grease that may come from the pumps (physical hazard).
- Entrance of dirt accumulated in the oil tanks, due to limited cleaning (physical hazard).
- Oxidation and spoilage of the organoleptic characteristics of olive oil, when the appropriate storage conditions are not kept (chemical hazard).
- Wrong handling from the staff.

Prevention:

- Optical cleanliness inspection of the tanks and other storage means.
- Usage of stainless-steel means for transferring the olive oil into the tanks.
- Usage of oil tanks with appropriate conical shape, for easier sediment removal.
- Proper closing of the tanks, preventing the entrance of insects or other foreign objects as well as the contact of olive oil with air and light leading to its oxidation.
- Avoidance of mixing olive oils with different physicochemical and organoleptic characteristics.
- Implementation of correct hygiene rules and industrial practice from the staff.
- Chemical analysis of the product.

Critical limit:

- Zero contamination from mineral oils, dirt, organic residues, or foreign bodies.
- Storage temperature up to 10 °C.

Inspection:

- Correct application of the maintenance programs of the piping and pumps.
- Cleaning and disinfecting programs.
- Staff handling.
- Periodical visual inspection of the pumps and oil tanks.

Record:

- Maintenance, cleaning, and disinfecting programs of the pumps and oil tanks.
- Any corrective action applied.
- Storage temperature.

In addition, in order to avoid the above and any other contaminations, the following must be done:

- The burner used for warming the water should be established outside of the olive oil plant, because olive oil can easily absorb smoke and other odors from the environment.
- The workers must not smoke in the oil plant.
- A special waiting area for the olive producers should be provided, so they do not hang around the oil plant.
- There should be a proper place for any waste.
- Garbage must be collected regularly.
- The toilets must be located outside the oil plant and always kept clean.
- The temperature and lighting in the olive oil plant are properly controlled.
- The entrance should be limited or restricted to visitors.

8.4 Application of the HACCP program in the packaging unit

The application of a HACCP program in an olive oil packaging unit is also imperative. The main hazards that have to be controlled during the implementation of the HACCP program in the packaging of olive oil are analyzed in this section.

Supply of the packaging materials
Hazards:

- Presence of dangerous chemical substances (chemical hazard) or foreign bodies in raw materials (physical hazard).

Prevention:

- Setting specifications.
- Supply of packaging materials from trustworthy suppliers.
- Presentation of quality certificates for all the materials from the supplier.
- Evaluation and approval of the supplier.

Inspection:

- Reviewing the suppliers.
- Sample analysis at intervals.

Transportation of packaging materials
Hazards:

- ♦ Contamination by dangerous chemical substances (chemical hazard).

Prevention:

- ♦ Use of transporting vehicles that will be exclusively for food.
- ♦ Placing different raw materials separately.
- ♦ Cleaning of the transportation means.
- ♦ Recording the loads transported by the same transportation mean.
- ♦ Filling the cleaning records of the transportation mean.

Inspection:

- ♦ Implementation of the above measures.

Receipt of the materials
Hazards:

- ♦ Dangerous chemical substances (chemical hazard).
- ♦ Foreign materials irrelative with the quality and safety specifications (physical hazard).

Prevention:

- ♦ Recording the codes of all batches of the packaging materials delivered.
- ♦ Control of the safety certificates.
- ♦ Control of the delivery conditions of the raw materials.

Inspection:

- ♦ Application of all the prevention measures.
- ♦ Records control.

Unloading and storage of the materials
Hazards:

- ♦ Dangerous chemical substances (chemical hazard).
- ♦ Foreign bodies.

Prevention:

- ♦ Use of appropriate filters and unloading pumps.
- ♦ Use of closed elastic piping.
- ♦ Check the filters after unloading.

Inspection:

- ♦ Application of all the preventive measures.
- ♦ Records control.

Work before oil packaging
Hazards:

- Dangerous chemical substances (chemical hazard).
- Insects (physical hazard).
- Olive oil of inferior quality (chemical hazard).
- Contaminated tanks (chemical hazard).

Prevention:

- Application of cleaning programs.
- Use of appropriate uniforms from the staff.
- Rodent and pest control program.
- Filters on the air duct.
- Window grilles.
- Plastic strip curtains at the doors.
- Use of traps.
- Marking of the chemical substances and controlled access to the storage areas.

Inspection:

- Application of the preventive measures.
- Records control.

Packaging
Hazards:

- Dangerous chemical substances (chemical hazard).
- Foreign bodies (e.g., broken glass, etc.) (physical hazard).

Prevention:

- Cleaning and maintenance of the packaging unit.
- Staff training.
- Appropriate uniforms for all the staff.
- Rodent and pest control program.
- Filters on the air duct.
- Window grilles.
- Plastic strip curtains at the doors.
- Use of traps.
- Marking of the toxic substances and controlled access to the storage areas.
- Use of appropriate and durable pressure containers.
- Control of the suitability of the compressor that shapes the plastic bottles.

Inspection:

- Application of the preventive measures.
- Records control.
- Application of a traceability program.

Storage of the packaged product
Hazards:

- Degradation of the olive oil quality because of high storage temperatures (chemical hazard).

Prevention:

- Appropriate storage conditions (temperature around 10 °C).

Dispatch and delivery of the packaged product
Hazards:

- Dangerous chemical hazards (chemical hazard).
- Intrusion of foreign substances or leakage due to insufficient closing (physical hazard).

Prevention:

- Safe closing of the containers.
- Careful loading and carriage.

Inspection:

- Correct application of the preventive measures.

Finally, when the product comes to the market, appropriate guidance must be given to the dealers for its correct storage and handling.

8.5 The context of traceability

Golan *et al.* (2004) mentioned that the definition of traceability is necessarily broad, because traceability is a tool for achieving a number of different objectives and food is a complex product. Accordingly, several definitions of traceability and its classifications, which come from organizations, legislations, and research literature, can be found. According to ISO 8402 (1994) quality standards, traceability is defined as "the ability to trace the history, application or location of an entity by means of recorded identification." In ISO 9000 (2005) standards, the definition is extended into "the ability to trace the history, application or location of that which is under consideration."

The revised definition of Bosona and Gebresenbet (2013) is a very informative and comprehensive definition of food traceability. Food traceability is defined as a part of logistics management that captures, stores, and transmits adequate information about a food, feed, food-producing animal, or substance at all stages in the food supply chain so that the product can be checked for safety and quality control, traced upward, and tracked downward at any time.

The definition of food traceability varies depending on the sector of the food industry. For the agro-based food chain, Wilson and Clarke (1998) defined food traceability as the information necessary to describe the production history of a food crop, and any subsequent transformations or processes that the crop might be subjected to on its journey from the grower to the consumer's plate. In contrast, traceability is defined as a system able to maintain a credible custody of identification for animals or animal products through various steps within the food chain, from the farm to the retailer (McKean, 2001).

Frequently, the terms "tracking" and "traceability" are used synonymously. However, these two terms identify two inverse processes (Perri *et al.*, 2012):

- Tracking identifies the location of a product from upstream to downstream in the chain and at every stage of the journey.
- Traceability or tracing is the inverse process, which allows us to gather the information previously issued.

Traceability does not refer to the production of a generic good. It makes each unit of production physically identifiable, managing production processes that are determined by "lots"; managing traceability means identifying each group of products and following the path.

It is necessary to record information relating to inputs (products and companies), processing (product lots, which lots, and what end products), and outflows (products, companies, and clients). The key is to define the composition of a set of products that have undergone the same process of transformation. Moreover, the amount of information that identifies a batch may vary, and of course the complexity of the whole system increases according to what information the company chooses to include in the identification of a lot. Internal traceability, then, helps to express the internal procedures of each company to trace the origin of materials used.

The label is the instrument through which information is transferred to consumers. Finally, the information given in the production lot should be able to trace all the links along the chain, back to the first producer and/or supplier of the product or substance to whom it belongs. The traceability chain is an intercompany process, resulting from internal processes of every operator in the industry. Such a system should be linked by efficient information. Traceability is not governed by a single person in the chain; thus, the relations between the operators allow the tracing of the chain.

An independent food safety watchdog, Food Standard Agency (FSA, 2002), identified three basic characteristics for traceability systems:

- identification of units/batches of all ingredients and products,
- information on when and where they are moved and transformed, and
- a system linking these data.

To enable traceability, an entity to trace has to be a Traceable Resource Unit (TRU). There are three types of traceable units: batch, trade unit, and logistic unit. A batch is defined as a quantity going through the same processes. A trade unit is a unit that is sent from one company to the next company in a supply chain (e.g., a box, bottle, or pack of bottles). The logistic unit is a type of trade unit, and it designates the grouping that a business creates before transportation or storage (e.g., pallet, container, etc.) (Aung & Chang, 2014).

Firms have three primary objectives in using traceability systems:

- improve supply management,
- facilitate traceback for food safety and quality, and
- differentiate and market foods with subtle or undetectable quality attributes.

The benefits associated with these objectives include lower cost distribution systems, reduced recall expenses, and expanded sales of products with attributes that are difficult to discern (Golan *et al.*, 2004). Besides being a way to improve food safety systems, traceability can also be seen as a strategic tool to improve the quality of raw materials, to improve inventory management, and finally as a source of competitive advantages (Alfaro & Rábade, 2009; Galvão *et al.*, 2010; Aung & Chang, 2014).

8.6 Traceability of olive oil

Olive oil is one of the most valuable products of the agro-food industry. It is an important component of the Mediterranean diet, not only because of its taste but also for its unique contribution to a healthy diet. As a foodstuff, its quality is governed by food safety legislation, with a particular focus on the exclusion of contaminants such as pesticides, metals, toxins, and allergens (Agrimonti *et al.*, 2011). In recent years, the European Union (EU) has supported efforts to protect producers and consumers from fraudulent activities involved in the production of this highly prized food. In particular, it has introduced regulations aimed at ensuring traceability, allowing the possibility of tracking batches of this product throughout its production, processing, and distribution, to define the origin of a food product and to govern its labeling.

Traceability of olive oil is of growing interest among producers, since it leads to supply chain optimization, increase of producers' competitiveness, and prevention of mislabeling of oils of different geographical origin; consequently, it assures correct information for the consumers (Regattieri *et al.*, 2007). Furthermore, EC regulation on marketing standards for olive oil enables producers to market their extra virgin and

virgin olive oils on the basis of geographic origin (Chatziantoniou *et al.*, 2014). The complete field-to-fork traceability of olive oil involves the characterization of the oils obtained from the main cultivars in each production zone, since the chemical composition of olive oil is well known to be influenced by genotype as well as different agronomic, environmental, and technological factors (Cajka *et al.*, 2010).

The factors that affect the traceability of olive oils can be clustered into four broad groups: (a) environmental (soil, climate), (b) agronomic (irrigation, fertilization), (c) cultivation and harvesting (cultivar, ripeness), and (d) technological factors (postharvest storage and extraction). The diversity and interrelationship of these factors make it very difficult to carry out a complete traceability of olive oils by either their chemical composition or sensory descriptors (Aparicio & García-González, 2013).

Over the past several years, various techniques for routine oil analyses, in combination with multicultivar statistical analysis (MSA), have been used to assure the traceability and the authenticity of olive oils by determining physical-chemical parameters such as fatty acids (Tsimidou *et al.*, 1987; Alonso-Garcia & Aparicio-Lopez, 1993; Stefanoudaki *et al.*, 1999; Bucci *et al.*, 2002), fatty acids and triacylglycerols (Aranda *et al.*, 2004; Ollivier *et al.*, 2006), and sterols (Leardi & Paganuzzi, 1987). Other instrumental techniques, such as nuclear magnetic resonance (NMR) (Mannina *et al.*, 2001; Vlahov *et al.*, 2003), Fourier transform infrared (FT-IR) spectroscopy (Bertran *et al.*, 2000), mass spectrometry (MS) (Cajka *et al.*, 2010), and isotopic ratios mass spectrometry (IRMS) (Angerosa *et al.*, 1999), have been adopted for olive oil analyses studying signals, related to specific compounds or isotopic ratios. More recently, NMR has been used for the prediction of the olive oil geographical origin using a fingerprinting approach, where the spectra are used as a whole (fingerprint) without assigning particular resonances, and thus obtaining a comprehensive, multivariate description of the analyzed samples (Longobardi *et al.*, 2012).

However, the content of metabolites is able to discriminate geographical origin of oil, but it is affected by the environmental conditions of plant growth; thus, it is unable to assess the composition of oil (Montealegre *et al.*, 2010). DNA, being not environmentally labile, has a great potential to be used as a means of identifying the cultivars of the trees purporting to be the source of a given sample of olive oil. Current technology for DNA analysis has developed rapidly over recent years, and now it includes an array of platforms, which altogether have given birth to the concept of "food genomics." The underlying principle of this approach is that the amount of DNA remaining in processed foodstuffs is sufficient for analytical purposes (Agrimonti *et al.*, 2011).

The first step for correct traceability is to define the strategy for olive oil sampling. General objectives look for intercluster differences, taking into consideration the maximum intercluster variability. A geographical origin characterization, for example, requires samples from all sources of variation (cultivar, altitude, extraction systems, harvesting time, and ripeness, among others). The ripeness characterization, for example, should avoid olive oil samples obtained by different extraction systems, but could collect samples from different altitudes and climates.

The way the information is managed is crucial for avoiding obvious or wrong conclusions. In general, the mathematical problems are limited to the improper use of procedures or the nonexistence of a validation process. Another big problem concerns the distribution of the values of the chemical parameters over the years. A high degree of data dispersion for some chemical parameters over time might decrease the coefficients of correct classification obtained by statistical procedures applied to a crop from a single year. This is because studies using information from only one crop can yield a high value of misclassifications when they are compared with data from other crops.

Finally, the quantification of chemical compounds is crucial for arriving at plausible results, although there are classical problems affecting the analyses. Thus, the use of unidentified compounds can lead to the selection of artifacts as potential sources of discrimination. The use of redundant information, such as chemical compounds and their sum, or fatty acids and certain triacylglycerols (TAGs), can be a useless activity, particularly because it may also produce noise in the course of discrimination. A real problem is also the use of datasets obtained by different chemical methodologies (e.g., capillary, semicapillary, and packed chromatographic columns), which leads to problems when the information is cross-tabulated.

8.7 Legislation for olive oil traceability

The applied legislation is highly complex, with lawmakers giving different functions to traceability depending on the application and environment of the law. With the event of the food crisis in the 1990s, it was evident

that new guidelines were urgently required to simplify and streamline the processes that could also be applied to food contamination. For this reason, the new laws of the EU were considered as an umbrella, termed “General laws for food safety,” and were applied not only to food safety but also to introduce the concept of traceability. Food companies (producers, manufacturers, and importers) need to be able to demonstrate the traceability of every food, animal feed, and ingredient, showing the chain from producer to consumer. The European Food Safety Authority (EFSA) was created to unify the various committees, to publicize the scientific process and risks, to strengthen the early warning system adopted by European governments and the European Commission, and to enable rapid interventions in cases of food safety in the human and animal food chains.

It is mandatory for companies distributing food to implement a tracing system to ensure food safety and to enable corrective action at the point of production where you have problems or risks. The EU regulation 178/2002 (EU, 2002) narrows the definition to the food industry by defining traceability as the ability to trace and follow a food, feed, food-producing animal, or substance intended to be, or expected to be, incorporated into a food or feed, through all stages of production, processing, and distribution. The Codex Alimentarious Commission (CAC, 2005) defines a more concise definition of traceability as the ability to follow the route of a food through specified stage(s) of production, processing, and distribution.

Since January 2005, a rule has required that EU countries implement labeling and identification procedures for products sold by farmers, producers, and first importers to the EU to enable and facilitate their traceability when they are put on the market. The main purpose is for someone to be able to initiate a withdrawal and/or recall procedure for products in the event of a food problem. The quality of traceability will enable targeted and precise withdrawals. It will also limit the extent of recalls and ensure the removal of holds on products that are not involved.

The appearance of denominations and protected indications of origin has promoted the existence of oils labeled according to these criteria. Regulation 2081/92 created the systems known as Protected Designation of Origin (PDO), Protected Geographical Indication (PGI), and Traditional Specialty Guaranteed (TSG) to promote and protect food products (see Table 8.3).

For example, an olive oil with a PDO denomination requires meeting precise definition of several parameters such as cultivar, geographical origin, agronomic practice, production technology, and organoleptic qualities (Gimenez *et al.*, 2010), and all of these parameters have to be investigated to study its traceability and to certify its quality. Among the above-mentioned factors, the first two are the most important (Montealegre *et al.*, 2010). Additionally, a Database of Origin and Registration (DOOR) was created to support these denominations (Montealegre *et al.*, 2010). The introduction of certifications of origin and quality for virgin olive oil as PDO makes the implementation of traceability procedures necessary.

In recent years, there has been increasing legislation to protect the rights of both the consumers and honest producers. In 2009, the EU Member States agreed to require origin labeling for virgin and extra virgin olive oils (EC Regulation 182/2009) to defend consumers’ needs about true characteristics and origin. To enforce these laws, a measure of the authenticity of samples must be applied, most often in the form of proving the presence or absence of adulterants or verifying geographical or cultivar origin by comparison with well-known and reliable samples.

Table 8.3 General regimen for food and certain other agricultural products based on Regulation 510/2006.

General regimen	Origin	Characteristics	Restriction
Protected Designation of Origin (PDO)	In that region, specific place, or country	Quality essentially or exclusively due to a particular geographical area	Produced, processed, and prepared in a given geographical area
Protected Geographical Indication (PGI)	In that region, specific place, or country	Slightly less strict; food reputation of a product from a given region is sufficient	One of the stages of production, processing, or preparation takes place in the area

8.8 Compositional markers of traceability

There are several parameters, major and minor compositional markers, with varied discriminant power used for olive oil traceability according to the cultivar of olive fruit processed for the production of the oil. Major components such as sterols, phenolic compounds, volatile compounds, pigments, hydrocarbons, tocopherols, fatty acids, and triglycerides may provide basic information on olive cultivars. Minor components can provide more useful information and have been more widely used to differentiate the botanical origin of olive oils.

8.8.1 Fatty acids

Olive oil fatty acids stand for very important components because some of olive oil's most beneficial properties are attributed to them. Furthermore, it was repeatedly demonstrated that the fatty acids profile varies considerably between olive oil and seed oil, thus making this determination very crucial. TAGs consist of a major part of naturally occurring fats and oils, and the analysis of intact TAGs was succeeded with the application of capillary gas–liquid chromatography, high-performance chromatography in normal and reversed-phase modes, thin-layer chromatography, and supercritical fluid chromatography, with emphasis on detection systems widely used in laboratories, that is, flame ionization detector (FID), ultraviolet detector (UVD), refractive index detector (RID), and evaporating light-scattering detector (ELSD) (Buchgrader *et al.*, 2004). The most widespread unsaturated fatty acids in vegetable oils (oleic, linoleic, and linolenic acids) could be quantified with ^{1}H-nuclear magnetic resonance spectroscopy (^{1}H-NMR-400 MHz).

High-resolution ^{13}C NMR spectroscopy was applied by Mavromoustakos *et al.* (1997) for the quantitative analysis of the most abundant fatty acids in the triacylglycerols of 22 virgin olive oil samples harvested at different geographic areas of Greece (Peloponessos, Aegean Islands, Euboea Island, central Greece, Ionian Islands, and north Greece), in an attempt to cover the important olive oil producing areas of the country. The obtained results were compared with data obtained with gas chromatography. Samples from the same area gave repeatably the same results within a 3-year period. Fatty acids composition of three Mediterranean virgin olive oils (Italy [I], Spain [S], and Morocco [M]) was determined with dynamic headspace gas chromatography by Reiners and Grosch (1998). The fatty acids profiles of oils I and S were similar, with the minor component stearic acid being higher, and linoleic acid lower in S than in I. Oil M differed from I and S by a lower content of oleic acid, which was compensated by a higher linoleic acid content.

Compound-Specific Isotope Analysis (CSIA) of fatty acids at natural abundance levels provides information on biogenetic and geographic origin of lipids and oils that is invaluable and almost indispensable nowadays for authenticity control and fraud detection in food analysis (Meier-Augestein, 2002). Ranalli *et al.* (2002) applied high-resolution gas chromatography (HRGC) for the determination of the contents of triacylglycerols, diacylglycerols, and fatty acids for three specific kinds of olive fruit oils (pulp, seed, and whole fruit) coming from seven major Italian olive cultivars. The three kinds of olive oil contained identical fatty acid species but of different concentration. The olive cultivar factor affected the quantitative but not the qualitative fatty acid composition of each fruit oil kind. In particular, the fruit oil kinds from the 'Caroleo' cultivar were richer in oleic acid and poorer in linoleic acid, whereas those from the 'Nebbio' and 'Leccino' cultivars were poorer in palmitic acid. Mannina *et al.* (2003) studied olive oil in a very limited geographical region with no consideration of the pedoclimatic factor (soil characteristics such as temperature and humidity). A relationship between the fatty acid composition and some specific cultivars has been also observed by Montealegre *et al.* (2010).

8.8.2 Phenolic compounds

The content of phenolic compounds is an important factor to be considered when evaluating the quality of olive oil (Servili & Montedoro, 2002), since these compounds have potent antioxidant activity and contribute significantly to the extraordinary stability of olive oils against oxidation (Tura *et al.*, 2007). Traditionally, separation and determination of phenolic compounds in extracts obtained from olive oil by liquid–liquid extraction or solid phase extraction have been carried out by high-performance liquid chromatography (HPLC) analysis coupled mostly with UV detection (Andrewes *et al.*, 2003; Bendini *et al.*, 2003; Baccouri *et al.*, 2008; Allalout *et al.*, 2009), electrochemical (Brenes

et al., 2000), fluorescence (García *et al.*, 2003), and MS detection systems (Andejelkovic *et al.*, 2008). GC (Saitta *et al.*, 2009) and capillary electrophoresis (CE) (Bendini *et al.*, 2003; Carrasco-Pancorbo *et al.*, 2006) coupled with different detection systems have also been used for this purpose. More recently, other techniques such as voltammetric sensors (Rodríguez-Méndez *et al.*, 2008) and high-resolution ^{1}H-NMR spectroscopy (Christophoridou & Dais, 2009) have also been applied for determination of composition of phenolic compounds in olive oil, which is strongly affected by agronomical and technological factors, such as olive cultivar (Tura *et al.*, 2007), place of cultivation (Cerretani *et al.*, 2006), climate, degree of maturation (Baccouri *et al.*, 2008), crop season (Gómez-Alonso *et al.*, 2002), and production process (Cerretani *et al.*, 2006). Using mainly HPLC and phenolic compound profiles, several works have been reported on the study of olive oils according to their cultivar or geographical origin (Andejelkovic *et al.*, 2008; Allalout *et al.*, 2009; Ocakoglu *et al.*, 2009).

Monocultivar 'Picual' olive oils were analyzed in terms of their phenolic compounds and tocopherols obtained during two successive crop seasons (1999–2000 and 2000–2001) in the south of Spain (Garcia *et al.*, 2002). Oils obtained at the beginning of each season had a higher content of each phenolic compound than those obtained at the end, although this effect was more pronounced for ortho-diphenols than for non-ortho-diphenols. The main phenolic compound detected was the aglycon of oleuropein followed by the other secoiridoid aglycons. Lignan concentration in oil showed low variability with season and harvesting time, and pinoresinol, in particular, was present at a concentration of about 40 ppm. Therefore, the total polyphenol content of this monocultivar oil ranged from 300 to 700 ppm. Similarly, significant differences in these compounds were not detected between geographic areas.

Twenty-one different phenols and polyphenols were determined in extra virgin olive oils with capillary zone electrophoresis (CZE) (Bonoli *et al.*, 2003). The distribution of the phenolic compounds revealed quantitative differences relative to the degree of olive ripening as well as to the manufacturing process. Bonoli *et al.* (2004) detected three simple phenols (tyrosol, hydroxytyrosol, and vanillic acid), a secoiridoid derivative (deacetoxy oleuropein aglycone), and two lignans (pinoresinol and acetoxypinoresinol) as main compounds in extra virgin olive oils with HPLC and CZE. The concentration of total phenols in extra virgin olive oil showed a decrease with ripeness of olive fruits from 6464.1 ± 739.0 to 4263.1 ± 30.6 (in mAU-peak area) and from $77{,}379.3 \pm 4588.2$ to $53{,}000.3 \pm 3019.3$ (in AU-peak area), respectively. Moreover, the decrease of o-diphenols from 212.2 ± 14.1 down to 127.5 ± 11.9 (in mg of gallic acid equivalents/kg of oil) is considerable.

Lerma-García *et al.* (2008) developed a simple and reliable capillary electrochromatography (CEC) method for the analysis of the phenolic fraction of olive oils using lauryl acrylate (LA) ester-based monolithic columns. Using CEC data and linear discriminant analysis (LDA), the olive oil samples belonging to three different geographical origins (Croatia, Italy, and Spain) were correctly classified with an excellent resolution among all the categories.

8.8.3 Volatile compounds

The unique and delicate flavor of olive oil is attributed to a series of volatile components like aldehydes, alcohols, esters, hydrocarbons, ketones, furans, and other compounds (Kiritsakis, 1998). Most of these volatiles were quantitated and identified in high-quality olive oil by gas chromatography–mass spectrometry (GC-MS), thus indicating the close relation to its sensory quality. Hexanal, trans-2-hexenal, 1-hexanol, and 3-methylbutan-1-ol are the major volatile compounds of olive oil. Since volatile flavor compounds are formed in the olive fruit through an enzymatic process, they are affected by the olive cultivar, the origin, the maturity stage of the fruit, the fruit storage conditions, and the olive fruit processing, as well as its taste and aroma (Kalua *et al.*, 2006).

Only a subset of volatile compounds and a combination among them could provide valuable information for olive cultivar differentiation (Montealegre *et al.*, 2010). Three volatile compounds (hexyl acetate, hexanal, and (E)-hex-2-enal) and the total concentration of ketones have been nominated to distinguish the olive cultivars (Tena *et al.*, 2007) and could also be used for olive oil. However, it has been found that the level of (E)-hex-2-enal in the analyzed samples showed variability that suggests an influence of genetic factors on the biosynthesis of this compound. In fact, genetic (Tura *et al.*, 2008) and geographic (Mahjoub-Haddada *et al.*, 2007) factors influence the production of volatile compounds in the olive fruits and affect the differentiation of olive oils according to the olive cultivar. The content of volatile compounds allowed differentiation

among monocultivar olive oils and even identification of the technique used for olive oil production (Torres Vaz-Freire *et al.*, 2009).

Dynamic headspace components, thermally desorbed and trapped in a fused-silica cold trap previously cooled to −110 °C and subsequently passed to a capillary column, connected to a mass spectrometer to identify the most important polar compounds (Morales *et al.*, 1994). Sniffing of the components eluted from the chromatographic column was performed for different virgin olive oil samples showing different chromatographic profiles, mainly quantitative, because most compounds were present in all oils analyzed and only the proportions in which these compounds were present varied. Apart from the 31 non-volatile compounds (fatty acids, sterols, alcohols, and methylsterols), Aparicio *et al.* (1997) determined 65 volatiles (aldehydes, alcohols, furans, hydrocarbons, acids, ketones, and esters) in 12 olive oils samples (four cultivars) from Greece, Italy, and Spain, with the principal purpose to show that virgin olive oil authentication is closely related with the olive cultivar from which oil is obtained by strictly physical means.

Stella *et al.* (2000) made a selection of an array of conducting polymer sensors and tested it with extra virgin olive oil samples as a first step toward the development of an electronic nose dedicated to the detection of olive oil aroma. Three different extra virgin Italian olive oils could be easily distinguished with an array of four sensors, and it is also possible to detect changes in the aromatic content of the headspace after handling of the samples. Meanwhile, Guadarrama *et al.* (2000) presented eight polymeric sensors on a thin film base of conducting polymers in order to discriminate among different virgin olive oils. Vichi *et al.* (2003) employed a method based on headspace solid-phase microextraction (HS-SPME) in conjunction with GC-MS to characterize the volatile profile of virgin olive oils produced in two geographical areas of northern Italy, the region of the Gulf of Trieste and the area near Lake Garda. The results suggest that besides the genetic factor, environmental conditions influence the volatile formation.

The instrumental performance of a Thermo Desorption-Cooled Injection System coupled with GC-MS was improved by a Plackett-Burman experimental design for direct thermal extraction of volatile compounds from extra virgin olive oils (Zunin *et al.*, 2004). Three linear combinations of the amounts of the lipoxygenase oxidation products proved to be decisive and sufficient in the differentiation of the two groups of samples. The obtained results have stressed that the new instrumental analysis of oils' volatile fraction allows a strict connection between the fine composition of oil aroma and olive cultivars. Gan *et al.* (2005) developed a novel approach using a surface acoustic wave sensing electronic nose (zNose) for flavor analysis to characterize 16 types of vegetable oils.

8.8.4 Pigments

The color of a virgin olive oil is due to the solubilization of the lipophilic chlorophyll and carotenoid pigments present in the olive. The green-yellowish color is due to various pigments, including chlorophylls, pheophytins, and carotenoids (Cichelli & Pertesana, 2004). Chlorophyll a is the major pigment, followed by chlorophyll b. The carotenoid fraction includes lutein, violaxanthin, neoxanthin, β-carotene, β-cryptoxanthin, and luteoxanthin (Montealegre *et al.*, 2010). Several researchers have reported the same qualitative composition in chlorophyll and carotenoids pigments independent of the olive cultivar and the time of harvest (Giuffrida *et al.*, 2007).

Minguez-Mosquera *et al.* (1992) separated fatty acids from five monovarietal Spanish olive oils ('Picual', 'Picudo', 'Subbetica', 'Hojiblanca', and 'Pajarero'), yielding a fat-free concentrate of pigments, by solid-phase extraction (SPE) on octadecyl (C18) columns. A total of 17 pigments was separated from this extract and quantified with HPLC using a reversed-phase C_{18} column, and the pigment content among these monocultivar virgin olive oils was found to be very different. Psomiadou and Tsimidou (2001) examined 52 samples of virgin olive oil from various regions of Greece for the presence and levels of chlorophyll and carotenoid pigments using normal-phase HPLC (NP-HPLC) and spectrophotometry and found that the lutein/β-carotene ratio was characteristic (<1) for samples from 'Koroneiki', the major Greek cultivar for oil production. The total Pheo-α (Pheo alpha+peak A-peak B)/total carotenoid (lutein+beta-carotene) ratio ranged between 2 and 11 due to prevailing green hues. This characteristic ratio may be used as an indicator of oil typicality along with other analytical parameters.

Cichelli *et al.* (2004) determined quasi-quantitatively the pigment contents (chlorophylls, pheophytins, and carotenoids) of 94 monocultivar olive oils samples of the harvest years 2000, 2001, and 2002 by HPLC,

and from the multivariate statistical analysis (principal component analysis [PCA]) of these parameters tried to evaluate the possibility to distinguish within the cultivars. A comprehensive evaluation between olive oil pigments was based on the amounts of pheophytin a and lutein, which represent the most substantial fraction (more than 80% for all the samples), with their values ranging between 2.06 and 37.06 ppm for pheophytin a and between 3.96 and 14.78 ppm for lutein. The observations concerning different processing technologies and degree of ripening were very important. Thus, higher amounts of pigments were found in olive oil samples using a newer extraction technology (centrifugal or continuous system) compared to the traditional one (pressure system). Cerretani *et al.* (2006) showed that the carotenoid and chlorophyll content determination using UV-Vis spectrophotometry was not useful in discriminating oils produced from different olive cultivars. The lutein/β-carotene ratio, however, has been reported as a tool for differentiating oils from a single cultivar.

8.8.5 Heavy metals

Graphite furnace atomic absorption spectroscopy (GFAAS) was employed by Jimenez *et al.* (2002) as a simple and rapid method for direct determination of iron in olive oil samples from different areas of Spain, without pretreatment. Several olive oil samples from different Spanish origin denominations were analyzed by means of automated determination of Al, Ba, Bi, Ca, Cu, Mg, Mn, Na, Pb, and Sn with on-line emulsion formation by flow injection analysis and determination by ICP-MS and FAAS (Jimenez *et al.*, 2004). The data obtained were treated with the PCA in order to assess the feasibility of using the concentration of the different elements in olive oil for classification according to geographical origin and type. The statistical analysis revealed that Al, Ba, and Mn have a strong differentiating ability, allowing separation of the olive oils analyzed according to their geographical origin and the type of olive employed, whereas Bi, Cu, Sn, and Pb were related to contamination and did not display differentiating capacity.

Dugo *et al.* (2004) determined the content of selenium in 50 samples of different Sicilian virgin olive oils produced in six olive-growing zones from 10 different cultivars of olives and found that both genetic (cultivar) and geographic (olive-growing zone) factors could substantially affect selenium concentration in oils. Zeiner *et al.* (2005) used both inductively coupled plasma atomic emission spectrometry (ICP-AES) and atomic absorption spectrometry (AAS) in conjunction with the use of microwave for metal decomposition of the olive oil samples. Metals such as Al, Co, Cu, K, Mn, and Ni were measured by AAS, and their concentration ranged from 0.15 to 1.5 μg/g, differing according to the geographical origin of the oils. The levels of four of the major pollutant heavy metals were determined with ICP-OES in virgin olive oil monocultivar samples of nine different cultivars ('Bosana', 'Pizze Carroga', 'Mallocrina', 'Manna', 'Nera di Gonnos', 'Nera di Oliena', 'Ogliastrina', 'Semidana', and 'Tonda di Cagliari') in Sardinia (Angioni *et al.*, 2006). High variability within cultivars was observed in lead and zinc data in contrast to cadmium and copper, which existed in similar levels. The amount of lead determined showed a significant variability, both in different cultivars and in different samples from the same cultivar. Moreover, zinc showed a high variability, with minimum and maximum values obtained for 'Bosana' and 'Tonda di Cagliari', respectively. The time of harvest did not affect significantly the contents of lead, copper, and cadmium in the analyzed oils, while some samples of 'Tonda di Cagliari' showed higher values for lead and zinc in the first harvest than in the second. Levels of zinc were, for all cultivars, higher in the first harvest than in the second, except for 'Ogliastrina' and 'Semidana'.

8.9 DNA-based markers of traceability

Molecular markers are investigated as a diagnostic tool for food authenticity, and for traceability and type composition of complex food matrices, in an increasing number of projects (Palmieri *et al.*, 2004). DNA-based methods make an important contribution to protect high-quality olive oils.

Significant amounts of DNA are present in olive oil obtained by cold pressing (Consolandi *et al.*, 2008). However, the filtration process lowers DNA concentrations, which tend to disappear due to nuclease degradation (De la Torre *et al.*, 2004). On the other hand, the length of storage can affect the use of DNA as an analyte for molecular traceability. Pafundo *et al.* (2010) observed a significant decrease of quality of DNA extracted from olive oil, with a consequent loss of information a month after the olive oil production.

A range of marker platforms has been applied for validating the oil varietal composition, including randomly amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), microsatellites (SSRs), single-nucleotide polymorphisms (SNPs), and sequence characterized amplified regions (SCARs). SSRs are particularly favored as they tend to be relatively polymorphic and can be readily adapted to high-throughput genotyping platforms, whenever large numbers of samples need to be evaluated, so they have been largely employed to characterize oil (Breton & Bervill, 2009). AFLP profiles consist of a range of (50–400 bp) fragments and are substantially more reproducible than RAPDs, which was the previous choice for multilocus profiling. A limit in AFLP application is the size of fragments. Pafundo *et al.* (2005) reported that AFLP fragments longer than 250 bp were not detectable in the oil samples, while Montemurro *et al.* (2008) reported that longer fragments can also be amplified. AFLP profiles are information rich, but the technique is complex, costly, and hardly applicable to oil mixtures. However, where cultivar-specific AFLPs can be identified, these can be fairly easily converted into a single locus assay (SCAR). SNPs are advantageous markers because their high density in genomes can permit discrimination of even very similar individuals. Moreover, SNPs could be useful to trace degraded DNA extracted from complex matrix as olive oil, since they can be detected within low-size-range amplicons (Agrimonti *et al.*, 2011).

DNA recovery methods from olive oil have been developed by many authors (Busconi *et al.* 2003; Doveri *et al.* 2006; Pasqualone *et al.* 2007; Consolandi *et al.* 2008). The first research was carried out using genomic DNA extracted from drupes. That DNA had a good potential to amplify correctly using RAPD markers (Cresti *et al.* 1997). By means of SCAR and AFLP markers, Busconi *et al.* (2003) were able to show that DNA recovered from olive oil had both organellar and nuclear origin. Pafundo *et al.* (2005) traced the cultivar composition of monovarietal olive oils by AFLPs, suggesting that DNA extraction is the most critical step affecting the procedure. Pafundo *et al.* (2007) also performed amplification of DNA isolated from olive oil using AFLPs. Pasqualone *et al.* (2007), using SSR analysis, demonstrated that microsatellites are useful in checking the presence of a specific cultivar in a PDO oil, thus verifying the identity of the product. Montemurro *et al.* (2008) analyzed ten virgin monocultivar olive oils prepared in the laboratory by AFLP markers. Martins-Lopes *et al.* (2008) evaluated the efficiency of RAPD, ISSR, and SSR molecular markers for olive oil cultivar identification and their possible use in certification purposes. Consolandi *et al.* (2008) developed a ligation detection reaction (LDR)/universal array (UA) platform by using several olive SNPs to verify the origin and the authenticity of extra virgin olive oils. Pafundo *et al.* (2010) investigated the effect of the storage time on the degradation of the DNA purified from the oil; a negative correlation existed between storage time and quality–quantity of recovered DNA.

So far, application of real time as a tool for olive oil authentication has been explored by Giménez *et al.* (2010). The authors evidenced that real-time PCR (RT-PCR) is useful to quantify DNA extracted from oil and, thus, to assess the yields of different methods of extraction. However, the size of amplicon is critical for the success of analysis. A possibility of utilizing quantitative RT-PRC (qRT-PCR) to quantify cultivars in PDO oils rests on the use of taqMan probes designed on SNPs specific of cultivars entering the oil composition. Several sequences from noncoding spacer regions between psbA-trnH and partial coding region of matK of a plastid genome provided a good discrimination between pure olive oil and its admixture by other vegetable oils such as canola and sunflower. The plastid-based molecular DNA technology has a great potential to be used easily for rapid detection of adulteration up to 5% in olive oil (Kumar *et al.*, 2011).

In a recent publication reported by Papadia *et al.* (2011), a systematic effort to obtain genetic characterization by SSR amplification, soil analyses, and ^{1}H-NMR spectra is carried out in order to make a direct connection between the olive tree (genetic information) and the NMR spectra (chemical information) of the extra virgin olive oil produced. The results reported show that a multidisciplinary approach, through the application of multivariate statistical analysis, could be used to set up a method for cultivar and/or geographic origin certification based on the construction of a suitable database.

8.10 Sensory profile markers of traceability

The sensory profile of the oil is deeply influenced by the genetic matrix: the combined effect of the taste, odor, and chemical response that give rise to the sensation perceived as "flavor" (Kilcast, 2003). Volatile aromatic compounds responsible for the sensory characteristics perceived by assessors are present in the unsaponifiable fraction of extra virgin olive oils. Volatile and phenolic substances are responsible for aroma

and taste, respectively (Angerosa *et al.*, 2000). Organoleptic characteristics of olive oil represent the sum of sensory parameters, which integrate its complex and specific qualitative profile including various intrinsic and extrinsic factors, such as cultivar (genetic), weather conditions, stage of ripeness, methods and/or system of harvest, and storage. Regulations and standards related to olive oil sensory evaluation include trained assessors of different numbers and nationalities in panel tests, which score certain attributes in a given scale (structured vs. unstructured). Collaborative international studies (COIs) have developed the quantitative descriptive analysis (QDA) sensory methodology for virgin olive oils, known as the "COI-panel test." The latter defines an agreed-on specific vocabulary of sensory attributes, performs a uniform tasting technique, and eliminates all troubles that can compromise the sensory trial (Arvanitoyannis & Vlachos, 2007).

According to Angerosa *et al.* (1996), a high number (204) of Italian olive oil samples of various cultivars, ripeness, sanitary state, and geographical origin were evaluated from the sensory and chemical points of view, simultaneously. The sensory test took place according to the EC standardized method (2568/9/CEE) by at least eight fully trained panel testers. The chemical information based on dynamic headspace analysis of volatile fractions was monitored with an artificial neural network (ANN), using the back-propagation algorithm and applied to the headspace results with the aim of predicting panel test scores. Four European virgin olive oil cultivars ('Arbequina', 'Coratina', 'Koroneiki', and 'Picual') cultivated in Greece, Italy, and Spain were analyzed for their sensory attributes, among others. The evaluation took place by six panels of assessors of different nationalities (Aparicio *et al.*, 1997). The attributes studied were flavor, aroma, odor, taste, mouth feel, and after-mouth feel/aftertaste.

According to Stefanoudaki *et al.* (2000), olive oils obtained from three European olive cultivars, 'Coratina' (Italy), 'Picual' (Spain), and 'Koroneiki' (Greece), at a certain stage of maturity, were subjected to a sensory evaluation test by a trained panel. The panel consisted of 12 trained individuals, who carried out the sensory evaluation according to the standard COI/T20/Doc 3. Sixteen sensory attributes contributing to "green" perception were evaluated on an additional profile sheet. Virgin olive oil sampled in different production areas of Italy and obtained from a new olive cultivar, I-77, was analyzed giving quantitative results comparable to those of several traditional cultivars (Ranalli *et al.*, 2000). The quantitative descriptive sensory profiling was performed by a 12-member fully trained analytical taste panel according to the International Olive Oil Council (IOOC) and EC Regulation no. 2568/1991. Attribute perceptions were flavor, aroma, odor, taste, and after-mouth feel/aftertaste. The correlation of the sensory score with the total volatile content did not prove to be equally satisfactory. Caporale *et al.* (2006) explored the appropriateness of several sensory descriptors in evaluating the typical nature of certain extra virgin olive oils, how they assess the impact of information about the origin of the product on the sensory profile perception, and how the effect of sensory expectations can influence liking and typical nature of responses for the experimental oils obtained from a defined cultivar.

8.11 Conclusion

Consumers, nowadays, are very well informed about the benefits they may ensure for their health through their dietary habits. Therefore, it is extremely important for the food companies and particularly the olive oil companies to comply with the requirements of the international quality standards concerning quality assurance and product safety. The proper application of HACCP and traceability rules, as described in ISO 22000, provides quality assurance and safety of the virgin olive oil and preserves the functional ingredients of the product. These functional ingredients are the most important factors that influence the quality standards of an olive oil, helping it excel from the other edible oils. Some olive cultivars are recognized as producing higher quality of oil because they derive from well-defined geographical areas. These oils command better prices, and generally are legally protected. Indeed, the aim of PDOs, PGIs, and TSG is to add value to certain specific high-quality products from a particular origin. Instrumental advances have led to greater success in the development of traceability. The development of accurate analytical fingerprinting methods for the authentication of olive oils and for the certification of the geographical origin is an actual issue and an important challenge. However, geographical traceability is still in need of intense research to incorporate new "omic" procedures for better characterization and evaluation of olive oil functional compounds. The information obtained from these and other procedures should be compiled to build a large database that would make it possible to determine the geographical origin of the most representative olive oils around the

world with great functionality and avoid the in-vogue tree structure designs that lack mathematical support and might lead to more problems than solutions.

References

Agrimonti, C., Vietina, M., Pafundo, S., & Marmiroli, N. (2011) The use of food genomics to ensure the traceability of olive oil. Trends in Food Science & Technology 22, 237–244.

Alfaro, J.A., & Rábade, L.A. (2009) Traceability as a strategic tool to improve inventory management: a case study in the food industry. International Journal of Production Economics 118 (1), 104–110.

Allalout, A., Krichène, D., Methenni, K., Taamalli, A., Oueslati, I., & Daoud D. (2009) Characterization of virgin olive oil from super intensive Spanish and Greek grown in northern Tunisia. Scientia Horticulturae 120, 77–83.

Alonso-Garcia, M.V., & Aparicio-Lopez, R. (1993) Characterization of European virgin olive oils using fatty acids. Grasas y Aceites 44 (1), 18–24.

Andejelkovic, M. Van Camp, J., Pedra, M., Renders, K., Socaciu, C., & Verhé, R. (2008) Correlations of the phenolic compounds and the phenolic content in some Spanish and French olive oils. Journal of Agricultural and Food Chemistry 56, 5181–5187.

Andrewes, P., Busch, J.L.H.C., De Joode, T., Groenewegen, A., & Alexandre, H. (2003) Sensory properties of virgin olive oil polyphenols: identification of deacetoxyligstroside aglycon as a key contributor to pungency. Journal of Agricultural and Food Chemistry 51, 1415–1420.

Angerosa, F., Breas, O., Contento, S., Gillou, C., Reniero, F., & Sada, E. (1999) Application of stable isotope ratio analysis to the characterization of the geographical origin of olive oils. Journal of Agricultural and Food Chemistry 47, 1013–1017.

Angerosa, F., Di Giacinto, L., Vito, R., & Cumitini, S. (1996) Sensory evaluation of virgin olive oils by artificial neural network processing of dynamic head-space gas chromatographic data. Journal of the Science of Food and Agriculture 72, 323–328.

Angerosa, F., Mostallino, R., Basti, C., & Vito, R. (2000) Virgin olive oil odour notes: their relationships with volatile compounds from lipoxygenase pathway and secoiridoid compounds. Food Chemistry 68, 283–287.

Angioni, A., Cabitza, M., Russo, M.T., & Caboni, P. (2006) Influence of olive cultivars and period of harvest on the contents of Cu, Cd, Pb, and Zn in virgin olive oils. Food Chemistry 99(3), 525–529.

Aparicio, R., & García-González D.L. (2013) Olive oil characterization and traceability. In: Handbook of olive oil: analysis and properties, ed. R. Aparicio & J. Harwood. Springer Science, New York.

Aparicio, R., Morales, M.T., & Alonso, V. (1997) Authentication of European virgin olive oils by their chemical compounds, sensory attributes, and consumers' attitudes. Journal of Agriculture and Food Chemistry 45, 1076–1083.

Aranda, F., Gòmez-Alonso, S., Rivera Del Alamo, R.M., Salvador, M.D., & Fregapane, G. (2004) Triglyceride, total and 2-position fatty acid composition of cornicabra virgin olive oil: comparison with other Spanish cultivars. Food Chemistry 86, 485–492.

Arvanitoyannis, I.S. (2009) HACCP and ISO 22000 application to foods of animal origin. Blackwell Publishing Ltd., Oxford.

Arvanitoyannis, I.S., & Vlachos, A. (2007) Implementation of physicochemical and sensory analysis in conjunction with multivariate analysis towards assessing olive oil authentication/adulteration. Critical Reviews in Food Science and Nutrition 47, 441–498.

Aung, M.M., & Chang, Y.S. (2014) Traceability in a food supply chain: safety and quality perspectives. Food Control 39, 172–184.

Baccouri, O., Guerfel, M., Baccouri, B., Cerretani, L., Bendini, A., Lercker, G., Zarrouk, M., & Daoud Ben Miled, D. (2008) Chemical composition and oxidative stability of Tunisian mono-varietal virgin olive oils with regard to fruit ripening. Food Chemistry 109, 743–754.

Bendini, A., Bonoli, M., Cerretani, L., Biguzzi, B., Lercker, G., & Gallina-Toschi, T. (2003) Liquid–liquid and solid-phase extractions of phenols from virgin olive oil and their separation by chromatographic and electrophoretic methods. Journal of Chromatography A 985, 425–433.

Bertran, E., Blanco, M., Coello, J., Iturriaga, H., Maspoch, S., & Montolin, I. (2000) Near-infrared spectrometry and pattern recognition as screening methods for the authentification of virgin olive oils of very close geographical origin. Journal of Near Infrared Spectroscopy 8(1), 45–52.

Bonoli, M., Bendini, A., Cerretani, L., Lercker, G., & Toschi, T.G. (2004) Qualitative and semiquantitative analysis of phenolic compounds in extra virgin olive oils as a function of the ripening degree of olive fruits by different analytical techniques. Journal of Agriculture and Food Chemistry 52, 7026–7032.

Bonoli, M., Montanucci, M., Gallina Toschi, T., & Lercker, G. (2003) Fast separation and determination of tyrosol, hydroxytyrosol and other phenolic compounds in extra-virgin olive oil by capillary zone electrophoresis with ultraviolet-diode array detection. Journal of Chromatography A 1011, 163–172.

Bosona, T., & Gebresenbet, G. (2013) Food traceability as an integral part of logistics management in food and agricultural supply chain. Food Control 33, 32–48.

Brenes, M., García, A., García, P., & Garrido, A. (2000) Rapid and complete extraction of phenols from olive oil and determination by means of a coulometric electrode array system. Journal of Agricultural and Food Chemistry 48, 5178–5183.

Breton, C., & Berville, A. (2009) The life history of the olive tree examined through molecular marker data. In: Advances in olive resources, ed. L. Berti & J. Maury. InTech, Kerala, India.

Bucci, R., Magri, A.D., Magri, A.L., Marini, D., & Marini, F. (2002) Chemical authentification of extra virgin olive oil by supervised chemometric procedures. Journal of Agricultural and Food Chemistry 50, 413–418.

Buchgraber, M., Ulberth, F., Emons, H., & Anklam, E. (2004) Triacylglycerol profiling by using chromatographic techniques. European Journal of Lipid Science and Technology 106, 621–648.

Busconi, M., Foroni, C., Corradi, M., Bongiorni, C., Cattapan, F., & Fogher, C. (2003) DNA extraction from olive oil and its use in the identification of the production cultivar. Food Chemistry 83 (1), 127–134.

Cajka, T., Riddellova, K., Klimankova, E., Cerna, M., Pudil, F., & Hajslova, J. (2010) Traceability of olive oil based on volatiles pattern and multivariate analysis. Food Chemistry 121, 282–289.

Caporale, G., Policastro, S., Carlucci, A., & Monteleone, E. (2006) Consumer expectations for sensory properties in virgin olive oils. Food Quality and Preference 17, 116–125.

Carrasco-Pancorbo, A., Gomez-Caravaca, A.M., Cerretani, L., Bendini, A., Segura-Carretero, A., & Fernandez-Gutierrez, A. (2006) Rapid quantification of the phenolic fraction of Spanish virgin olive oils by capillary electrophoresis with UV detection. Journal of Agricultural and Food Chemistry 54, 7984–7991.

Cerretani, L., Bendini, A., Del Caro, A., Piga, A., Vacca, V., Caboni, M.F., & Gallina Toschi, T. (2006) Preliminary characterisation of virgin olive oils obtained from different cultivars in sardinia. European Food Research and Technology 222, 54–61.

Chatziantoniou, S.E., Triantafillou, D.J., Karayannakidis, P.D., & Diamantopoulos, E. (2014) Traceability monitoring of Greek extra virgin olive oil by differential scanning calorimetry. Thermochimica Acta 576, 9–17.

Christophoridou, S., & Dais, P. (2009) Detection and quantification of phenolic compounds in olive oil by high resolution ^{1}H nuclear magnetic resonance spectroscopy. Analytica Chimica Acta 633, 283–292.

Cichelli, A., & Pertesana, G.P. (2004) High-performance liquid chromatographic analysis of chlorophylls, pheophytins and carotenoides in virgin olive oils: chemometric approach to classification. Journal of Chromatography A 1046, 141–146.

Consolandi, C., Palmieri, L., Severgnini, M., Maestri, E., Marmiroli, N., Agrimonti, C., Baldoni, L., Donini, P., De Bellis, G., & Castiglioni, G. (2008) A procedure for olive oil traceability and authenticity: DNA extraction, multiplex PCR and LDR-universal array analysis. European Food Research and Technology 227, 1429–1438.

Cresti, M., Linskens, H.F., Mulcahy, D.L., Bush, S., Di Stilio, V., Xu M.Y., Vigani, R., & Cimato, A. (1997) Comunicación preliminar sobre la identificación del DNA de las hojas y el aceite de oliva de *Olea europaea*. Olivae 69, 36–37.

De La Torre, F., Bautista, R., Cánovas, F.M., & Claros, G. (2004) Isolation of DNA from olive oil and oil sediments: application in oil fingerprinting. Journal of Food Agriculture and Environment 2(1), 84–89.

Di Giovacchino, L., Costantini, N., Serraiocco, A. Surricchio, G., & Basti, C. (2001) Natural antioxidants and volatile compounds of virgin olive oils obtained by two or three-phases centrifugal decanters. European Journal of Lipid Science & Technology 103(5), 279–285.

Di Giovacchino, L., Sestili, S., & Di Vincenzo, D. (2002) Influence of olive processing on virgin olive oil quality. European Journal of Lipid Science & Technology 104(5), 587–601.

Doveri, S., O'Sullivan, D.M., & Lee, D. (2006) Non-concordance between genetic profiles of olive oil and fruit: a cautionary note to the use of DNA markers for provenance testing. Journal of Agricultural and Food Chemistry 54(24), 9221–9226.

Dugo, G., La Pera, L., Giuffrida, D., Salvo, F., & Lo Turco, V. (2004) Influence of the olive and the zone of provenience on selenium content determined by cathodic stripping potentiometry (CSP) in virgin olive oil. Food Chemistry 88(1), 135–140.

El-Sayed, W.M., Abou-Zaid, F.O.F., El-Kalyoubi, M.H., & Abd El-Razik, M.M. (2015) Hazard Analysis Critical Control Points (HACCP) application during olive oil centrifugal extraction. Journal of Environmental Science, Toxicology and Food Technology 9(1), 50–56.

Escanciano, C., & Santos-Vijande M.L. (2014) Reasons and constraints to implementing an ISO 22000 food safety management system: evidence from Spain. Review. Food Control 40(6), 50–57.

Fernández-Segovia, I., Pérez-Llácera, A., Peidrob, B., & Fuentesa, A. (2014) Implementation of a food safety management system according to ISO 22000 in the food supplement industry: a case study. Food Control 43(9), 28–34.

Galvão, J.A., Margeirsson, S., Garate, C., Viðarsson, J.R., & Oetterer, M. (2010) Traceability system in cod fishing. Food Control 21, 1360–1366.

Gan, H.L., Che Man, Y.B., Tan, C.P., Noraini, I., & Nazimah, S.A.H. (2005) Characterisation of vegetable oils by surface acoustic wave sensing electronic nose. Food Chemistry 89, 507–518.

García, A., Brenes, M., García, P., Romero, C., & Garrido, A. (2003) Phenolic content of commercial olive oils. European Food Research and Technology 216, 520–525.

García, A., Brenes, M., Romero, C., García, P., & Garrido, A. (2002) Study of phenolic compounds in virgin olive oils of the picual variety. European Journal on Food Research Technology 215(5), 407–412.

Giménez, M.J., Pistón, F., Martín, A., & Atienza, S.G. (2010) Application of real-time PCR on the development of molecular markers and to evaluate critical aspects for olive oil authentication. Food Chemistry 118, 482–487.

Giuffrida, D., Salvo, F., Salvo, A., La Pera, L., & Dugo, G. (2007) Pigments composition in monovarietal virgin olive oils from various Sicilian olive varieties. Food Chemistry 101, 833–837.

Golan, E., Krissoff, B., Kuchler, F., Calvin, L., Nelson, K., & Price, G. (2004) Traceability in the U.S. food supply: economic theory and industrial studies. Agricultural Economic Report no. 830.

Guadarrama, A., Rodríguez-Méndez, M.L. De Saj, J.A., Ríos, J.L., & Olías, J.M. (2000) Array of sensors based on conducting polymers for the quality control of the aroma of the virgin olive oil. Sensors and Actuators: B Chemical 69, 276–282.

Ho, S.K.M. (1994) Is the ISO 9000 series for total quality management? International Journal 11 (9), 74–89.

ISO. (1994) ISO 8402. Retrieved from http://www.scribd.com/doc/40047151/ISO-8402-1994-ISO-definitions

ISO. (2005) ISO 9000. Retrieved from http://www.pqm-online.com/assets/files/standards/ISO-9000-2005.pdf

Jimenez, M.S., Lopez, A., & Castillo, J.R. (2002) Automatic emulsion formation as a sample introduction system for the GFAAS determination of iron in edible and mineral oils. Atomic Spectroscopy 23(6), 183–189.

Jimenez, M.S., Velarte, R., Gomez, M.T., & Castillo, J.R. (2004) Multi-element determination using on-line emulsion formation and ICP-MS/FAAS for the characterization of virgin olive oils by principal component analysis. Atomic Spectroscopy 25(1), 1–12.

Kalogeropoulos, N., Kaliora, A.C., Artemiou A., & Giogios, I. (2014) Composition, volatile profiles and functional properties of virgin olive oils produced by two-phase vs three-phase centrifugal decanters. Food Science and Technology 58(1), 272–279.

Kilcast, D. (2003) Sensory science. Chemistry in Britain 39, 62–64.

Kiritsakis, A. (2007) Olive oil: conventional and organic, 4th ed. Copy City Digital, Thessaloniki Greece, 247–321. (In Greek)

Kumar, S., Kahlon, T., & Chaudhary, S. A (2011) Rapid screening for adulterants in olive oil using DNA barcodes. Food Chemistry 127, 1335–1341.

Leardi, R., & Paganuzzi, V. (1987) Characterization of the origin of extra virgin olive oils by chemometric methods applied to the sterols fraction. Rivista Italiana delle Sostanze Grasse 64, 131–136.

Lerma-García, M.J., Simó-Alfonso, E.F., Ramis-Ramos, G., & Herrero-Martínez, J.M. (2008) Rapid determination of sterols in vegetable oils by CEC using methacrylate ester-based monolithic columns. Electrophoresis 29, 4603–4611.

Longobardi, F., Ventrella, A., Napoli, C., Humpfer, E., Schütz, B., Schäfer, H., Kontominas, M.G., & Sacco, A. (2012) Classification of olive oils according to geographical origin by using ^{1}H NMR fingerprinting combined with multivariate analysis. Food Chemistry 130, 177–183.

Mahjoub-Haddada, F., Manai, H., Daoud, D., Fernandez, X., Lizzani-Cuvelier, L., & Zarrouk, M. (2007) Profiles of volatile compounds from some monovarietal Tunisian virgin olive oils. Comparison with French PDO. Food Chemistry 103, 467–476.

Mannina, L., Dugo, G., Salvo, F., Cicero, L., Ansanelli, G., Calcagni, C., & Segre, A. (2003) Study of the cultivar composition relationship in Sicilian olive oils by GC, NMR and statistical methods. Journal of Agriculture and Food Chemistry 51, 120–127.

Mannina, L., Patumi, M., Proietti, N., Bassi, D., & Segre, A.L. (2001) Geographical characterization of Italian extra virgin olive oil using high field ^{1}H NMR Spectroscopy. Journal of Agricultural and Food Chemistry 49, 2687–2696.

Mavromoustakos, T., Zervou, M., Theodoropoulou, E., Panagiotopoulos, D., Bonas, G., Day, M., & Helmis, A. (1997) ^{13}CNMR Analysis of the triacylglycerol composition of Greek virgin olive oils. Magnetic Resonance in Chemistry 35, S3–S7.

Mckean, J.D. (2001) The importance of traceability for public health and consumer protection. Scientific and Technical Review 20(2), 363–371.

Meier-Augenstein, W. (2002) Stable isotope analysis of fatty acids by gas chromatography-isotope ratio mass spectrometry. Analytica Chimica Acta 465, 63–79.

Minguez-Mosquera, M.I., Gandul-Rojas, B., & Lourdes Gallardo-Guerrero, M. (1992) Rapid method of quantification of chlorophylls and carotenoids in virgin olive oil by high-performance liquid chromatography. Journal of Agriculture and Food Chemistry 40, 60–63.

Moe, T. (1998) Perspectives on traceability in food manufacture. Trends in Food Science & Technology 9(5), 211–214.

Montealegre, C., Alegre, M.L., & Garcia-Ruiz, C. (2010) Traceability markers to the botanical origin in olive oils. Journal of Agricultural and Food Chemistry 58, 28–38.

Montemurro, C., Pasqualone, A., Simeone, R., Sabetta, W., & Blanco, A. (2008) AFLP molecular marker to identify virgin olive oils from single Italian cultivars. European Food Research and Technology 226, 1439–1444.

Moral, J., De Oliveira, R., & Trapero, A. (2009) Elucidation of the disease cycle of olive anthracnose caused by colletotrichum acutatum. Ecology and Epidemiology 99(5), 548–556.

Morales, M.T., Aparicio, R., & Rios, J.J. (1994) Dynamic headspace gas chromatographic method for determining volatiles in olive oil. Journal of Chromatography A 668, 455–462.

Ocakoglu, D., Tokatli, F., Ozen, B., & Korel, F. (2009) Distribution of simple phenols, phenolic acids and flavonoids in Turkish monovarietal extra virgin olive oils for two harvest years. Food Chemistry 113, 401–410.

Ollivier, D., Artaud, J., Pinatel, C., Durbec, J.P., & Guérère, M. (2006) Differentiation of french virgin olive oil rdos by sensory characteristics, fatty acid and triacylglycerol compositions and chemometrics. Food Chemistry 97, 382–393.

Ordano, M., Engelhard, I., Rempoulakis, P., Nemny-Lavy, E., Blum, M., & Yasin, S. (2015) Olive fruit fly (*Bactrocera oleae*) population dynamics in the Eastern Mediterranean: influence of exogenous uncertainty on a monophagous frugivorous insect. PLoS ONE 10(5), 1–18. Available from: http://journals.plos.org

Pafundo, S., Agrimonti, C., & Marmiroli, N. (2005) Traceability of plant contribution in olive oil by amplified fragment length polymorphisms. Journal of Agricultural and Food Chemistry 53, 6995–7002.

Pafundo, S., Agrimonti, C., Maestri, E., & Marmiroli, N. (2007) Applicability of SCAR markers to food genomics: olive oil traceability. Journal of Agriculture and Food Chemistry 55, 6052–6059.

Pafundo, S., Busconi, M., Agrimonti, C., Fogher, C., & Marmiroli, N. (2010) Storage-time effects on olive oil DNA assessed by amplified fragments length polymorphisms. Food Chemistry 123, 787–793.

Palmieri, L., Doveri, S., Marmiroli, N., & Donini, P. (2004) Molecular marker characterisation of olive oil variety composition and SNP development in olive. Proceedings of XII international plant and animal genome conference. 2004, San Diego, CA.

Papadia, P., Del Coco, L., Muzzalupo, I., Rizzi, M., Perri, E., Cesari, G., Simeone, V., Mondelli, F., Schena, F.P., & Fanizzi, F.P. (2011) Multivariate analysis of ^{1}H-nmr spectra of genetically characterized extra virgin olive oils and growth soil correlations. Journal of the American Oil Chemists' Society 88 (10), 1463–1475.

Pardo, J.E., Pérez, J.J., Andrés, M., & Alvarruiz, A. (2002) Application of the hazard analysis and critical control point (HACCP) system in the processing line of virgin olive oil. Grasas y Aceites 53 (3), 309–318.

Pardo, J.E., Perez, J.I., Sanchez, J.E., Andres, M., & Alvarruiz, A. (2003) Application of the hazard analysis and critical control point (HACCP) system in a bottling line of virgin olive oil. Grasas y Aceites 53 (1), 58–64.

Pasqualone, A., Montemurro, C., Summo, C., Sabetta, W., Caponio, F., & Blanco, A. (2007) Effectiveness of microsatellite DNA markers in checking the identity of protected designation of origin extra virgin olive oil. Journal of Agriculture and Food Chemistry 55, 3857–3862.

Perri, E., Benincasa, C., & Muzzalupo, I. (2012) Olive oil traceability. In: Olive germplasm: the olive cultivation, table olive and olive oil industry in Italy, ed. I. Muzzalupo. InTech, Kerala, India.

Psomiadou, E., & Tsimidou, M. (2001) Pigments in Greek virgin olive oils: occurrence and levels. Journal of the Science of Food and Agriculture 81, 640–647.

Ranalli, A., Modesti, G., Patumi, M., & Fontanazza, G. (2000) The compositional quality and sensory properties of virgin olive oil from a new olive cultivar i-77. Food Chemistry 69, 37–46.

Ranalli, A., Pollastri, L., Contento, S., Di Loreto, G., Iannucci, E., Lucera, L., & Russi, F. (2002) Acylglycerol and fatty acid components of pulp, seed and whole olive fruit oils. Their use to characterize fruit cultivar by chemometrics. Journal of Agriculture and Food Chemistry 50, 3775–3779.

Regattieri, A., Gamberi, M., & Manzini, R. (2007) Traceability of food products: General framework and experimental evidence. Journal of Food Engineering 81, 347–356.

Reiners, J., & Grosch, W. (1998) Odorants of virgin olive oils with different flavor profiles. Journal of Agricultural and Food Chemistry 46, 2754–2763.

Rijswijk, W.V., & Frewer, L.J. (2006) How consumers link traceability to food quality and safety: An international investigation. 98th EAAE seminar, Marketing dynamics within the global trading system: new perspectives, Greece.

Rodríguez-Méndez, M.L., Apetrei, C., & De Saja, J.A. (2008) Evaluation of the polyphenolic content of extra virgin olive oils using an array of voltammetric sensors. Electrochimica Acta 53, 5867–5872.

Saitta, M., Salvo, F., Di Bella, G., Dugo, G., & La Torre, G.L. (2009) Minor compounds in the phenolic fraction of virgin olive oils. Food Chemistry 112, 525–532.

Servili, M., & Montedoro, G. (2002) Contribution of phenolic compounds to virgin olive oil quality. European Journal of Lipid Science and Technology 104, 602–613.

Servili, M., Esposto, S., Veneziani, G., Urbani, S., Taticchi, A., Di Maio, I., Selvaggini, R., Sordini, B., & Montedoro, G. (2011) Improvement of bioactive phenol content in virgin olive oil with an olive-vegetation water concentrate produced by membrane treatment. Food Chemistry 124(7), 1308–1315.

Stefanoudaki, E., Kotsifaki, F., & Koutsaftakis, A. (1999) Classification of virgin olive oils of the two major Cretan cultivars based on their fatty acid composition. Journal of the American Oils Chemists' Society 76(5), 623–626.

Stefanoudaki, E., Kotsifaki, F., & Koutsaftakis, A. (2000) Sensory and chemical profiles of three European olive varieties (*Olea europea* L.): an approach for the characterization and authentication of the extracted oils. Journal of the Science of Food and Agriculture 80, 381–389.

Stella, R., Barisci, J.N., Serra, G., Wallace, G.G., & De Rossia, D. (2000) Characterisation of olive oil by an electronic nose based on conducting polymer sensors. Sensors and Actuators: B Chemical 63, 1–9.

Tena, N., Lazzez, A., Aparicio-Ruiz, R., & Garcia-Gonzalez, D.L. (2007) Volatile compounds characterizing Tunisian Chemlali and Chetoui virgin olive oils. Journal of Agriculture and Food Chemistry 55, 7852–7858.

Torres Vazfreire, L., Gomes Da Silva, M.D.R., & Costa Freitas, A.M. (2009) Comprehensive two-dimensional gas chromatography for fingerprint pattern recognition in olive oils produced by two different techniques in Portuguese olive varieties Galega vulgar, Cobrancosa and Carrasquenha. Analytica Chimica Acta 633, 263–270.

Tsimidou, M., Macrae, M., & Wilson, I. (1987) Authentification of virgin olive oils using principal component analysis of triglyceride and fatty acid profiles. Part I. Classification of Greek oils. Food Chemistry 25, 227–239.

Tura, D., Failla, O., Bassi, D., Pedo, S., & Serraiocco, A. (2007) Cultivar influence on virgin olive (*Olea europaea* L.) Oil flavor based on aromatic compounds and sensorial profile. Science Horticulture 2008(118), 139–148.

Tura, D., Gigliotti, C., Pedo, S., Failla, O., Bassi, D., & Serraiocco, A. (2007) Influence of cultivar and site of cultivation on levels of lipophilic and hydrophilic antioxidants in virgin olive oils (*Olea europea* l.) and correlations with oxidative stability. Scientia Horticulturae 112, 108–119.

UK Food Standard Agency (FSA). (2002) Traceability in the food chain: A preliminary study. Retrieved from www.food.gov.uk/multimedia/pdfs.

Vichi, S., Pizzale, L., Conte, L.S., Buxaderas, S., & López-Tamames, E. (2005) Simultaneous determination of volatile and semi-volatile aromatic hydrocarbons in virgin olive oil by headspace solid-phase microextraction coupled to gas chromatography/mass spectrometry. Journal of Chromatography A 1090, 146–154.

Vlahov, G., Del Re, P., & Simone, N. (2003) Determination of geographical origin of olive oils using ^{13}C Nuclear Magnetic Resonance Spectroscopy. I – Classification of olive oils of the Puglia region with denomination of protected origin. Journal of Agricultural and Food Chemistry 51, 5612–5615.

Wilson, T.P., & Clarke, W.R. (1998) Insights from industry food safety and traceability in the agricultural supply chain: Using the internet to deliver traceability. Supply Chain Management 3(3), 127–133.

Zeiner, M., Steffan, I., & Juranovic Cindric, I. (2005) Determination of trace elements in olive oil by ICP-AES and ETA-AAS: a pilot study on the geographical characterization. Microchemical Journal 81, 171–176.

Zunin, P. (2004) Direct thermal extraction and gas chromatographic–mass spectrometric determination of volatile compounds of extra virgin olive oils. Journal of Chromatography A 1023, 271–276.

9 Integrated olive mill waste (OMW) processing toward complete by-product recovery of functional components

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9.1 Introduction

Olive oil extraction is an ancient agricultural industry all over the Mediterranean area, and to date it is of much economic importance for many countries. This agro-industrial activity generates large amounts of highly offensive waste (olive mill waste, or OMW) that is usually unexploited and in most cases poses a severe threat to the environment.

There are two ways of extracting the oil: traditional pressing, which has been used for many centuries with only minor modifications; and centrifugation, which the olive oil industry has taken over in recent decades. There are also two centrifugation systems, called three-phase and two-phase systems, which are described in Figure 9.1. Even though traditional pressing is a relatively obsolete technology, it is still in use by some olive oil producers. After extraction by pressing, a solid fraction called “olive husk” is obtained as a by-product along with an emulsion containing the olive oil that is separated by decanting from the remaining olive mill wastewater (OMWW). The continuous three-phase extraction process, which is the predominant process in modern olive mills, generates two streams of waste: a wet solid cake (~30% of raw material weight) called “orujo” or “olive cake,” and a watery liquid (50% of raw material weight) called “alpechin” or OMWW (Tsagaraki *et al.*, 2007). In spite of the clear advantages of this system compared to pressing (i.e., complete automation, better oil quality, and smaller area required), it also presents some negative aspects, such as greater water and energy consumption, higher wastewater production, and more expensive installations (Roig *et al.*, 2006). At the end of the 1991–1992 olive oil campaign, a new centrifugation system was developed that reduced the OMW by 75%. This system was launched to the market as “ecological” because of the reduction in water consumption, and as a “two-phase” system because it produced two fractions: a solid one called “alperujo” (also called “olive wet husk,” “wet pomace,” or “two-phase olive mill waste” [TPOMW]) and a liquid one (olive oil). The different by-products resulting from the olive oil extraction, depending on the extraction system utilized, are summarized in Table 9.1.

At present, olive cake is processed in seed oil factories in order to extract the small amount of oil remaining in the waste. Both crude and exhausted olive cake can be used as solid fuels, added to animal feed as a supplement, or returned to the olive grove as mulch. While economic concerns regarding the profitability of seed oil production now are being questioned, the problem of disposal of olive cake is adequately addressed by one of these three alternatives (Tsagaraki *et al.*, 2007). However, when TPOMW was intended to be treated similarly, great difficulties appeared because of its high moisture and carbohydrate concentration. Thus, the alperujo tends to stick to the furnace walls, blocking the gaseous stream and causing an explosion hazard (Arjona *et al.*, 1999). Besides, because of its high moisture, the drying process demands a lot of energy that significantly increases costs (Roig *et al.*, 2006).

As far as OMWW treatment and disposal are concerned, the situation is more complicated. Attempts to alleviate the problem started more than 50 years ago and as of yet there has been little success in finding an

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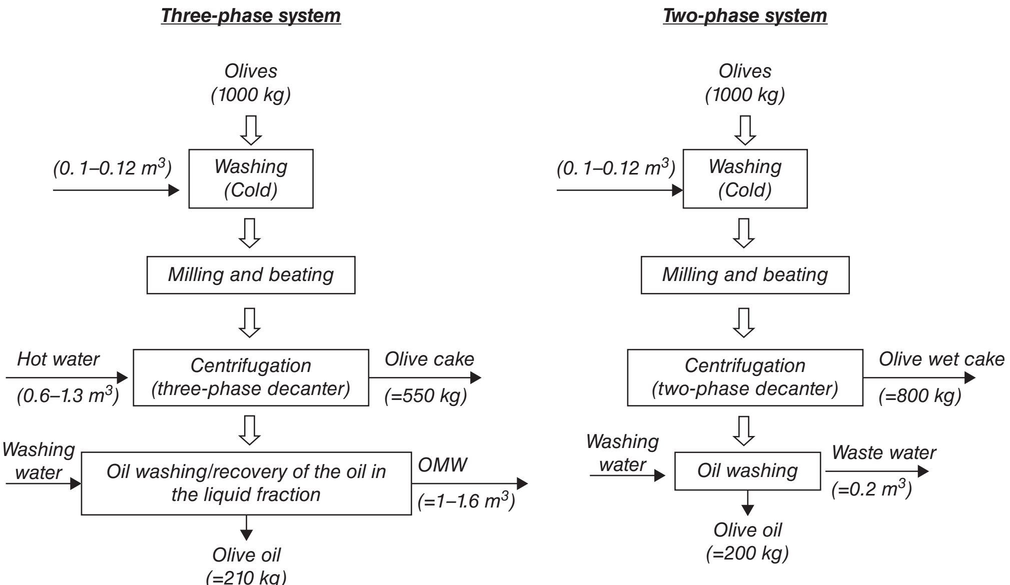

Figure 9.1 Three- and two-phase centrifugation systems. *Source*: Alburquerque *et al.* (2004). Reproduced with permission of Elsevier.

environmentally friendly and economically viable solution to be generally adopted. OMWW is an aqueous, dark, foul-smelling, and turbid liquid, which includes emulsified grease; it is easily fermentable and has a high organic content. It is an extremely offensive food waste stream due to several negative effects, such as:

- high phytotoxicity with strong negative impact on soil quality and plant growth, due to phenolic compounds, low pH, and toxic fatty acids;
- strong discoloration and pollution of natural waters, resulting in surface water and groundwater pollution;
- threatening the aquatic life; and
- problems with offensive odors.

The management of OMWW is a very important issue in Mediterranean countries, where more than 2.4 million tons of olives are produced per year (95% of the total world production), 90% of which is for olive oil production. For three-phase olive mills, the wastewater produced is estimated to be 1.1–1.5 times the weight of milled olives (Paraskeva *et al.*, 2007). The olive producers operate on a seasonal basis, and the production units are small; therefore, as a rule, they do not process the liquid effluents from

Table 9.1 Olive oil extraction by-products.

Production system	Inputs	Outputs
Traditional pressing	Olives (1000 kg) Washing water (100–120 kg)	Oil (200 kg) Solid waste (400 kg) Wastewater (600 kg)
Two–phase system	Olives (1000 kg) Washing water (100–120 kg)	Oil (200 kg) Solid waste (800–950 kg)
Three–phase system	Olives (1000 kg) Washing water (100–120 kg) Mixing water (500–1000 kg)	Oil (200 kg) Solid waste (500–600 kg) Wastewater (1000–1200 kg)

the production process. The most common practice followed today is the disposal of OMWW in nearby aquatic receivers like streams, rivers, lakes, and even the sea. More specifically, 58% of the olive oil mills dispose their wastewater into streams, which end up in larger water reservoirs; 12% dispose it directly into the sea; while 19% ends up on the soil. As a result, severe environmental problems are caused. Studies focusing on the characterization and evolution of a soil affected by OMWW disposal showed that, even after two years of sediment removal, residual contamination levels are still considerable in the upper 40 cm of soil (Sierra *et al.*, 2001). The fact that olive mills are mostly located close to the sea, where most of the olive orchards are, indicates the magnitude of negative socioeconomic impacts to areas of high touristic interest, such as sea resorts.

Several methods have been proposed for treating OMW. These methods can be grouped in three categories:

1. *physical and physicochemical processes*: thermal processes (heat concentration, gasification, and combustion), flocculation, adsorption, separation processes, and ozonation
2. *biological treatments*: aerobic and anaerobic processes
3. *coupled physicochemical and biological treatments*.

The above treatment methods belong to a one-dimensional waste treatment approach, which is depollution. They also share the same obstacle of the high financial burden of depollution, especially considering the small size of most olive mills. A practical way to overcome this obstacle is to develop waste treatment schemes that combine depollution with recovery of valuable by-products or ingredients. In such processes, the income from by-products could pay for the cost of waste treatment (depollution).

9.2 Characterization of olive mill waste

9.2.1 Chemical composition

The properties of wastewaters and solid residues are summarized in Table 9.2.

Olive mill wastewaters from the olive oil industry have the following general properties (Azbar *et al.*, 2004):

- ✓ High organic content
- ✓ Acidic character with pH values between 3.0 and 5.9
- ✓ High concentrations of phenolic compounds (up to 80 g/L)
- ✓ High content of solid matter (total solids up to 20 g/L).

Generally, the main characteristic of OMWW is the presence of organic compounds such as organic acids, lipids, alcohols, and polyphenols that turn OMWW into phytotoxic material, representing a great environmental hazard when it is not properly managed. Chemical oxygen demand (COD) values normally range from 1.9 to 220 kg/m^3 (Alba, 1994; Davies *et al.*, 2004); the biochemical oxygen demand (BOD_5) is not commonly determined, but it is found to vary from 16.0 to 93.5 kg/m^3 (Fernandéz & García, 1989; Saviozzi *et al.*, 1991). In the same context, the total solids (TS) varied from 5.9 to 103.2 kg/m^3, whereas the volatile solids (VS) varied from 2.4 to 89.9 kg/m^3 (Hamdi & Garcia, 1991; Alba, 1994). However, OMWW also contains valuable matters such as a high organic matter concentration and a number of nutrients, especially potassium that could be recycled as a potential fertilizer.

OMWW also contains various amounts of sugars depending on the cultivar of olives, the climatic conditions during growth, and the extraction methods used. Sugar levels are generally 1.6–4.0% (w/v), but they can be higher in rare cases. The sugars constitute up to 60% of the dry substance, and in decreasing order they consist of fructose, mannose, glucose, saccharose, and traces of sucrose and pentose (Fernandez-Bolanos *et al.*, 1983).

Phenolic compounds are present in OMWW at concentrations of 0.5 to 24.0 g/L and are strictly dependent on the processing system used for olive oil production (Russo, 2007). So far, more than 30 phenolic compounds have been identified in OMWW. According to El-Abbassi *et al.* (2012), flavonoids correspond to about 45–65% of OMWW phenolic content. Main flavonoid subgroups in OMWW are flavanols and

Table 9.2 Characteristics of olive mill wastewaters (OMWWs) and solid residues.

	Olive mill by-product			
Characteristic	**OMWW**	**Olive cake**	**TPOMW**	**References**
pH	2.2–5.9		4.9–6.8	Galiatsatou *et al.* (2002), Dermeche *et al.* (2013)
Total carbon (%)	2.0–3.3	29.0–42.9	25.4	Vlyssides *et al.* (1998), Garcia-Castello *et al.* (2010)
Organic matter (%)	57.2–62.1	85.0	60.3–98.5	Aktas *et al.* (2001), Vlyssides *et al.* (2004)
Total nitrogen (%)	0.63	0.2–0.3	0.25–1.85	Saviozzi *et al.* (2001), Di Giovacchino *et al.* (2006), Dermeche *et al.* (2013)
Ash (%)	1.0	1.7–4.0	1.4–4.0	Vlyssides *et al.* (1998), Di Giovacchino *et al.* (2006), Lafka *et al.* (2011)
Lipids (%)	0.03–4.25	3.50–8.72	3.76–18.00	Vlyssides *et al.* (1998), Paredes *et al.* (1999), Di Giovacchino *et al.* (2006), Dermeche *et al.* (2013)
Total sugars (%)	1.50–12.22	0.99–1.38	0.83–19.30	Vlyssides *et al.* (1998), Caputo *et al.* (2003), Vlyssides *et al.* (2004)
Total proteins (%)		3.43–7.26	2.87–7.20	Vlyssides *et al.* (1998), Alburquerque *et al.* (2004)
Total phenols (%)	0.63–5.45	0.200–1.146	0.40–2.43	Vlyssides *et al.* (1998), Caputo *et al.* (2003), Dermeche *et al.* (2013)
Cellulose (%)		17.37–24.14	14.54	Vlyssides *et al.* (1998)
Hemicellulose (%)		7.92–11.00	6.63	Vlyssides *et al.* (1998)
Lignin (%)		0.21–14.18	8.54	Vlyssides *et al.* (1998)

TPOMW, Two-phase olive mill waste.

proanthocyanidins. Hydroxytyrosol is the most abundant phenolic compound in OMWW and represents about 55–70% of the total phenolic concentration of OMWW (El-Abbassi *et al.*, 2012), whereas gallic acid accounts for 0.3–0.6 g of tyrosol equivalents/L in OMWW samples (Obied *et al.*, 2008).

As far as the inorganic compounds are concerned, the content, composition, and physicochemical status of metal cations and inorganic anions present in three-phase OMWW have been reported in literature (Arienzo & Capasso, 2000). The following concentrations of cations and anions were determined by Borja *et al.* (2006):

– *Cations*: K^+, 9.80 g/L; Mg^{2+}, 1.65 g/L; Ca^{2+}, 1.35 g/L; Na^+, 0.162 g/L; Fe^{2+}, 0.033 g/L; Zn^{2+}, 0.0301 g/L; Mn^{2+}, 0.0091 g/L; Cu^{2+}, 0.0098 g/L
– *Anions*: Cl^-, 1.3 g/L; $H_2PO_4^-$, 0.85 g/L; F^-, 0.53 g/L; SO_4^{2-}, 0.42 g/L; NO_3^-, 0.0109 g/L.

Crude olive cake (orujo) contains crushed stones, skin, pulp, water (~25%), and some residual oil (4.5–9%). Crude fat and neutral detergent fiber are the most variable components. Lignin content is particularly high. Crude protein content is generally low, and a substantial part is linked to cell wall components. Amino acid composition is similar to that of barley grain with a deficit in glutamic acid, proline, and lysine (Nefzaoui *et al.*, 1985). In the water-soluble fraction, the main compounds are carbohydrates. Glucose represents a significant part of the total monosaccharide content, especially under conditions of mild severity, followed by arabinose, but the solubilization of sugars occurs predominantly in the oligomeric fraction. Mannitol is also found in significant amounts (1.5%) (Niaounakis & Halvadakis, 2006).

The characteristics of olive pomace (TPOMW) are very different from those of olive cake. TPOMW is a thick sludge that contains pieces of stone and pulp from the olive fruit and vegetation water. Its moisture content is in the range of 65–75%, compared to 22–25% in traditionally pressed olive pomace and 40–45% in three-phase systems (Dermeche *et al.*, 2013). This greater moisture, together with the sugars and fine solids that in the three-phase system are contained in the vegetation water, gives the two-phase sludge a doughy consistency and make its transport, storage, and handling difficult (Arjona *et al.*, 2005). According to Torrecilla *et al.* (2006), olive pomace is composed of approximately 55% water (all the vegetation water and, sometimes, the water added in the olive oil mill during olive fruit processing), 22% pulp, 20% olive pits, and 3% oil. Dermeche *et al.* (2013) reported that, compared to OMWW, the dry matter content is higher in olive cake, and the ash content is more significant in olive cake and TPOMW (1.70–4 and 1.42–4%, respectively). Especially interesting are the low content of sulfur (in view of further uses of the de-oiled olive pomace as clean fuel), the content of potassium and phosphorous (in view of the use of combustion ash in fertilization), and the high content of sugars, which leads to problems inside dryers.

Since TPOMW contains fragments of pits and skin, it is high in lignin. The lignin itself and its ability to bind to other organic constituents hinder the ability of microorganisms and their enzymes to degrade TPOMW (Milczarek *et al.*, 2011). This makes TPOMW difficult to dispose of as a composting substrate without pretreatment (Alburquerque *et al.*, 2004). Different studies have investigated the sugar content of alcohol-insoluble residue isolated from olive pomace and its soluble and insoluble fractions. The sugar composition of stones and seed husk has also been determined for several cultivars of olive fruit using a neutral detergent fiber method (Heredia *et al.*, 1987). The main components detected are cellulose in the stone and hemicellulose in the seed. Coimbra and Waldron (1995) isolated and characterized the cell wall polysaccharides of olive stone and found that it contains 62% total carbohydrates that are rich in Xyl (from hemicellulose) and Glc (from cellulose). A delignification treatment then makes it possible to extract polysaccharides from the (olive stone) cell wall. Glucuronoxylans were the major non-cellulosic polysaccharide identified.

Olive stones and seeds contain significant amounts of phenolic compounds. Three glucosides, including salidroside (tyrosol–glucose), nuezhenide (glucose–elenolic acid–glucose–tyrosol), and nuezhenide–oleoside, have been identified in olive stones (Maestro-Durán *et al.*, 1994). Nuezhenide is found only in seeds as a predominant phenol, whereas verbascoside only appears in significant quantities in the seeds and pulp (Ryan *et al.*, 2003). Tyrosol and hydroxytyrosol have been detected in olive stones, whereas decarboxymethyl oleuropein has been found in the pulp, seeds, and stones (Fernández-Bolanos *et al.*, 1998). The most abundant phenolic compounds in TPOMW are tyrosol and hydroxytyrosol, together with *p*-coumaric acid and, to a lesser extent, vanillic acid (Lesage-Meessen *et al.*, 2001; Fernández-Bolanos *et al.*, 2002). Other minor compounds identified include verbascoside, rutin, caffeoylquinic acid, luteolin-4-glucoside, 11-methyloleoside, hydroxytyrosol-10-b-glucoside, luteolin-7-rutinoside, and oleoside (Dermeche *et al.*, 2013).

9.2.2 Physical properties

The particle size distribution of OMWW shows a bimodal distribution, due to the two main components of OMWW: (a) the pulp particles, with a mean particle diameter of about 0.9 mm; and (b) a fraction of fine particles, with a mean particle size of about 0.5 mm, which represents only a small fraction (Goula & Adamopoulos, 2013).

Figure 9.2 shows the flow curves of OMWW at 25, 35, 50, and 60 °C. As can be seen, the wastewater shows a non-Newtonian behavior, which can be attributed to the presence of high-molecular-weight materials such as pectins. OMWW samples require a certain amount of yield stress before flow could begin. This suggests that wastewater has a coherent network structure, which requires a certain amount of force to rupture it before flow could occur (Goula & Adamopoulos, 2013). Figure 9.2 also provides the flow curve of OMWW at 25 °C in the backward direction.

Moisture sorption data for OMWW at 20, 30, 40, 50, and 60 °C are shown in Figure 9.3. The isotherms demonstrate an increase in equilibrium moisture content with increasing water activity (a_w), at constant temperature, and are sigmoid in shape, characteristic of amorphous materials rich in hydrophilic components (Al-Muhtaseb *et al.*, 2004). At low and intermediate water activities, the so-called multilayer sorption region, moisture content increased linearly with a_w; whereas at high water activity levels, the so-called capillary condensation region, water content rapidly increased with a_w.

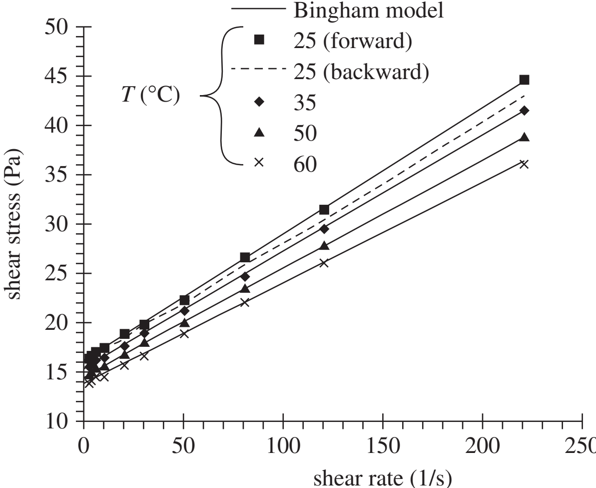

Figure 9.2 Flow curves of OMWW at 25, 35, 50, and 60 °C. *Source*: Goula and Adamopoulos (2013). Reproduced with permission of Taylor & Francis.

According to Torrecilla *et al.* (2006), a fraction of fine and medium particles in the range of 0.25–1 mm, with a maximum around 0.6 mm, is observed in olive pomace. Another maximum appears at the mean particle size of about 1.4 mm; and, finally, a wide distribution of coarse particles is seen between 2 and 5 mm. The authors attributed this type of distribution to the fact that dry olive pomace consists of particles of different nature (skin, pulp, and pits). Liebanes *et al.* (2006) distinguished husk, pulp, and peel in dried two-phase solid olive oil by-product. The husk particles, with a mean particle diameter of 2 mm, represent the predominant and heaviest fraction (about 85% w.b.) in the sample. The pieces of husk consist of cellulose, hemicellulose, and lignin and have a compact appearance with no macroscopic porous structure; therefore, most of the water is supposed to be adsorbed on their external surface. In contrast, the pulp particles are light and small, and represented only 15% (w.b.). The small pieces of peel are needle shaped, and their proportion is negligible in the sample.

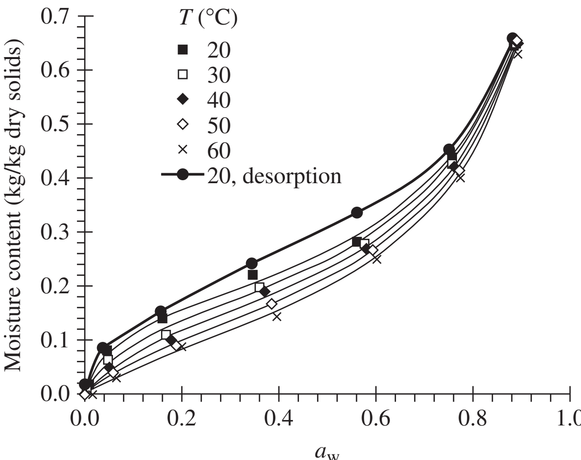

Figure 9.3 Moisture isotherms of OMWW at various temperatures. *Source*: Goula and Adamopoulos (2013). Reproduced with permission of Taylor & Francis.

The two-phase olive pomace has a very complex nature because of its high moisture content, stickiness, and viscosity. Its rheological properties critically depend on the moisture content, and it can be considered as a granular solid only below 50% (w.b.) water content. The olive pomace pseudoparticles are agglomerations of hard pieces of olive husk and pulp, which act as joining matrix and provide high plasticity to the material texture. According to Torrecilla *et al.* (2006), olive pomace is a non-Newtonian fluid with characteristics of either viscoelastic or Bingham-plastic materials, depending on the water content and the strain conditions inside a given apparatus.

Liebanes *et al.* (2008) measured the moisture sorption isotherms of two-phase solid olive oil mill by-product at different temperatures (15–50 °C) in order to thoroughly know the hygroscopic properties of this material. The shape of the isotherms at low temperatures, $T < 25$ °C, is characteristic of type II solids according to the Brunauer classification. At low water activities, the equilibrium moisture content grows as it is described by the monolayer theory of Langmuir, in which the curve growth is limited by the saturation effect because of the limited number of water positions on the solid surface. Nevertheless, the solid equilibrium moisture rises with the air relative humidity in most of the cases. There is an inflection point in the isotherm that leads to an exponential growth at higher water activities, which is characteristic of the multilayer sorption explained by the BET and GAB theories. According to the shape of the isotherms obtained, the two-phase pomace is a macroporous solid with a small specific surface and low hygroscopicity. Only at high water activities of 0.90 does the equilibrium moisture significantly increase, due to some capillarity effects on the water condensation.

9.2.3 Microbial content

The concentration of microorganisms in OMWW ranges from 10^5 to 10^6 colony-forming units (CFU)/mL, comprising several types of bacteria, yeasts, and the molds *Aspergillus* and *Penicillium* (Niaounakis & Halvadakis, 2006). The presence of lactic acid bacteria, yeasts, and molds was also reported for OMWW in Morocco (Mouncif *et al.*, 1993). These authors identified a variety of yeast species, but they only found three species of filamentous fungi (*Aspergillus*, *Penicillium*, and *Geotrichum*). According to Borja *et al.* (2006), among the strains identified were several species of *Acinetobacter*, *Pseudomonas*, and *Enterobacter*. However, much of the microbial activity was represented by 71 strains, showing different metabolic patterns (Niaounakis & Halvadakis, 2006). Millan *et al.* (2000) studied the microbial composition of OMWW from four disposal ponds. Among the fungal members, 12 different genera (*Acremonium*, *Alternaria*, *Aspergillus*, *Chalara*, *Fusarium*, *Lecytophora*, *Paecilomyces*, *Penicillium*, *Phoma*, *Phycomyces*, *Rhinocladiella*, and *Scopulariopsis*) were found. Members of five genera (*Chalara*, *Fusarium*, *Paecilomyces*, *Penicillium*, and *Scopulariopsis*) were widely distributed and were able to grow efficiently in undiluted OMWW as a sole source of nutrients. Strains of *Fusarium*, *Paecilomyces*, *Penicillium*, and *Scopulariopsis* showed a marked capacity for OMWW detoxification, depleting its antibacterial activity almost completely. The pathogenic *Klebsiella pneumoniae* ss. *pneumoniae* has also been isolated from untreated and treated OMWW (Niaounakis & Halvadakis, 2006).

The biodegradation potential of these strains was also studied (Millan *et al.*, 2000). In this respect, four of the strains used were able to decrease markedly the antimicrobial activity of undiluted OMWW. The antibacterial activity of OMWW is well documented (Vazquez *et al.*, 1974; Moreno *et al.*, 1987; Rodriguez *et al.*, 1988). OMWW contains a variety of antimicrobial substances such as oleuropein and phenolic acids, and it inhibits growth of many Gram-positive bacteria as well as methanogenic consortia. These effects not only are responsible for alteration of soil microbiota following irrigation with OMWW (Paredes *et al.*, 1986; Moreno *et al.*, 1987), but also are a problem because the biomethanization step is inhibited during anaerobic digestion (Millan *et al.*, 2000). To solve this problem, a combination of aerobic and anaerobic processes has been used with a certain degree of success. Certain fungal species such as *Aspergillus terreus* and *Geotrichum candidum* have been used during the first stage of treatment both to reduce the COD of the effluent and to eliminate the phenolic inhibitors (Borja *et al.*, 1993).

Vivas *et al.* (2009) found that TPOMW was dominated by members of the phylum *Proteobacteria*, followed by *Actinobacteria* (*Streptomyces*), *Firmicutes* (*Staphylococcus*), and uncultured *Acidobacteria* strains as minor constituents of olive waste microbiota. Members of *Hydrocarboniphaga*, *Pseudoxanthomonas*, and *Stenotrophomonas* (*Gammaproteobacteria*) were identified, while *Comamonas* (*Betaproteobacteria*) was

the main microbial group detected. Moreover, a *Brevundimonas* species was the only representative within *Alphaproteobacteria* (Ntougias *et al.*, 2013).

Some of the yeast isolates from TPOMW exhibited cellulase, β-glucanase, β-glucosidase, peroxidase, and polygalacturonase activities, which could contribute to the degradation of complex compounds, including olive pomace phenolics (Romo-Sanchez *et al.*, 2010).

Pichia, *Candida*, and *Saccharomyces*-like species are the predominant yeasts in OMW. Reduction of both phenolics and sugars is the main metabolic function of yeasts in OMW. Moreover, the acidic pH of OMW may be advantageous for this microbial group to outcompete bacteria (Ntougias *et al.*, 2013). Filamentous fungi, such as *Aspergillus* and *Penicillium* spp., are common habitants of OMW (Millan *et al.*, 2000; Aissam *et al.*, 2007), while white-rot fungi have been isolated to a lesser extent. It appears that the high salt and sugars concentrations of OMW as well as the acidic pH favor the growth of osmotolerant yeasts in OMW (Giannoutsou *et al.*, 2004).

9.3 Current technologies for olive mill waste treatment

9.3.1 Olive mill wastewater (OMWW) treatment

9.3.1.1 Physical and physicochemical processes

In practice, the most common OMWW elimination method is through *evaporation in storage ponds* in the open, because of the low investment required and the favorable climatic conditions in Mediterranean countries. However, this method needs large areas and produces several problems such as bad odor, infiltration, and insect proliferation. The evaporation of OMWW produces sludge. The majority of the sludge produced in evaporation ponds is disposed of in landfill sites, although it may also be used either in agriculture or as a heat source due to its oil content. Vitolo *et al.* (1999) proposed the preparation of a fuel by mixing the solid residue of OMWW with olive husk. According to Roig *et al.* (2006), most of the studies about revalorization of OMWW sludge focus on *composting*. Tomati *et al.* (1995) found that a fertilizer with a high level of humidification and no phytotoxic effects was obtained by composting OMWW with wheat straw. Paredes *et al.* (2000) tested different substrates and evaluated the evolution of organic matter and nitrogen during the composting process. Cegarra *et al.* (1996) applied OMWW composts to cultivate horticultural and other crops. Another imaginative way of recycling this waste was proposed by Hytiris *et al.* (2004), who investigated the potential of using OMWW sludge as an additive for the development of construction materials.

Vacuum evaporation is another alternative disposal method for olive mill wastewaters. A vacuum of 5 kPa allows the wastewater to evaporate at low temperatures, such as 38 °C. The distillate is a colorless liquid but has a COD of 3000–4000 mg/L, which still requires further treatment. Biological oxidation of the distillate preceded by pH adjustment and C:N:P correction gives a treated water complying with the wastewater discharge regulations. The concentrate can be mixed with the solid residues from the olive oil mill. This mixture can be either de-oiled and burned or used for various purposes, such as animal fodder or fertilizer, as it contains 14% protein and 5% potassium (Azbar *et al.*, 2004).

Ultrafiltration (UF) and nanofiltration (NF) are pressure-driven, membrane filtration processes that are used to separate and concentrate macromolecules and colloids from wastewater. The UF process is applicable for particles in the molecular range of 0.01–0.1 μm, while the reverse osmosis (RO) process is used for particles in the ionic range of less than 0.001 μm. Thus, the UF process cannot separate constituents from water as effectively as NF or RO. Turano *et al.* (2002) presented a treatment of OMWW that consisted of (a) a preliminary centrifugation step, in which the suspended solids were removed, and (b) an actual selective separation phase, carried out by UF of the centrifuge supernatant. This method allows a COD reduction of about 90% and a complete separation of fats, rejected by the membrane, from salts, sugars, and polyphenols contained in the permeate. Paraskeva *et al.* (2007) reported that UF in combination with NF and/or RO were very efficient for the treatment and fractionation of OMWW. The UF process resulted in the separation of high-molecular-weight constituents including suspended solid particles, whereas, after NF, phenols were removed to an extent exceeding 95%.

Ozonation is a treatment in which ozone is applied to the water for disinfection or for taste and odor control. Several studies have shown that the extent of COD removal achieved during olive mill effluent

ozonation, even at the most favorable conditions, could not exceed 20–30% (Benitez *et al.*, 1999; Beltran-Heredia *et al.*, 2001).

Electrolysis is based on in situ production of strong oxidizing hydroxyl radicals. Israilides *et al.* (1997) reported, after 10 h of electrolysis, COD removal of 93%, TOC removal of 80.4%, VSS reduction of 98.7%, and phenolic compounds removal of 99.4%. In another electrochemical treatment study for OMWW, the possibility of oxidizing the phenols and polyphenols at a PbO_2 anode was tested (Longhi *et al.*, 2001).

Precipitation is the chemical conversion of soluble substances into insoluble particles. *Coagulation* and *flocculation* initiate a chemical reaction that promotes the formation, agglomeration, or clumping of such particles, thereby facilitating their removal from solution. The use of direct flocculation with polyelectrolytes for the treatment of OMWW showed that two polyelectrolytes, one anionic and one cationic, failed to yield separation, whereas for three others a minimum dose of 2.3–3 g/L was required. Nearly complete reduction of solids was observed in subsequent analysis, while COD and BOD reductions were up to 55 and 23%, respectively (Sarika *et al.*, 2005). Ferric chloride treatment after acid cracking resulted in 95% COD removal and 90% phenol removal at a dose of 3 g/L, whereas alum achieved similar reductions (94 and 91% for COD and phenols, respectively) at a dose of 6 g/L (Kestioglu *et al.*, 2005).

Electrocoagulation is an electrochemical method, in which the coagulant is generated in situ by electrolytic oxidation of an appropriate anode material that, at appropriate pH, leads to the formation of insoluble metal hydroxide that is able to remove pollutants by surface complexation or electrostatic attraction. Electrocoagulation can remove more than 70% of COD, polyphenols, and the dark color present in OMWW, due to the in situ electrogeneration of aluminum hydroxide, electrochemical oxidation, and reaction with soluble aluminum species (Hanafi *et al.*, 2010). Adhoum and Monser (2004) also worked on electrocoagulation using aluminum electrodes to investigate the treatment of OMWW and achieved a fast and effective reduction of pollutants (76% of COD, 91% of polyphenol, and 95% of dark color) present in fresh and stored OMWW.

A group of researchers collaborated to develop the *evaporation–hydrolysis–oxidation* (EHO) method for the treatment of OMWW (Israilides *et al.*, 1997). In this method, following a preconcentrating step by evaporation, hydrolysis under controlled heat input and subsequent oxidation by air take place.

9.3.1.2 Biological processes

Aerobic treatment is a commonly used technology in wastewater treatment. For OMWW, an acclimatization period for the microorganisms is required. Several authors have performed microbiological treatments of OMWW for production of biopolymers such as xanthan (Lopez *et al.*, 1996), pullulan (Ramos-Cormenzana *et al.*, 1996), and polyhydroxyl alkanoates (Roig *et al.*, 2006). Aerobic treatment with microorganisms has also been used to remove the pollution effect of OMWW. These studies are nowadays focused on the degradation of phenolic compounds, the main ones responsible for phytotoxicity. Many microorganisms have been tested: the fungus *Pleurotus ostreatus*, *Bacillus pumilus*, the yeast *Yarrowia Lipolytica*, and others (Scioli *et al.*, 1997). The use of OMWW as substrate for *Azotobacter vinelandii* growth and application of the treated effluent to cultivated soils as fertilizer was proposed by Ehaliotis *et al.* (1999) and Piperidou *et al.* (2000).

Studies on activated sludge treatment report COD removal rates of 80–85% and hydraulic retention times in the range of 20–25 days (Rozzi & Malpei, 1996). More recent studies examined the aerobic degradation of OMWW in a completely mixed-batch activated sludge reactor after microorganism adaptation (Paraskeva & Diamadopoulos, 2006). COD and phenol removals in the range of 58–84% and 90% were observed for initial CODs of 22–98 g/L. BOD removal rates of 45–77% for retention times of 2.5–4.5 days have also been reported elsewhere (Velioglou *et al.*, 1992). Although all of these methods are very interesting from a scientific point of view, they are not usually used at the industrial scale.

The seasonal production and the high organic load of OMWW make *anaerobic treatment* a very attractive option for these wastes. A variety of anaerobic processes such as anaerobic contact, upflow anaerobic sludge blanket (UASB), and anaerobic filters have been applied to diluted OMW treatment. According to Erguder *et al.* (2000), OMWW can be treated anaerobically with high efficiencies (85–93%), and treatment of 1 L OMWW by anaerobic methods results in production of about 57 L of methane. In studies with laboratory UASB reactors, COD reductions of 70–80% were achieved for an initial COD range of 22.6–97 g/L (Raposo

et al., 2004). Application of an anaerobic sequencing batch reactor showed COD reductions of up to 80% with a hydraulic retention time (HRT) of 3 days (Ammary, 2005). Anaerobic degradation of a thermally pretreated OMW in two magnetically stirred batch reactors using sepiolite and bentonite, respectively, as microbial support showed that over 90% of the initial COD was removed (Borja *et al.*, 1992). When using an anaerobic reactor with bentonite as support medium, the minimum retention time required was 4 days and COD reductions were up to 88.8% for HRTs of up to 25 days (Raposo *et al.*, 2004).

9.3.1.3 Combined processes

Coupling chemical and biological processes has received a lot of attention in recent years as a promising treatment alternative for effluents that are too toxic to be treated biologically (Mantzavinos & Kalogerakis, 2005). Ferreira *et al.* (2008) applied a biological treatment with *Pleurotus* spp. and a chemical treatment, through oxidation by Photo-Fenton, to OMWW. The Photo-Fenton reaction was very efficient (>90%) in color reduction. Combining ozonation and aerobic treatment, Benitez *et al.* (1999) reported a total COD reduction of 82.5%, a percentage higher than either of the two technologies could achieve alone. Khoufi *et al.* (2007) examined the effect of a physico-electrochemical method to detoxify OMWW prior an anaerobic biotreatment process. The proposed pretreatment process consisted of a preliminary electrocoagulation step, in which most phenolic compounds were polymerized, followed by a sedimentation step.

9.3.2 Solid olive mill waste treatment

9.3.2.1 Physical and physicochemical processes

Solid OMW can be valorized by *drying* and performing a second *extraction of oil* with solvents. Numerous research works have studied drying of both types of solid OMW – olive cake and olive pomace – using a cabinet-type dryer (Doymaz *et al.*, 2004; Akgün & Doymaz, 2005), a rotary dryer (Arjona *et al.*, 2005), a tray dryer (Göğüş & Maskan, 2006; Jumah *et al.*, 2007), a spout-fluid bed dryer (Marmo, 2007), a fluidized bed dryer (Liébanes *et al.*, 2006; Torrecilla *et al.*, 2006; Meziane, 2011), a solar dryer (Montero *et al.*, 2011), a microwave-convection dryer (Göğüş & Maskan, 2001; Milczarek *et al.*, 2011), and a drying tunnel (Arjona *et al.*, 1999). The most usual method used was the rotary drying, which, however, revealed some problems and disadvantages. According to Arjona *et al.* (1999), the high moisture content of the solid olive waste demands much more energy, and the sugars present in it make it sticky and difficult to dry. Thus, low-temperature drying processes, such as spout-fluid bed drying and fluidized bed drying, were proposed (Liebanes *et al.*, 2006; Marmo, 2007). In addition, the high-temperature applications, such as 250 °C of drying medium, cause pomace hydrolysis and reduce the quality of oil produced (Freire *et al.*, 1999).

Dry solid waste can be extracted using hexane to reclaim the residual oils. In some countries, this extracted oil is a good starting material for the soap-stock manufacturing industry. Further refining and blending with high-quality “virgin” oils may result in good edible oils, provided that the chemical composition is suitable. After the second extraction, the exhausted solid waste is usually used as fuel to obtain thermal or electric energy through *combustion* (Caputo *et al.*, 2003). This method is currently used in most of the olive mills to help with the malaxation process on cold winter days (Roig *et al.*, 2006).

Biomass *gasification* is a new physicochemical method, especially for the de-oiled TPOMW. This process transforms solid biomass into synthetic gas that can be used for obtaining important chemical products such as CH_3OH or NH_3 and for preparation of synthetic fuel (Jurado *et al.*, 2003; Roig *et al.*, 2006).

TPOMW has been assayed directly as a *soil amendment*, considering its high potassium concentration, its low economic value, and the fact that it is produced near application lands and, therefore, no transport is required. However, it causes great nutritional imbalances since it modifies the nitrogen cycle in soil due to its high C/N ratio (Roig *et al.*, 2006). Saviozzi *et al.* (2001) evaluated the suitability of TPOWM as soil amendment by adding mineral nitrogen, and they concluded that its mineralization largely depended on the type of soil, being temporarily inhibited in acidic soils. A number of laboratory studies assessed the suitability, as a vermicomposting substrate, of exhausted TPOMW either alone or mixed with cattle manure and/or municipal biosolids (Nogales *et al.*, 1999).

In a recent review, Nasopoulou and Zabetakis (2013) suggested that olive pomace could be exploited as an alternative *dietary lipid source* in compounded fish feeds, resulting in the formulation of functional

aquacultured fish feeds according to the EU legislation (EC 1924/2006). Moreover, olive pomace and olive cake can also be used fresh, dry, ensiled, or as a component of concentrate pellets and multinutrient feed blocks in animal feeds without attenuating animal performance and meat quality, thus allowing farmers to decrease the daily cost of feeding. Terramocia *et al.* (2013) used dried stoned olive pomace in the feeding of lactating buffalos and observed that the dietetic-nutritional characteristics of the milk were improved due to the greater content in tocopherols and retinol and the presence of hydroxytyrosol.

9.3.2.2 Biological processes

Several research groups have studied the *co-composting* of TPOMW with other agricultural wastes. Some suitable materials used as bulking agents were straw (Madejon *et al.*, 1998), cotton waste (Cegarra *et al.*, 2000), and poplar sawdust and bark chips (Filippi *et al.*, 2002). According to Roig *et al.* (2006), physical characteristics of TPOMW make it difficult to compost by forced aeration systems. Baeta-Hall *et al.* (2005) compared two different aeration processes for the co-composting of TPOMW and grape stalks, and recommended mechanical turning-over for forced aeration. These composts have been used as fertilizers in horticultural crops (Madejon *et al.*, 2001).

Biogas and partially stabilized organic matter can be obtained through *anaerobic digestion*. Tekin and Dalgic (2000) proposed biogas production, whereas Borja *et al.* (2003) conducted a laboratory-scale experiment on the mesophilic anaerobic digestion of TPOMW. According to Niaounakis and Halvadakis (2006), anaerobic treatment as the only process is not suited for TPOMW, because of its low water content compared to OMWW. Moreover, anaerobic treatment requires further treatment measures that lead to additional costs. Another problem is the long starting-up of the process after a longer shutdown period.

9.4 Recovery of functional components from olive mill waste

The large amount of wastes and by-products generated during the olive oil production process, which traditionally have been considered as a problem, constitute in fact a great source of value-added or functional compounds that have the potential to be used as food additives and/or nutraceuticals (De Leonardis *et al.*, 2007; Rosello-Soto *et al.*, 2015). Thus, a more recent approach to exploiting OMW has involved the use of processing technologies to fractionate potential high-value components from olive-processing residues. The recovered compounds may be broadly classified into insoluble, water-soluble, and lipid soluble. The most popular value-added ingredients of OMW are phenols (e.g., simple phenolic compounds, tannins, flavonols, anthocyanins, etc.), dietary fibers (pectins, oligosaccharides, and mannitol), and squalene.

9.4.1 Phenolic compounds

Only recently, in relation to the major interest for the natural compounds with biological activities, researchers have begun to consider the recovery of polyphenols, as high value compounds, transforming OMW from effluents to raw material with high potential economic value (Russo, 2007). Polyphenols, as substances with biological activity (antioxidative, antimicrobial, etc.), can be used in numerous applications in the pharmaceutical, cosmetics, and food industries. Synthetic antioxidants (butylated hydroxytoluene [BHT], butylated hydroxyanisole [BHA], tertiary-butylhydroquinone [TBHQ], etc.) have been used substantially as antioxidants in foods, but concerns over their safety have led researchers toward interest in natural antioxidants. These kinds of synthetic antioxidants are substituted phenolic compounds, and subsequently much of the research on natural antioxidants has also been focused on phenolic compounds, particularly the hydroxycinnamic acids and flavonoids.

Recently, the European Food Safety Authority (EFSA) issued a health claim regulation (EC Reg. no. 432/2012) that "olive oil polyphenols contribute to the protection of blood lipids," whereas it may be used "only for olive oil, which contains at least 5 mg of hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) per 20 g of olive oil." This regulation has initiated a boost in the market demand for

olive polyphenols, as functional ingredients, to be used in beverages, health shots, and smoothies as well as antioxidant additives in various foods, nutritional supplements, and cosmetics.

Many studies on the recovery of polyphenols from OMWW have been conducted on a small scale, and several techniques are used individually or in combination. These techniques largely comprise membrane separation, extraction, adsorption, and chromatographic procedures. Processes of phenols recovery involve typically a condensing step (i.e., thermal concentration, UF, or lyophilization) prior to carrying out the sequential extraction steps with organic solvents (e.g., methanol, ethanol, or hydro-alcoholic solutions). Other practices include the application of selective concentration by liquid membranes, resin chromatography, or supercritical fluid extraction. These processes aim to recover either a particular phenol (i.e., hydroxytyrosol) in pure form or a phenol mixture as a crude product. However, there are only a few references on the use of olive solid wastes as a substrate for the recovery of polyphenols.

9.4.1.1 Membrane separation

OMW comprises phenolic compounds of different molecular weights (MWs) ranging from low-molecular-weight phenolics such as benzoic acid derivatives (MW up to 198) to high-molecular-weight phenolics such as secoiridoid aglycons (MW up to 378) and lignins (MW up to 416) (Bendini *et al.*, 2007). The wide range of MWs of OMW compounds complicates their recovery with high purities, which may be achieved by using membrane technologies. Selective separation and concentration of phenolic compounds would greatly facilitate their further isolation by other methods (Figure 9.4) (Sierra *et al.*, 2001). UF in combination with nanofiltration and/or reverse osmosis is usually used for fractionation of OMWW components (Figure 9.5). The end product is a phenol concentrate that needs further processing for isolation of valuable fractions (Paraskeva *et al.*, 2007). However, if pretreatment is not applied, recovery of membrane permeability with regular cleaning is not possible. Thus, various pretreatment methods have been proposed, such as enzymatic, chemical, and physico-chemical pretreatments; neutralization; sedimentation; and centrifugation (Turano *et al.*, 2002).

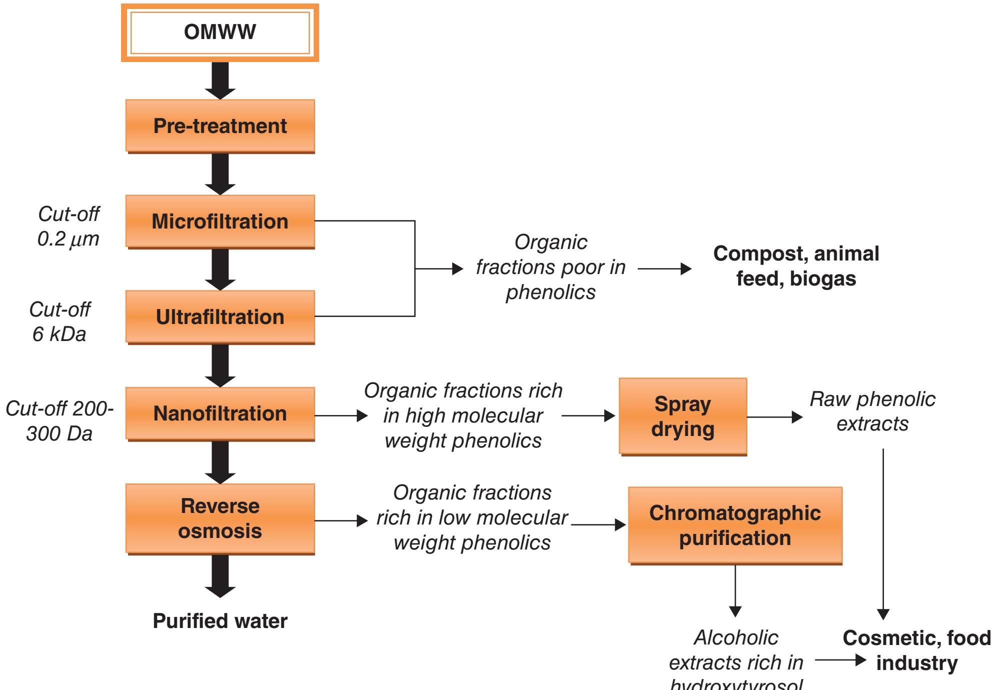

Figure 9.4 A treatment scheme for recovery of phenolic compounds from OMWW.

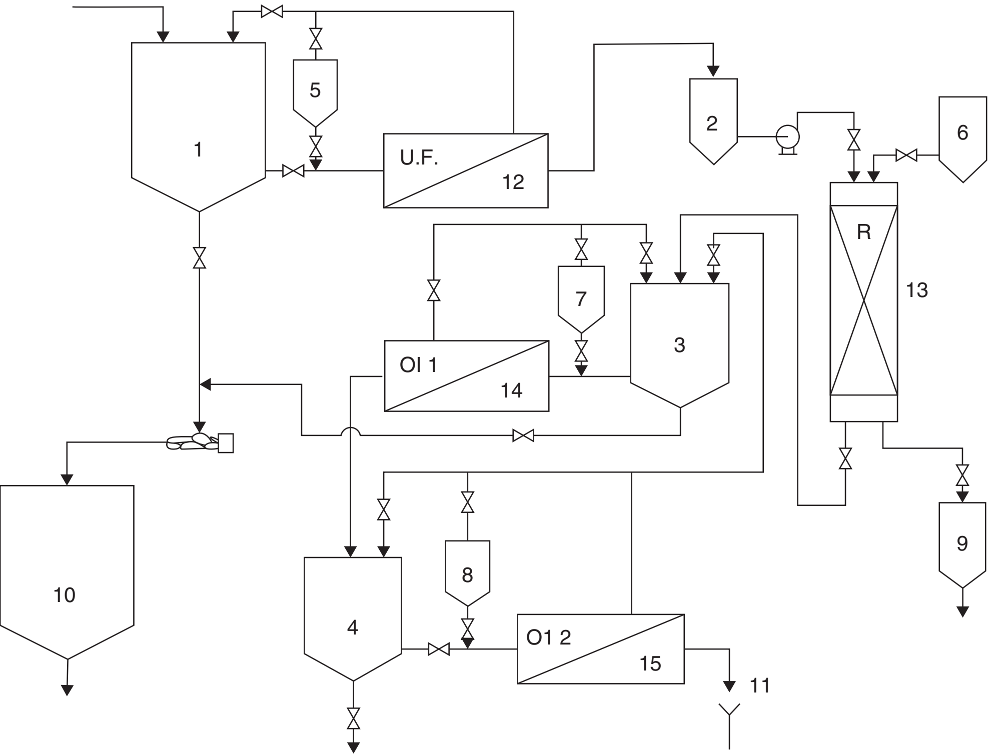

Figure 9.5 Schematic drawing of an integrated membrane pilot plant. (1–4) service tanks; (5–8) washing tanks; (9) polyphenols recovery tank; (10) concentrated recovery tank; (11) freshwater discharge; (12) UF pilot plant: (13) resins column; (14–15) reverse osmosis pilot plants. *Source*: Canepa (1988). Reproduced with permission of Elsevier.

Canepa *et al.* (1988) described a combination of reverse osmosis (RO) and adsorbing on porous polymers for the phenol recovery. Pizzichini and Russo (2005) used microfiltration (MF) and UF for COD reduction, followed by NF and RO for phenol recovery. The final RO operation was characterized by spiral-shaped polymeric membranes made of composite polyamide and yielded a concentrate rich in polyphenolic compounds and a permeate consisting of purified water. Castanas *et al.* (2005) disclosed a system of filters composed of substrata of natural products consisting of turf, sand, and sawdust, and optionally one or more filters of resins consisting of cationic, anionic, mixed-type, or polyvinylpolypyrrolidone resins. Pizzichini and Russo (2005) proposed a method in which acidified wastewater is subjected to enzymatic hydrolysis by a pectinase enzyme complex to remove the cellulose, hemicellulose, and pectin microdispersed components from OMWW. The liquid fraction separated from the degradation products is then used in a membrane system, including tangential MF, tangential NF, and RO units in sequence. The ultimate retentate of the RO system is rich in polyphenols. The retentate of the MF unit can also be used for extracting polyphenolic compounds thereafter. The experimental study of Russo (2007) concerning removal efficiencies of several MF and UF membranes concentrated the recovered polyphenols by using a final RO consisting of a polymeric hydronautics membrane (composite polyamide). With a concentration factor of 2.5, the RO membrane produced a retentate containing 464.870 ppm free low-MW polyphenols composed of 390.449 ppm hydroxytyrosol, 27.400 ppm tyrosol, 14.968 ppm oleuropein, 14.329 ppm caffeic acid, and 17.724 ppm protocatechuic acid.

Paraskeva *et al.* (2007) compared the concentration efficiencies of NF and RO after a UF process. The NF step showed reduction of efficiencies to 99% for TSS, 97% for TOC, 97% for COD, and 98% for phenols; the NF retentate had a concentration of 9.96 g/L of phenols. Operating the UF permeate with the RO membrane obtained similar reduction results, whereas the phenols concentration in the RO concentrate was 6.78 g/L. Nunes *et al.* (2007) described the production of a hydroxytyrosol concentrate by use of nanofiltration and

RO procedures. An aqueous extract (olive cake plus water) was given to a NF membrane Desal DK type yielding a recovery of hydroxytyrosol of about 70%. The following RO process further concentrated the obtained NF retentate using a Dow Filmtec SW 30 membrane. Villanova (2008) described one NF and one RO module for the concentration of pretreated OMWW. Placed in the third unit after two UF modules, the NF membrane and following RO membrane yielded more than 1 g/L of hydroxytyrosol and more than 0.6 g/L of tyrosol.

El-Abbassi *et al.* (2009, 2012) investigated the use of direct contact membrane distillation (DCMD) for the concentration of phenolic compounds employing a Gelman TF200 membrane made of PTFE. The obtained retentate had a polyphenols content of about 6.88 g/L. Garcia-Castello *et al.* (2010) investigated the concentration abilities of osmotic distillation (OD) and vacuum membrane distillation (VMD) after a NF process. The whole membrane system delivered a liquid concentrate containing about 0.5 g/L low-MW polyphenols. Servili *et al.* (2011) described a RO process using a spiral thin-film membrane made of Durasan and polysulfone for the concentration after pretreatments with MF and UF. The content of total phenols rose from 4.9 to 19.3 g/L in the RO concentrate. Cassano *et al.* (2013) investigated a process based on two UF processes followed by a NF treatment. This permitted to operate a fractionation of OMWW separating organic substances of different MWs. The NF retentate was a concentrated solution enriched in polyphenolic compounds suitable for cosmetic, food, and pharmaceutical industries as liquid, frozen, dried, or lyophilized formulations.

El-Abbassi *et al.* (2014) used membrane filtration as a pretreatment prior to application of cloud point extraction to OMWW. The method resulted in a mixture of natural antioxidants suitable for pharmaceutical, cosmetic, or food applications. Zaklis *et al.* (2015) used reverse osmosis, after a nanofiltration, to concentrate the low-MW compounds, prior to further treatment with resin adsorption and desorption for the purification of the phenolic compounds of OMWW. Comandini *et al.* (2015) treated OMWW with a semi-industrial membrane filtration system, including UF and RO modules. UF reduced phenol concentration by about 40% with respect to the initial level; in the permeate of reverse osmosis, the phenol concentration ranged from 0 to 1% of the initial content. In contrast, the content of phenolic compounds was increased about 2.6-fold in RO concentrate. 3,4-(dihydroxyphenyl) ethanol and *p*-(hydroxyphenyl) ethanol were the main compounds detected of a total of 32.

During filtration of wastewaters, severe fouling of the membrane occurs, thus affecting the process performance. Fouling reduces the permeate fluxes and determines both efficiency decrease and variation of membrane selectivity; it also makes the process highly expensive due to repeated plant shutdown for cleaning and washing the membranes (Belfort *et al.*, 1994; Turano *et al.*, 2002). The critical flux method seems to be one of the most used approaches to overcome fouling problems. At the critical flux point, the drag forces on the solute molecules concentrated over the membrane surface are equal to the dispersive forces, leading to a steady-state layer in a gel state. Under these conditions, only reversible fouling can occur, which can be periodically soft-cleaned (Field *et al.*, 1995). However, some authors have pointed out that operation below the critical flux may not be sufficient to avoid long-term fouling (Cho & Fane, 2002; Stoller & Chianese, 2006). These authors introduced the concept of sustainable flux, at which the desired separation can be operated in a profitable manner, only minimizing but not eliminating fouling entirely. Thus, the best permeate flux cannot be theoretically predicted but only experimentally measured. Recently, Ochando-Pulido *et al.* (2015) achieved successfully complete restoration of the membrane permeability by performing a cleaning procedure upon turbulent tangential velocity over the membrane (4.01 m/s, equivalent to $N_{Reynolds}$ = 21,000) at a cleaning operating temperature ranging from 30 to 35 °C during 20–25 min. Dammak *et al.* (2015) studied a model of oleuropein solution filtration using NF membrane and different feed concentrations and showed that the permeate flux was governed mainly by the osmotic pressure of oleuropein. The flux declined following the accumulation of oleuropein at the membrane surface, forming a concentration polarization layer with lower hydraulic resistance than membrane resistance.

9.4.1.2 Extraction

Solvent extraction is the most widely used technique to recover phenolic compounds from OMWW in spite of its high cost resulting from the requirement of large amounts of organic solvents. In recent studies, the disadvantages of organic solvents, such as toxicity and flammability, are avoided by using supercritical fluids.

Solvent extraction is used individually or preferably employed prior to membrane processes for the recovery of polyphenols from olive oil by-products.

Allouche *et al.* (2004) achieved high recovery (85.46%) of hydroxytyrosol from OMWW using a three-stage continuous countercurrent liquid–liquid extraction unit. Emmons and Guttersen (2005) described a process involving addition of citric acid to the raw material, subsequent heating in order to precipitate the solids, and extraction of oleuropein aglycon from a water-immiscible constituent with a nonpolar organic solvent mixture, preferably mixture of 50/50 hexane and acetone. De Martino *et al.* (2011) proposed a method of obtaining an organic extract containing hydroxytyrosol by means of a Soxhlet, continuous-countercurrent, or batch extraction system using ethyl acetate, methyl isobutyl ketone, methyl ethyl ketone, diethyl ether, methanol, or n-butanol.

Tornberg and Galanakis (2008) disclosed a method for isolating dietary fibers and valuable polyphenols from OMWW, in which first of all OMWW is defatted by centrifugation and concentrated by removing the water content. Afterwards, it is extracted by using ethanol up to 7% (v/v) and an organic acid in the range of 0.5–3% by weight of the extraction solution. The polyphenols remaining within the dietary fibers were then extracted with at least 85% (v/v) ethanol and separated by filtration. After dilution with 15–40% (v/v) ethanol, the liquid phase containing polyphenols may be clarified by filtration. Takac and Karakaya (2009) used ethanol up to 70% and an organic acid in the range of 0.5–3% to extract polyphenols from OMWW. Lafka *et al.* (2011) subjected OMWW to conventional liquid solvent extraction and supercritical fluid extraction using different solvents and carbon dioxide, respectively. The optimum extraction conditions were 180 min using a 5:1 (v/w) ratio of ethanol to sample at pH 2. Jerman-Klen and Mozetic-Vodopivec (2011) used ultrasound-assisted extraction for the recovery of phenols from OMWW. The ultrasound-assisted extraction of freeze-dried OMWW in 100% methanol (1.5 g/25 mL, w/v) offered high qualitative–quantitative phenol yields, and it may be utilized as a valuable source of phenols, especially hydroxytyrosol and tyrosol.

Firlbeck (2013) reported that vanillic acid might be extracted from OMWW quantitatively by a second run of foam fractionation. Foam fractionation of vanillic acid under the optimized process parameters (pH of 6–7) led to an enrichment factor of 22.24 in the foamate and 55.88% recovery in the residue. Yu *et al.* (2014) tested the possibility of obtaining functional ingredients, such as phenolic compounds, from olive fruit dreg (a waste from olive soft drink processing) using subcritical water extraction. The subcritical water extracts contained higher amounts of phenolic compounds, such as chlorogenic acid, homovanillic acid, gallic acid, hydroxytyrosol, quercetin, and syringic acid.

9.4.1.3 Adsorption

The profitability of an industrial process for the adsorptive purification and concentration of phenolic compounds from OMW mainly depends on the adsorption efficiency and on the recovery rates during desorption. So far, few studies have been carried out using sorbent materials for the removal of polyphenolic compounds contained in OMW.

Al-Malah *et al.* (2000) used activated clay and reported that sorption of phenols was reversible and mainly due to hydrophobic interactions. Cuomo and Rabovskiy (2001) described a method of extracting antioxidant components from olive by-products, which includes the steps of passing OMWW through a solid matrix to trap antioxidants on the matrix and washing the matrix with a polar organic solvent to remove the antioxidants in the polar organic solvent. Suitable solvents include polar alcohols, acetone, ethyl acetate, acetonitrile, dioxane, and mixtures thereof. The polar organic solvent can be partially removed to form a liquid concentrate or, preferably, substantially and completely removed to produce a solid antioxidant composition. The antioxidant activity of the composition can be enhanced, either by acidifying OMWW or by dissolving the solid antioxidant composition in an acid and then redrying it. The extracted antioxidant composition has a total phenolic content of about 10–30% gallic acid equivalents by weight on a dry weight basis and consists of about 1–5% hydroxytyrosol, 0.4–1.5% tyrosol, and 0.05–1% oleuropein, all based on dry weight. A particularly preferred solid matrix material is a polymeric adsorbent marketed under the trademark Amberlite®.

Activated carbon obtained from treated olive pomace has been used for OMWW polyphenol sorption, succeeding efficient phenol removal (Galiatsatou *et al.*, 2002). Wilhelm *et al.* (2003) described the isolation

of antioxidants from OMWW by fluidized bed adsorption, especially using polymeric ion-exchanging adsorbents (e.g., Amberlite XAD or Lewatit EP), followed by elution of the adsorbed antioxidants and removal of the solvent. Sabbah *et al.* (2004) proposed a method using sand filtration and subsequent treatment with powdered activated carbon, yielding a 95% removal of phenolic compounds. The recovery yield was lower (60%) using a solid phase extraction by employing Amberlite XAD16 resin as the adsorbent and ethanol as the biocompatible desorbing phase (Scoma *et al.*, 2011). Santi *et al.* (2008) treated OMWW with mineral adsorbents and concluded that the zeolite, compared to other substrates (clay soil and bentonite), appeared to be a useful mineral in reducing the organic load from the OMWW. Bertin *et al.* (2011) suggested that Amberlite XAD7, XAD16, IRA96, and Isolute ENV+ are the four most promising adsorption resins. The highest recovery of hydroxytyrosol (77%) was achieved when non-acidified ethanol was used as the desorbing phase.

Ferri *et al.* (2011) reported the highest phenol adsorption (76%) using IRA96 polar resin, whereas Ena *et al.* (2012) stated that granular activated carbon can be more efficient than Azolla in terms of phenols adsorption and desorption. Zaklis *et al.* (2015) used nonionic XAD4, XAD16, and XAD7HP resins for the recovery of phenols from low-MW compounds of OMWW and for their separation from carbohydrates. The recovered phenolic compounds were concentrated through vacuum evaporation, reaching a final concentration of 378 g/L in gallic acid equivalents containing 84.8 g/L hydroxytyrosol. Recently, Frascari *et al.* (2016) developed a continuous-flow adsorption/desorption process for the recovery of phenolic compounds from OMWW (pretreated with centrifugation and microfiltration to remove suspended solids) using an Amberlite XAD16 resin packed in a 0.53 m column and acidified ethanol as desorption solvent.

Stasinakis *et al.* (2008) investigated total phenol removal efficiency from OMWW by several types of treated olive pomace (dried olive pomace; dried and solvent-extracted olive pomace; and dried, solvent-extracted, and incompletely combusted olive pomace as biosorbents). Singh *et al.* (2008) investigated the adsorption of both phenol and 2,4-dichlorophenol through the acid treatment of coconut shells, whereas Achak *et al.* (2008) used banana peel as a low-cost solution biosorbent for removing phenolic compounds from OMWW, yielding an 88% removal with a peel dosage of 30 g/L.

9.4.1.4 *Chromatographic separation*

Fernandez-Bolanos *et al.* (2002) suggested a method for obtaining hydroxytyrosol from OMWW by a two-step chromatographic treatment. In the first column, the polystyrene-based non-activated ion exchange resins, in the form of a gel or macro-reticular, are used to provide partial purification of hydroxytyrosol. After elution with water, the hydroxytyrosol solution from the previous stage is fed to the second column involving nonionic, polystyrene-based XAD resins to enable hydroxytyrosol to be completely purified after eluting with a 30–33% methanol–water or ethanol–water mixture. Liu and Wang (2008) developed a method for the recovery of hydroxytyrosol involving dilution with water, passage through a styrene resin chromatographic column, washing with distilled water, extraction with ethyl acetate, and finally distillation at low temperatures.

9.4.2 Pectins, oligosaccharides, and mannitol

Pectins are natural hydrocolloids found in plants that are widely used as gelling agents, stabilizers, and emulsifiers in the food industry. Commercial pectins are only available from two important sources: apple pomace and citrus peels. The waste beet solids from sugar extraction and the sunflower heads residues obtained after the oil extraction are very promising sources as they contain 10–20% pectic material; however, pectins obtained from these sources have poor gelling ability. Mannitol is used in pharmacy; and it is used as an anticaking and free-flow agent, lubricant, stabilizer, thickener, and low-calorie sweetener in the food industry.

Garcia-Granados (1994) disclosed a process for obtaining mannitol and derived products from OMWW, olive twigs, leaves, and stalks. The process comprises the steps of partial or complete drying; then, mannitol is extracted by means of alcohols or hydroalcohols, the extracts obtained are defecated by the addition of basic lead acetate, the extracts are concentrated, and the mannitol is isolated by crystallization with alcohol. Fernández-Bolanos *et al.* (2000) investigated the use of a steam explosion process to extract mannitol from

olive cake coming from a three-phase centrifugation system. With this process, the olive cake is treated in a 2 L steam explosion unit at temperatures around 200 °C for time periods of 2–4 min. All the mannitol present in the olive cake is recovered with a high degree of purity by means of various purification stages (ultrafiltration, ion exchange, and fractionated crystallization).

According to Cardoso *et al.* (2003), TPOMW can be a potential source of gelling pectic material, with useful properties for practical applications. Pectic raw material was extracted from the alcohol-insoluble residue of TPOMW. The purified pectic extract contained 48% galacturonic acid and 31% arabinose, with a total sugar content of 72% and a degree of methylesterification of 43%. Compared with a commercial low methoxy citrus pectin, the olive pectic extract exhibited higher critical galacturonic acid and calcium concentration for gelation to take place, and gels showed lower viscoelastic moduli at corresponding galacturonic acid and calcium concentrations.

Fernández-Bolanos *et al.* (2004) suggested a hydrothermal treatment of alperujo, in which an autohydrolysis process occurs and the solid by-product is partially solubilized. From this water-soluble fraction, several compounds of high added value can be obtained besides the antioxidant hydroxytyrosol, such as the monosaccharides xylose, arabinose, and glucose; oligosaccharides; mannitol; and products of sugar destruction.

Galanakis *et al.* (2010a) also obtained a water-soluble polysaccharide fraction using thermally concentrated OMWW and a citric acid–ethanol precipitation process. This fraction, following a simple isolation and concentration procedure, was able to form gels, despite the high ionic concentration, the low pectin content, and the high methylation degree (59%) of pectic polysaccharides. The role of endogenous pectin methyl esterase during thermal concentration was also examined (Galanakis *et al.*, 2010b). The water-soluble polysaccharide fraction was successfully clarified using ultrafiltration (25 kDa) (Galanakis *et al.*, 2010c). Its utilization in meatballs improved their cooking properties by restricting the oil uptake during deep-fat frying (Galanakis *et al.*, 2010d). Recently, Rubio-Senent *et al.* (2015a) subjected alperujo to gentle heating at 50–80 °C for 1–2 h, and the aqueous by-product obtained by a new three-phase centrifugation was treated with ethyl alcohol precipitation with 40 and 80% (v/v) EtOH, followed by purification. The pectic material presented a high molecular size and a low percentage of methyl esterification and acetylation. In comparison with commercial pectins, the extract had better oil-holding capacity and emulsifying activity similar to that of citrus pectin, but exhibited a stronger antioxidant activity due to polyphenolic compounds. Furthermore, they collected the pectin fraction released from steam-treated alperujo at 160 °C for 30, 45, and 60 min (Rubio-Senent *et al.*, 2015b). The fraction presented a low MW in the range of 2–40 kDa, a high content of neutral sugars, and a high percentage of acetylation. In comparison with commercial pectins, the extract had low water-holding and high oil-holding capacities, a normal emulsifying activity, and a similar emulsion stability to that of apple pectin. The *in vitro* analyses showed considerable bile–acid binding activity and a glucose retardation index similar to the values obtained for citrus pectins.

9.4.3 Squalene

Squalene is a natural 30-carbon organic compound obtained primarily from shark liver oil, although plant sources (primarily vegetable oils) are now used as well, including amaranth seed, rice bran, wheat germ, and olives. Squalene is of great interest and required in large quantities in the health, food, and pharmaceutical industries due to its antioxidant activity and high biological values. Existing attempts to obtain squalene from olive oil residues by distillation methods have not resulted in producing squalene in economically viable quantities.

Casanelles (1986) described a process for the recovery of squalene from olive oil by-products that includes hydrogenation, successive crystallizations in organic solvents, to remove the insoluble impurities and distillation of the filtrates. Traveria (1988) proposed a process for obtaining squalene by subjecting by-products from the refining of olive oil to saponification, using a 5% excess of a strong alkali; extracting the unsaponifiable matters by means of distillation by dragging a steam; hydrogenating the distilled product with a nickel catalyst; and purifying the hydrogenated product by means of deodorization, deparaffination by crystallizing, and filtering at low temperature.

An alternative process for the recovery of squalene uses supercritical carbon dioxide extraction. The process has been carried out on a pilot plant scale with a column operating in the countercurrent mode. By the use of this process, squalene can be recovered in high purity and yields of about 90% (Bondioli *et al.*, 1993).

An improved process was developed by the partners of the EU project FAIR2-CT95-1075. The process comprises the following steps: (a) saponification of olive oil residues, (b) drying of the saponified material to a residual water content below 1%, (c) extraction of the resulting soap with CO_2 as solvent, and (d) purification of the extract by chromatography.

9.5 Integral recovery and revalorization of olive mill waste

Valorization of OMW requires exploitation of many components. Despite the studies cited and their potentially promising results, research on potential utilization routes including all levels of value, from high value (antioxidant, antiviral, anticarcinogenic, etc.) to relatively low value (compost, feeding, etc.), has not been completed yet. It is clear that reaching an adequate exploitation of these by-products will require their full utilization.

Zumbe (2004) described a process for producing an olive powder from an aqueous olive paste derived from olive cake or TPOMW, which is suitable for human consumption. A typical process comprises the following steps: (a) pretreating the olive cake or TPOMW with a stabilizing additive or preservative (e.g., citric acid, NaCl, ethanol, sodium metabisulfite, etc.) to avoid microbial deterioration; (b) blanching the stabilized olive cake or TPOMW at moderate heat to inactivate the polyphenol oxidase enzyme; (c) adding to the blanched paste an esterase enzyme (preferably, a thermostable β-glucosidase) to hydrolyze oleuropein; (d) drying the olive paste to remove water and provide a particulate intermediate; and (e) optionally, dry comminuting the particulate intermediate having a water content of less than 20% by weight (preferably even less than 5%) in a mill, while the temperature of the material is maintained at a temperature less than 10 °C to form a powder of which at least 99% by weight has a particle size less than 0.55 mm.

El-Shafey *et al.* (2005) developed an integrated process including, consecutively, lime precipitation, filtration with cake dewatering, and filtrate posttreatment via activated carbon adsorption. Lime treatment alone is not enough for the OMWW treatment, since the filtrate still contains large amounts of the unwanted organics. Separation of the solids by filtration using a novel technology of membrane filter press with cake dewatering via membrane squeezing followed by vacuum drying over hot cakes was performed. Diatomite precoating has decreased the specific cake resistance, and with diatomite body feed the specific cake resistance showed a further decrease with a higher filtration rate due to the increased permeability and porosity of the cake. Membrane squeezing using hot water followed by vacuum application over the hot cakes for 30 min is considered as a successful key for dewatering the produced cakes. Produced cakes can be used as an energy source due to their lower calorific value of 15.71 MJ/kg. A posttreatment for the filtrate is required, and activated carbon adsorption was examined for the removal of the remaining phenols and total organics. Activated carbon showed good removal efficiency for phenols and total organics, and total removal by the end of the combined treatment processes reached 99.7% for phenol and 80% for total organics (expressed as COD). The thermal energy produced by the burning of the produced cakes would be enough for the thermal needs of both the olive mill and the proposed treatment process.

Crea and Caglioti (2005) patented a method in which de-pitted olives are subjected to pressing to obtain a liquid-phase mixture including olive oil, vegetation water, and solid by-products. Vegetation water (which is substantially free of monophenolic compounds) is separated from the liquid-phase mixture by centrifugation. A treatment of the aqueous olive fraction with citric acid (1%) for 6 months followed by freeze drying yields a golden-brown crystalline powder. This product is composed of 98–99% dry solids, including 1–2% citric acid, 6% polyphenols, as well as proteins, fat, and carbohydrates. The phenolics fraction consists of hydroxytyrosol (50–70%), oleuropein (5–10%), tyrosol (0.3%), oleuropein aglycone, and gallic acid. Consumption of the product, under the name HIDROX®, is considered safe at levels up to 20 mg/kg day (Soni *et al.*, 2006).

The patented system reported by Fernández-Bolaños *et al.* (2006) allows the production of two forms of purified hydroxytyrosol (Hytolive®1 and Hytolive®2) from olive oil by-products, in a very simple, practical, and economic way. As a first step, it includes passing the liquid source of hydroxytyrosol through an ion-exchange resin to trap antioxidants and elution with water. The second step is adsorption on a XAD-type nonionic resin followed by washing with a mixture of methanol or ethanol and water (30–33%). A solution containing at least 75% of hydroxytyrosol present in the olive by-product is obtained (Hytolive2). The polar organic solvent is finally removed to produce a solid with about 95% by weight hydroxytyrosol (Hytolive1),

plus significant fractions that reach 99.6% purity. The extracted Hytolive1 has an antioxidant activity characterized by increasing the oxidative stability of refined olive oil 1.71-fold in the presence of 100 ppm of hydroxytyrosol. Both products (Hytolive1 and Hytolive2) may be used for a variety of applications: as natural food antioxidants, in the preparation of functional foods, or as pharmaceutical solutions or cosmetics. Currently, the company Puratos is using Hytolive as an ingredient in bakery (www.genosa.com/hytolive).

Ibarra and Sniderman (2008) developed a process for producing liquid and powdered olive polyphenols concentrate from by-products of two-phase and three-phase olive oil extraction processes. The process starts by an extraction procedure, where the solvent may be water, ethanol, or their mixtures at a ratio of by-product to solvent weight of 1/3 to 1/30. The polyphenols solution separated from solids in a decanter and/or by means of a filtration step is then clarified in a disk centrifuge or by using microfiltration membranes. Partial or complete fat removal was also claimed by means of solvent extraction, decanting, or cryogenic separation. Although vacuum evaporation or chromatographic methods can be used to concentrate the polyphenols solution obtained, membrane separation techniques are preferred. The loss of polyphenols in the retentate of ultrafiltration is reported to be less than 10% based on the total dissolved solids of the olive polyphenol extract. The ultrafiltration permeate is preferred to pass through nanofiltration membranes made of thin-film composites and cellulosic materials to remove the dissolved minerals from the solution. In the last step, the olive polyphenol concentrate may be dried by using a pan vacuum dryer or a spray dryer to obtain a powder. In different steps of the process, some enzymatic treatments are also carried out. Deactivation or inhibition of polyphenol oxidase, which is responsible for the undesirable polymerization of polyphenols, can be employed after the first step of the process. Hydrolysis of oleuropein and/or demethyoleuropein into hydroxytyrosol, tyrosol, eleanolic acid, and glucose by means of glucosidase or esterase is another enzymatic treatment suggested before or after the second step of the process. The olive polyphenol concentrate obtained is directed to be used as ingredient in many foods to impart a strong bitter profile to food products, to add antioxidant activity and antimicrobial properties to the foods, and consequently to increase the foods' shelf life.

Benavent (2008) presented an integral procedure for obtaining a concentrated liquid with a high content of polyphenols. The procedure starts with a solid–liquid separation or centrifugation to obtain the liquid fraction of alperujo or alpechin. The liquid fraction is subjected to centrifugation; biological treatment with pectolytic enzymes and amylases, with the aim of increasing its filterability; alcohol fermentation of sugars; and filtration procedures before being concentrated using a multiple-step vacuum evaporator. The phenolic compounds can be recovered by selective resins to obtain a compound rich in antioxidants for dietary uses. The product concentrated in an evaporator has the characteristics of being a colloidal suspension, dark grayish-brown, slightly acidic, and free of sedimentable solids.

The work of Tornberg and Galanakis (2008, section 7.4.2) and Galanakis *et al.* (2010a, 2010b, 2010c, 2010d), on the recovery of phenolics from OMWW, has led to a product with 4% olive polyphenols of a broad spectrum named PHENOLIVE AB (http://phenoliv.com/). This product is supplied as powder with a dry content of 98% and with solubility in water of 98%.

Petrotos *et al.* (2012) reported a system in which the collected OMWW is clarified with ceramic membrane microfiltration and subsequently concentrated by using an RO technique. The RO retentate is a polyphenol-enriched dark liquid, which is used in order to recover the polyphenols by preparative chromatography in the subsequent step with pure deionized water as permeate. To recover the polyphenols, the RO retentate is circulated through a bed of XAD4 Amperlite commercial polyphenol-selective macroporous resin for 24 hours and water–ethanol solution (50:50, v/v) as eluting medium for about 3-4 hours. At the end of the elution step, the alcoholic polyphenol solution is collected and concentrated by RO using the same scheme as for clarified OMWW. The concentrated alcoholic polyphenol solution is subsequently processed by freeze drying to obtain a dark-brown and bitter dry powder consisting of OMWW polyphenols. In a later version, Gkoutsidis *et al.* (2015) developed an integral scheme that involved diluted OMWW ultrafiltration, which converted the original OMWW slurry into a clear low-viscosity liquid at a high rate. In a second step, the polyphenols got recovered in powder form from the clear ultrafiltrate by selective adsorption/desorption on macroporous resins combined with spray drying. In addition, organic fertilizers were produced by innovative high-pressure concentration of the de-phenolized clear ultrafiltrate, either alone or in mixture with the ultrafiltration retentate. In this way, the total flow of OMWW was converted into valuable products. Pure (not encapsulated) and microencapsulated polyphenols produced were used for enrichment of dairy products (Petrotos *et al.*, 2012). The proposed production scheme was submitted for a patent under the name Olivex.

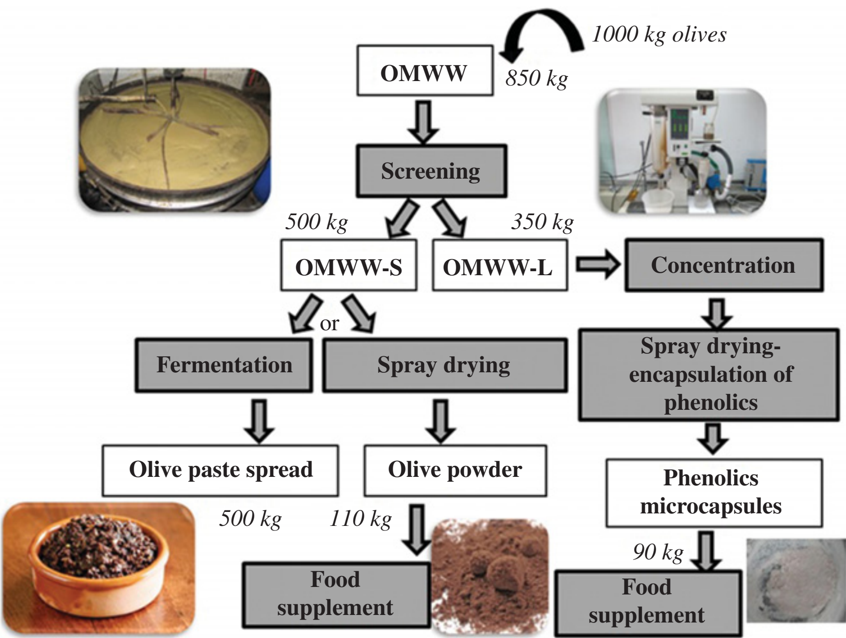

Figure 9.6 An integrated process for OMW utilization. *Source*: Goula and Lazarides (2015). Reproduced with permission of Elsevier.

An integrated method for OMWW utilization involving processes such as screening, fermentation, spray drying, and encapsulation has recently been proposed in our lab. The main mass balances are that 850 kg of OMWW (from a three-phase centrifugation system) derived from about 1000 kg of olives can lead to about 120 kg of dry olive powder, or 500 kg of olive paste spread and 90 kg of encapsulated polyphenols (Figure 9.6). The OMWW powder can be used in a wide variety of applications, such as in the preparation of pharmaceutical products, such as cholesterol-lowering agents and anticancer medicine; food products or food additives; and cosmetic products, such as creams. In addition, the OMWW powder proved to be efficient in improving the shelf life of different products, such as oil rusk, by inhibiting their oxidation.

According to the process scheme of Figure 9.6, the unit operations used are:

- *Screening of OMWW*: A woven vibrating screen with a nominal mesh size of 0.1 mm is used. The screen removed about 99% of total solids, and the so-called olive mill waste solids (OMWS) fraction had a solids content of 20.8 ± 1.5%.
- *Fermentation of OMWS*: An equivalent volume of 6% brine was added to the OMWS, and fermentation was complete when the pH stabilized to around 4.4 (in about 14 days). The excess brine was then washed off. Microbiological examination revealed that fermentation of OMWS relies on yeasts. The fermented OMWS proved microbiologically safe due to the absence of enterobacteria, pseudomonas, and staphylococcus. The most important biochemical activity was the appearance of increased β-glucosidase activity. The obtained by-product had 20 mg GAE/g dry weight olive polyphenols and complete absence of bitter or pungent taste. This by-product was used to prepare an "olive paste spread" product after mixing it with olive oil, vinegar, peppers, and herbs in various proportions. The OMWS paste was compared with different commercial olive pastes, and the sensory analysis showed that the highest values of the overall assessment were found in the OMWW-based paste (Nanis, 2014).
- *Spray drying of OMWS*: Three groups of experiments were conducted (Chasekioglou *et al.*, 2017). The first group included spray-drying experiments in which addition of drying aids (maltodextrin or skim milk powder) was used as a method to resolve the OMWS stickiness problem. During the second experimental group, the problem of residue formation was resolved by using dehumidified air as a drying medium.

In the third group of experiments, the combination of drying aid addition and use of dehumidified air was used as a way of reducing residue formation. In experiments conducted using a standard spray-drying system, where the drying medium was atmospheric air, product recovery was lower than 2–3%, whereas the use of drying aids increased the yield to 3.7–10.9% for skim milk powder and 1.3–23.7% for maltodextrins. In experiments conducted with dehumidified air, the recovery ranged from 58.6 to 80.5%. Finally, a combination of maltodextrin addition and use of dehumidified air as drying medium seemed to be an effective way of producing a free-flowing OMWW powder with a yield ranging from 87.5 to 93.0%.

– *Encapsulation of OMWL polyphenols*: The liquid fraction derived from OMW screening (OMWL) had a very low total solids content (~0.5%) consisting mainly of phenolic compounds (99%). The proposed scheme for OMWL treatment includes its concentration and the subsequent encapsulation of the phenolic fraction by the proposed modified spray-drying system. Alternatively, the OMWL fraction can be fed back to the decanter. By this procedure, a large quantity of water is reutilized in the olive mill plant, and a smaller amount of olive mill waste is dried, resulting in less energy consumption.

9.6 Conclusion

Olive mill waste treatment and disposal is a problem with great complexity due to the nature of the waste and several economical, technical, and organizational constraints involved in the olive oil sector. Practically, all treatment processes developed for domestic and industrial wastewater have been tested on OMW, but none of them appeared suitable to be generally adopted. All tested approaches so far, although technically successful, lack economic viability. This is because up to now, the emphasis has been on treating OMW like all other wastes (i.e., reduction of polluting loads to legally accepted levels for disposal to the environment). To achieve this goal, sophisticated technical solutions are required that the majority of the small-sized and geographically scattered olive mills cannot afford. Future olive oil waste management strategies should be toward a combination of detoxifying OMW and utilizing it, at the same time, for producing valuable by-products with high functional activity. In this way, high costs of detoxification could be compensated by the income from useful by-products.

References

Achak, M., Ouazzani, N., Yaacoubi, A., & Mandi, L. (2008) Caractérisation des margines issues d'une huilerie moderne et essai de leur traitement par coagulation-floculation par la chaux et le sulfate d'aluminium. Sciences de l'eau 21, 53–57.

Adhoum, N., & Monser, L. (2004) Decolourization and removal of phenolic compounds from olive mill wastewater by electrocoagulation. Chemical Engineering and Processing 43, 1281–1287.

Aissam, H., Penninckx, M.J., & Benlemlih, M. (2007) Reduction of phenolics content and COD in olive oil mill wastewaters by indigenous yeasts and fungi. World Journal of Microbiology and Biotechnology 23, 1203–1208.

Akgün, N.A., & Doymaz, I. (2005) Modelling of olive cake thin-layer drying process. Journal of Food Engineering 68, 455–461.

Aktas, E.S., & Imre, S., & Ersoym, L. (2001) Characterization and lime treatment of olive mill wastewater. Water Research 35, 2336–2340.

Alba, J. (1994) Nuevas tecnologías para la obtención del aceite de oliva. Fruticultura Profesional (Suplemento) 62, 85–95.

Alburquerque, J.A., Gonzalvez, J., Garcıa, D., & Cegarra, J. (2004) Agrochemical characterisation of "alperujo," a solid by-product of the two phase centrifugation method for olive oil extraction. Bioresource Technology 92, 195–200.

Allouche, N., Fki, I., & Sayadi, S. (2004) Toward a high yield recovery of antioxidants and purified hydroxytyrosol from olive mill wastewaters. Journal of Agricultural and Food Chemistry 52, 267–273.

Al-Malah, K., Azzam, M., & Abu-Lail, N.I. (2000) Olive mills effluent (OME) wastewater post-treatment using activated clay. Separation and Purification Technologies 20, 225–234.

Al-Muhtaseb, A.H., McMinn, W.A.M., & Magee, T.R.A. (2004) Water sorption isotherms of starch powders. Part 1: mathematical description of experimental data. Journal of Food Engineering 61, 297–307.

Ammary, B.Y. (2005) Treatment of olive mill wastewater using an anaerobic sequencing batch reactor. Desalination 177, 157–165.

Arienzo, M., & Capasso, R. (2000) Analysis of metal cations and inorganic anions in olive oil mill waste waters by atomic absorption spectroscopy and ion chromatography: detection of metals bound mainly to the organic polymeric fraction. Journal of Agricultural and Food Chemistry 48, 1405–1410.

Arjona, R., Garcia, A., & Ollero, P. (1999) The drying of alpeorujo, a waste product of the olive mill industry. Journal of Food Engineering 41, 229–234.

Arjona, R., Ollero, P., & Vidal, B. (2005) Automation of an olive waste industrial rotary dryer. Journal of Food Engineering 68, 239–247.

Arvanitoyannis, I.S., Kassaveti, A., & Stefanatos, S. (2007) Olive oil waste treatment: A comparative and critical presentation of methods, advantages & disadvantages. Critical Reviews in Food Science and Nutrition 47, 187–229.

Azbar, N., Bayram, A., Filibeli, A., Muezzinoglu, A., Sengul, F., & Ozer, A. (2004) A review of waste management options in olive oil production. Critical Reviews in Environmental Science and Technology 34, 209–247.

Baeta-Hall, L., Sàágua, M.C, Bartolomeu, M.L., Anselmo, A.M., & Rosa, M.F. (2005) Biodegradation of olive oil husks in composting aerated piles. Bioresource Technology 96, 69–78.

Belfort, G., Davis, R.H., & Zydney, A.L. (1994) The behaviour of suspensions and macromolecular solutions in crossflow microfiltration. Journal of Membrane Science 96, 1–58.

Beltran-Heredia, J., Torregrosa, J., Garcia, J., Dominguez, J.R., & Tierno, J.C. (2001) Degradation of olive mill wastewater by the combination of Fenton's reagent and ozonation processes with an aerobic biological treatment. Water Science and Technology 44, 103–108.

Benavent, M.C. (2008) Procedure for the industrialisation of olive oil press by-products and the product obtained. Patent US20080146828 A1.

Bendini, A., Bonoli, M., Cerretani, L., Biguzzi, B., Lercker, G., & Toschi, T.G. (2003) Liquid–liquid and solid-phase extractions of phenols from virgin olive oil and their separation by chromatographic and electrophoretic methods. Journal of Chromatography A 985, 425–433.

Benitez, F.J., Beltran-Heredia, J., Torregrosa, J., & Acero, J.L. (1999) Treatment of olive mill wastewaters by ozonation, aerobic degradation and the combination of both treatments. Chemical Technology and Biotechnology 74, 639–646.

Bertin, L., Ferri, F., Scoma, A., Marchetti, L., & Fava, F. (2011) Recovery of high added value natural polyphenols from actual olive mill wastewater through solid phase extraction. Chemical Engineering J 171, 1287–1293,

Bondioli, P., Mariani, C., Lanzani, A., Fedeli, E., & Muller, A. (1993) Squalene recovery from oil deodorizer distillates. Journal of the American Oil Chemists Society 70, 763–766.

Borja, R., Garrido, S.E., Martinez, L., Ramos-Cormenzana, A., & Martin, A. (1993) Kinetic study of anaerobic digestion of olive mill wastewater previously fermented with *Aspergillus terreus*. Process Biochemistry 28, 397–401.

Borja, R., Martin, A., Maestro, R., Alba, J., & Fiestas, J.A. (1992) Enhancement of the anaerobic digestion of olive mill wastewater by the removal of phenolic inhibitors. Process Biochemistry 27, 231–237.

Borja, R., Rincon, B., & Raposo, F. (2006) Anaerobic biodegradation of two-phase olive mill solid wastes and liquid effluents: kinetic studies and process performance. Journal of Chemical Technology and Biotechnology 81, 1450–1462.

Borja, R., Rincón, B., Raposo, F., Alba, J., & Martín, A. (2003) Kinetics of mesophilic anaerobic digestion of the two-phase olive mill solid waste. Biochemical Engineering Journal 15, 139–145.

Canepa, P., Marignetti, N., Rognoni, U., & Calgari, S. (1988) Olive mills wastewater treatment by combined membrane processes. Water Research 22, 1491–1494.

Caputo, A.C., Scacchia, F., & Pelagagge, P.M. (2003) Disposal of by-products in olive oil industry: waste-to-energy solutions. Applied Thermal Engineering 23, 197–214.

Cardoso, S.M., Coimbra, M.A., & da Silva, J.A.L. (2003) Calciummediated gelation of an olive pomace pectic extract. Carbohydrate Polymers 52, 125–133.

Casanelles, D.J. (1985) Process for preparing squalene and squalane from by-products from the refining of vegetable oils. Patent ES8602102.

Cassano, A., Conidi, C., Giorno, L., & Drioli, E. (2013). Fractionation of olive mill wastewaters by membrane separation techniques. Journal of Hazardous Materials 248–249, 185–193.

Castanas, E., Andricopoulos, N., Mposkou, G., & Vercauteren, J. (2005) A method for the treatment of olive mill waste water. Patent WO2005003037 (B1).

Cegarra, J., Amor, J.B., Gonzálvez, J., Bernal, M.P., & Roig, A. (2000) Characteristics of a new solid olive-mill-by-product ("alperujo") and its suitability for composting. In: Proceedings of the International Composting Symposium ICS'99, 1CBA Press Inc., ed. P.R. Warman & B.R. Taylor. 124–140.

Cegarra, J., Paredes, C., Roig, A., Bernal, M.P., & Garcia, D. (1996) Use of olive mill wastewater compost for crop production. International Biodeterioration and Biodegradation 38, 193–203.
Chasekioglou, A., Goula, A.M., Adamopoulos, K.G., & Lazarides, H.N. (2015) An approach to turn olive mill wastewater into a valuable food by-product based on spray drying in dehumidified air using drying aids. Powder Technology 311, 376–389.
Cho, B.D., & Fane, A.G. (2002) Fouling transients in nominally sub-critical flux operation of a membrane bioreactor. Journal of Membrane Science 2002(209): 391–403.
Coimbra, M.A., Waldron, K.W., & Selvendran, R.R. (1995) Isolation and characterization of cell wall polymers from the heavily lignified tissues of olive (*Olea europaea*) seed hull. Carbohydrate Polymers 27, 285–294.
Comandini, P., Lerma-Garcνa, M.J., Massanova. P., Simo-Alfonso, E.F., & Toschia. T.G. (2015) Phenolic profiles of olive mill wastewaters treated by membrane filtration systems. Journal of Chemical Technology Biotechnology 90, 1086–1093.
Crea, R., & Caglioti, L. (2005) Water-soluble extract from olives. Patent US 6936287 B1.
Cuomo, J., & Rabovskiy, A.B. (2001) Antioxidant compositions extracted from olives and olive by-products. Patent WO01455142001.
Dammak, M., Neves, A., Nabetanic, H., Isoda, H., Sayadi, S., & Nakajima, M. (2015) Transport properties of oleuropein through nanofiltration membranes. Food and Bioproducts Processing 94, 342–353.
Davies, L.C., Vilhena, A.M., Novais, J.M., & Martins-Dias, S. (2004) Olive mill wastewater characteristics: modelling and statistical analysis. Grasas y Aceites 55, 233–241.
De Leonardis, A., Macciola, V., Lembo, G., Aretini, A., & Nag, A. (2007) Studies on oxidative stabilisation of lard by natural antioxidants recovered from olive-oil mill wastewater. Food Chemistry 100, 998–1004.
De Martino, A., Arienzo, M., Iorio, M., Vinale, F., Lorito, M., Prenzler, P.D., Ryan, D., & Obied, H.K. (2011) Detoxification of olive mill wastewaters by zinc–aluminium layered double hydroxides. Applies Clay Science 53, 737–744.
Dermeche, S., Nadour, M., Larroche, C., Moulti-Mati, F., & Michaud, P. (2013) Olive mill wastes: biochemical characterizations and valorization strategies. Process Biochemistry 48, 1532–1552.
Di Giovacchino, L., & Prezioso, S. (2006) Utilization of olive mill by-products. In: Proceedings of Olive.bioteq 2006. Recent Advances in Olive Industry, 379–389.
Doymaz, I., Gorel, O., & Akgün, N.A. (2004) Drying characteristics of the solid by-product of olive oil extraction. Biosystems Engineering 88, 213–219.
Ehaliotis, C., Papadopoulou, K., Kotsou, M., Mari, I., & Balis, C. (1999) Adaptation and population dynamics of *Azotobacter vinelandii* during aerobic biological treatment of olive-mill wastewater. FEMS Microbiology Ecology 30, 301–311.
El-Abbassi, A., Hafidi, A., García-Payo, M.C., & Khayet, M. (2009) Concentration of olive mill wastewater by membrane distillation for polyphenols recovery. Desalination 245, 670–674.
El-Abbassi, A., Kiai, H., Hafidi, A., García-Payo, M., & Khayet, M. (2012) Treatment of olive mill wastewater by membrane distillation using polytetrafluoroethylene membranes. Separation Purification Technology 98, 55–61.
El-Abbassi, A., Kiai, H., & Hafidi, A. (2012) Phenolic profile and antioxidant activities of olive mill wastewater. Food Chemistry 132, 406–412.
El-Abbassi, A., Kiai, H., Raiti, J., & Hafidi, A. (2014) Cloud point extraction of phenolic compounds from pretreated olive mill wastewater. Journal of Environmental Chemical Engineering 2, 1480–1486.
El-Shafey, E.I., Correia, P.F.M., & de Carvalho, J.M.R. (2005) An integrated process of olive mill wastewater treatment. Separation Science and Technology 40, 2841–2869.
Emmons, W., & Guttersen, C. (2005) Isolation of oleuropein aglycon from olive oil vegetation water. Patent US20050103711.
Ena, A., Pintucci, C., & Carlozzi, P. (2012) The recovery of polyphenols from olive mill waste using two adsorbing vegetable matrices. Journal of Biotechnology 157, 573–577.
Ergüder, T.H., Güven, E., & Demirer, G.N. (2000) Anaerobic treatment of olive mill wastes in batch reactors. Process Biochemistry 36, 243–248.
Faaij, A., Hekkert, M., Worrell, E., & van Wijk, A. (1998) Optimization of the final waste treatment system in the Netherlands. Resources, Conservation and Recycling 22, 47–82.
Fernandéz, A.G., & García, P.G. (1989) Purificación de aguas residuales ricas en polifenoles: aplicación de la oxidación húmeda a los efluentes acuosos derivados de las industrias olivareras. Grasas y Aceites 40, 291–295.
Fernandez-Bolanos, J. (2000) Process for obtaining mannitol from pulp extracted from olives. Patent ES2143939.
Fernández-Bolanos, J., Felizón, B., Brenes, M., Guillén, R., & Heredia, A. (1998) Hydroxytyrosol and tyrosol as the main compounds found in the phenolic fraction of steam-exploded olive stones. Journal of the American Oil Chemists Society 75, 1–7.

Fernández-Bolaños, J., Felizón, B., Heredia, A., Rodríguez, R., Guillén, R., & Jiménez, A. (2001) Steam-explosion of olive stones: hemicellulose solubilisation and enhancement of enzymatic hydrolysis of cellulose. Bioresource Technology 79, 53–61.
Fernandez-Bolanos, J., Fernandez-Diez, M.J., Rivas Moreno, A., & Perez, T. (1983) A sucares y polioles en aceitunas verdes III. Grasas y Aceites 34, 168–171.
Fernández-Bolaños, J., Rodríguez, G., Gómez, E., Guillen, R., Jiménez, A., Heredia, A., & Rodríguez, R. (2004) Total recovery of the waste of two-phase olive oil processing: isolation of added-value compounds. Journal of Agricultural and Food Chemistry 52, 5849–5855.
Fernandez-Bolanos, J., Rodriguez, G., & Rodriguez, R. (2002) Production in large quantities of highly purified hydroxytyrosol from liquid-solid waste of two phase olive oil processing or "alperujo." Journal of Agricultural and Food Chemistry 50, 6804–6811.
Fernandez-Bolanos, J., Rodriguez, G., Rodriguez, R., Guillın, R., & Jimenez, A. (2006) Extraction of interesting organic compounds from olive oil waste. Grasas y Aceites 57, 95–106.
Ferreira, F., Carvalho, L., Pereira, R., Antunes, S.C., Marques, S.M., Gonhalves, F., Duarte, A.C., Rocha-Santos, T.A.P., & Freitas, A.C. (2008) Biological and photo-Fenton treatment of olive oil mill wastewater. Global NEST Journal 10, 419–425.
Ferri, F., Bertin, L., Scoma, A., Marchetti, L., & Fava, F. (2011) Recovery of low molecular weight phenols through solid-phase extraction. Chemical Engineering J 166, 994–1001
Field, R.W., Wu, D., Howell, J.A., & Gupta, B.B. (1995) Critical flux concept for microfiltration fouling. Journal of Membrane Science 100, 259–272.
Filippi, C., Bedini, S., Levi-Minzi, R., Cardelli, R., & Saviozzi, A. (2002) Cocomposting of olive oil mill by-products: chemical and microbiological evaluations. Compost Science and Utilization 10, 63–71.
Firlbeck, D., Faulstich, M., Urmann, C.A., Azaizeh, H., Tafesh, A., & Riepl, H. (2013) Central composite design for optimal technology of concentrating vanillic acid using foam fractionation. Separation and Purification Technology 119, 28–34.
Frascari, D., Bacca, A.E.M., Zama, F., Bertin, L., Fava, F., & Pinelli, D. (2016) Olive mill wastewater valorisation through phenolic compounds adsorption in a continuous flow column. Chemical Engineering Journal 283, 293–303
Freire, F., Figueiredo, A., & Ferrão, P. (1999) Thermal analysis and drying kinetics of olive bagasse. Drying Technology 17, 895–907.
Galanakis, C.M., Tornberg, E., & Gekas, V. (2010a) A study of the recovery of the dietary fibers from olive mill wastewater and the gelling ability of the soluble fiber fraction. LWT – Food Science and Technology 43, 1009–1017.
Galanakis, C.M., Tornberg, E., & Gekas, V. (2010b) The effect of heat processing on the functional properties of pectin contained in olive mill wastewater. LWT – Food Science and Technology 43, 1001–1008.
Galanakis, C.M., Tornberg, E., & Gekas, V. (2010c) Clarification of high-added value products recovered from olive waste. Journal of Food Engineering 99, 190–197.
Galanakis, C.M., Tornberg, E., & Gekas, V. (2010d) Dietary fiber suspensions from olive mill wastewater as potential fat replacements in meatballs. LWT – Food Science and Technology 43, 1018–1025.
Galiatsatou, P., Metaxas, M., Arapoglou, D., & Kasselouri-Rigopoulou, V. (2002) Treatment of olive mill waste water with activated carbons from agricultural by-products. Waste Manage (Oxford) 2002(22), 803–812.
Garcia-Castello, E., Cassano, A., Criscuoli, A., Conidi, C., & Drioli, E. (2010) Recovery and concentration of polyphenols from olive mill wastewaters by integrated membrane system. Water Research 44, 3883–3892.
García-Granados, A., & Martínez, L. (1994) Procedimiento para obtener manitol y productos derivados a partir de alpeorujo procedente del procesado de las aceitunas según el procedimiento de dos fases. Patent ES2060549.
Giannoutsou, E.P., Meintanis, C., & Karagouni, A.D. (2004) Identification of yeast strains isolated from a two-phase decanter system olive oil waste and investigation of their ability for its fermentation. Bioresource Technology 93, 301–306.
Gkoutsidis, P.K., Petrotos, K.B., Kokkora, M.I., Tziortziou, A.D., Christodouloulis, K., & Goulas, P. (2015) Olive mill wastewater treatment by diafiltration. 2015 FABE International Conference.
Göğüş, F., & Maskan, M. (2001) Drying of olive pomace by a combined microwave-fan assisted convection oven. Nahrung/Food 45, 129–132.
Göğüş, F., & Maskan, M. (2006) Air drying characteristics of solid waste (pomace) of olive oil processing. Journal of Food Engineering 72, 378–382.
Goula, A.M., & Adamopoulos, K.G. (2013) A method for preparing a novel solid product from olive mill wastewater: wastewater characterization and product recovery. Drying Technology 31, 339–349.
Goula, A.M., & Lazarides, H.N. (2015) Integrated processes can turn industrial food waste into valuable food by-products and/or ingredients: the cases of olive mill and pomegranate wastes. Journal of Food Engineering 167, 45–50.

Hamdi, M., & Garcia, J.L. (1991) Comparison between anaerobic filter and anaerobic contact process for fermented olive mill wastewater. Bioresource Technology 8, 23–29.
Hanafi, F., Assobheib, O., & Mountadara, M. (2010) Detoxification and discoloration of Moroccan olive mill wastewater by electrocoagulation. Journal of Hazardous Materials 74, 807–812.
Heredia, A., Guillen, R., Fernández-Bolanos, J., & Rivas, M. (1987) Olives stone as a source of fermentable sugars. Biomass 14, 143–148.
Hytiris, N., Kapellakis, I.E., de La Roij, R., & Tsagarakis, K.P. (2004) The potential use of olive mill sludge in solidification process. Resources, Conservation and Recycling 40, 129–139.
Ibarra, A., & Sniderman, Z.N. (2008) Olive polyphenols concentrate. Patent US20080014322.
Israilides, C.J., Vlyssides, A.G., Mourafeti, V.N., & Karvouni, G. (1997) Olive oil wastewater treatment with the use of an electrolysis system. Bioresource Technology 61, 163–170.
Jerman-Klen, T., & Mozetic-Vodopivec, B. (2011) Ultrasonic extraction of phenols from olive mill wastewater: comparison with conventional methods. Journal of Agricultural and Food Chemistry 59, 12725–12731.
Jumah, R., Al-Kteimat, E., Al-Hamad, A., & Telfah, E. (2004) Constant and intermittent drying characteristics of olive cake. Drying Technology 25, 1421–1426.
Jurado, F., Cano, A., & Carpio, J. (2003) Modelling of combined cycle power plants using biomass. Renewable Energy 28, 743–753.
Kestioglu, K., Yonar, T., & Azbar, N. (2005) Feasibility of physicochemical treatment and advanced oxidation processes (AOPs) as a means of pretreatment of olive mill effluent (OME). Process Biochemistry 40, 2409–2416.
Khoufi, S., Feki, F., & Sayadi, S. (2007) Detoxification of olive mill wastewater by electrocoagulation and sedimentation processes. Journal of Hazardous Materials 142, 58–67.
Lafka, T-I., Lazou, A.E., Sinanoglou, V.J., & Lazos, E.S. (2011) Phenolic and antioxidant potential of olive oil mill wastes. Food Chemistry 125, 92–98.
Lesage-Meessen, L., Navarro, D., Maunier, S., Sigoillot, J.C., Lorquin, J., Delattre, M., Simon, J.L., Asther, M., & Labat M. (2001) Simple phenolic content in olive oil residues as a function of extraction systems. Food Chemistry 75, 501–507.
Liébanes, M.D., Aragón, J.M., & Palancar, M.C. (2008) Modeling the moisture sorption isotherms of two-phase solid olive oil by-product. European Journal of Lipid Science and Technology 110, 413–421.
Liebanes, M.D., Aragon, J.M., Palancar, M.C., Arevalo, G., & Jimenez, D. (2006) Fluidized bed drying of 2-phase olive mill byproducts. Drying Technology 24, 1609–1618.
Liu, D., & Wang, M. (2010) Novel use of hydroxytyrosol and olive extracts/concentrates containing in it. Patent US0130620 A1.
Longhi, P., Vodopivec, B., & Fiori, G. (2001) Electrochemical treatment of olive oil mill wastewater. Analytica Chimica Acta 91, 169–174.
Lopez, M.J., & Ramos-Cormenzana, A. (1996) Xanthan production from olive-mill wastewaters. International Biodeterioration and Biodegredation 38, 263–270.
Madejón, E., Galli, E., & Tomati, U. (1998) Composting of wastes produced by low water consuming olive mill technology. Agrochimica 42, 135–146.
Madejón, E., López, R., Murillo, J.M., & Cabrera, F. (2001) Agricultural use of three (sugar-beet) vinasse compost: effect on crops and chemical properties of a cambisol soil in the guadalquivir river Valley (SW Spain). Agriculture, Ecosystems and Environment 84, 55–65.
Maestro-Durán, R., Leon-Cabello, R., Ruiz-Gutierrez, V., Fiestas, P., & Vazquez-Roncero, A. (1994) Glucosidos fenólicos amargos de las semillas del olivo. Grasas y Aceites 45, 332–335.
Mantzavinos, D., & Kalogerakis, N. (2005) Treatment of olive mill effluents: part I. Organic matter degradation by chemical and biological processes—an overview. Environment International 31, 289–295.
Marmo, L. (2007) Low temperature drying of pomace in spout and spout-fluid beds. Journal of Food Engineering 79, 1179–1190.
Meziane, S. (2011) Drying kinetics of olive pomace in a fluidized bed dryer. Energy Conversion and Management 52, 1644–1649.
Milczarek, R., Dai, A., Otonic, C., & McHugh, T. (2011) Effect of shrinkage on isothermal drying behavior of 2-phase olive mill waste. Journal of Food Engineering 103, 434–441.
Millan, B., Lucas, R., Robles, A., Garcia, T., de Cienfuegos, G.A., & Galvez, A. (2000) A study on the microbiota from olive-mill wastewater (OMW) disposal lagoons, with emphasis on filamentous fungi and their biodegradative potential. Microbiological Research 155, 143–147.
Montero, I., Miranda, T., Arranz, J.I., & Rojas, C.V. (2011) Thin layer drying kinetics of by-products from olive oil processing. International Journal of Molecular Sciences 12, 7885–7897.
Moreno, E., Perez, J., Ramos-Cornnenzana, A., & Martinez, J. (1987) Antimicrobial effect of wastewater from olive oil extraction plants selecting soil bacteria after incubation with diluted waste. Microbios 51, 169–174.

Mouncif, M., Tamoh, S., Faid, M., & Achkari-Begdouri, A. (1993) A study of chemical and microbiological characteristics of olive waste water in Morocco. Grasas y Aceites 44, 335–338.

Nanis, H. (2014) Microbiological and physicochemical changes during fermentation of solid residue of olive mill wastewaters, which is exploited towards the production of an olive paste–type product. M.Sc. thesis, Aristotle University of Thessaloniki, Greece.

Nasopoulou, C., & Zabetakis, I. (2013) Agricultural and aquacultural potential of olive pomace : a review. Journal of Agricultural Science 2013(5), 116–127.

Nefzaoui, A. (1985) Valorisation des residus lignocellulosiques dans l'alimentation des ruminants par les traitements aux alkalis : application aux grignons d'olive. PhD thesis, Universite Catholique de Louvain-la-Nueve.

Niaounakis, M., & Halvadakis, C.P. (2006) Olive processing waste treatment: literature review and patent survey. London: Elsevier Applied Science.

Nogales, R., Melgar, R., Guerrero, A., Lozada, G., Benítez, E., Thompson, R., & Gómez, M. (1999) Growth and reproduction of *Eisenia Andrei* in dry olive cake mixed with other organic wastes. Pedobiologia 43, 744–752.

Ntougias, S., Bourtzis, K., & Tsiamis, G. (2013) The microbiology of olive mill wastes. BioMed Research International, Article ID 784591.

Nunes, A.V.M.M., Crespo, J.P.S.G., Santos, J.C.D., Matias, A.F., & Duarte, C.M. (2007) Method of obtaining a natural hydroxytyrosol-rich concentrate from olive tree residues and subproducts using clean technologies. IP Monitor.

Obied, H.K., Prenzler, P.D., & Robards, K. (2008) Potent antioxidant biophenols from olive mill waste. Food Chemistry 111, 171–178.

Ochando-Pulido, J.M., Victor-Ortega, M.D., & Martinez-Ferez, A. (2015) On the cleaning procedure of a hydrophilic reverse osmosis membrane fouled by secondary-treated olive mill wastewater. Chemical Engineering Journal 260, 142–151.

Otles, S., & Selek, I. (2012) Treatment of olive mill wastewater and the use of polyphenols obtained after treatment. International Journal of Food Studies 1, 85–100.

Paraskeva, C.A., Papadakis, V.G., Tsarouchi, E., Kanellopoulou, D.G., & Koutsoukos, P.G. (2007) Membrane processing for olive mill wastewater fractionation. Desalination 213, 218–229.

Paraskeva, P., & Diamadopoulos, E. (2006) Technologies for olive mill wastewater (OMW) treatment: a review. Journal of Chemical Technology and Biotechnology 81, 1475–1485.

Paredes, C., Cegarra, J., Roig, A., Sanchez-Monedero, M.A., & Bernal, M.P. (1999) Characterization of olive-mill wastewater (alpechın) and its sludge for agricultural purposes. Bioresource Technology 67, 111–115.

Paredes, C., Roig, A., Bernal, M.P., Sanchez-Monedero, M.A., & Cegarra, J. (2000) Evolution of organic matter and nitrogen during co-composting of olive mill wastewater with solid organic wastes. Biology and Fertility of Soils 32, 222–227.

Paredes, M.J., Monteoliva-Sanchez, M., Moreno, E., Perez, J., Ramos-Cornenzana, A., & Martinez, J. (1986) Effect of waste waters from olive mill extraction plants on the bacterial population of soil. Chemosphere 15, 659–664.

Petrotos, K.B., Karkanta, F.K., Gkoutsidis, P.E., Giavasis, I., Papatheodorou, K.N., & Ntontos, A.C. (2012) Production of novel bioactive yogurt enriched with olive fruit polyphenols. World Academy of Science, Engineering and Technology 64, 967–872.

Piperidou, C.I., Chaidou, C.I., Stalikas, D., Soulti, K., Pilidis, G.A., & Balis, C. (2000) Bioremediation of olive mill wastewater: chemical alterations induced by *Azotobacter vinelandii*. Journal of Agricultural and Food Chemistry 48, 1941–1948.

Pizzichini, M., & Russo, C. (2005) Process for recovering the components of olive mill wastewater with membrane technologies. Patent WO2005123603.

Ramos-Cormenzana, A., Juarez-Jimenez, B., & Garcıa-Pareja, M.P. (1996) Antimicrobial activity of olive mill wastewaters (alpechin) and biotransformed olive oil mill wastewater. International Biodeterioration and Biodegradation 38, 283–290.

Raposo, F., Borja, R., Sánchez, E., Martı́n, M.A., & Martı́n, A. (2004) Performance and kinetic evaluation of the anaerobic digestion of two-phase olive mill effluents in reactors with suspended and immobilized biomass. Water Research 38, 2017–2026.

Rodriguez, M., Perez, J., Ramos-Cornenzan, A., & Martinez, J. (1988) Effect of extracts obtained from olive oil mill wastewaters on *Bacillus megaterium* ATCC 33085. Journal of Applied Bacteriology 64, 219–226.

Roig, A., Cayuela, M.L., & Sanchez-Monedero, M.A. (2006) An overview on olive mill wastes and their valorisation methods. Waste Management 26, 960–969.

Romo-Sanchez, S., Alves-Baffi, M., Arevalo-Villena, M., Ubeda-Iranzo, J., & Briones-Perez, A. (2010) Yeast biodiversity from oleic ecosystems: study of their biotechnological properties. Food Microbiology 27, 487–492.

Rosello-Soto, E., Koubaa, M., Moubarik, A., Lopes, R.P., Saraiva, J.A., Boussetta, N., Grimi, N., & Barba, F.J. (2015) Emerging opportunities for the effective valorization of wastes and by-products generated during olive oil production process: nonconventional methods for the recovery of high-added value compounds. Trends in Food Science & Technology 45, 296–310.

Rozzi, A., & Malpei, F. (1996) Treatment and disposal of olive mill effluents. International Biodeterioration and Biodegradation 38, 135–144.

Rubio-Senent, F., Rodríguez-Gutierrez, G., Lama-Munoz, A., García, A., & Fernandez-Bolanos, J. (2015a) Novel pectin present in new olive mill wastewater with similar emulsifying and better biological properties than citrus pectin. Food Hydrocolloids 50, 237–246.

Rubio-Senent, F., Rodríguez-Gutierrez, G., Lama-Munoz, A., García, A., & Fernandez-Bolanos, J. (2015b) Pectin extracted from thermally treated olive oil by-products: characterization, physico-chemical properties, in vitro bile acid and glucose binding. Food Hydrocolloids 43, 311–321.

Russo, C. (2007) A new membrane process for the selective fractionation and total recovery of polyphenols, water and organic substances from vegetation waters (VW). Journal of Membrane Science 288, 239–246.

Ryan, D., Prenzler, P.D., Lavee, S., Antolovich, M., & Robards, K. (2003) Quantitative changes in phenolic content during physiological development of the olive (*Olea europaea*) cultivar hardy's mammoth. Journal of Agriculture and Food Chemistry 51, 2532–2538.

Sabbah, I., Marsook, T., & Basheer, S. (2004) The effect of pretreatment on anaerobic activity of olive mill wastewater using batch and continuous systems. Process Biochemistry 39, 1947–1951.

Santi, C.A., Cortes, S., D'Acqui, L.P., Sparvoli, E., & Pushparaj, B. (2008) Reduction of organic pollutants in olive mill wastewater by using different mineral substrates as adsorbents. Bioresource Technology 99, 1945–1951,

Sarika, R., Kalogerakis, N., & Mantzavinos, D. (2005) Treatment of olive mill effluents: part II. complete removal of solids by direct flocculation with poly electrolytes. Environment International 31, 297–304.

Saviozzi, A., Levi-Minzi, R., Cardelli, R., Biasci, A., & Riffaldi, R. (2001) Suitability of moist olive pomace as soil amendment. Water, Air and Soil Pollution 128, 13–22.

Saviozzi, A., Levi-Minzi, R., Riffaldi, R., & Lupetti, A. (1991) Effeti dello spandimento di acque di vegetacione sul terreno agrario. Agrochimica 35(1–3), 135–147.

Scioli, C., & Vollaro, L. (1997) The use of *Yarrowia lilolytica* to reduce pollution in olive mill wastewaters. Water Research 31, 2520–2524.

Scoma, A., Bertin, L., Zanaroli, G., Fraraccio, S., & Fava, F. (2011) A physicochemical–biotechnological approach for an integrated valorization of olive mill wastewater. Bioresource Technology 102, 10273–10279,

Servili, M., Esposto, S., Veneziani, G., Urbani, S., & Taticchi, A. (2011) Improvement of bioactive phenol content in virgin olive oil with an olive vegetation water concentrate produced by membrane treatment. Food Chemistry 124, 1308–1315.

Sierra, J., Martí, E., Montserrat, G., Cruanas, R., & Garau, M.A. (2001) Characterisation and evolution of a soil affected by olive oil mill wastewater disposal. The Science of the Total Environment 279, 207–214.

Soni, M.G., Burdock, G.A., Christian, M.S., Bitler, C.M., & Crea, R. (2006) Safety assessment of aqueous olive pulp extract as an antioxidant or antimicrobial agent in foods. Food and Chemical Toxicology 44, 903–915.

Stasinakis, A.S., Elia, I., Petalas, A.V., & Halvadakis, C.P. (2008) Removal of total phenols from olive-mill wastewater using an agricultural by-product, olive pomace. Journal of Hazardous Materials 160, 408–413.

Stoller, M., & Chianese, A. (2006) Optimization of membrane batch processes by means of the critical flux theory. Desalination 191, 62–70.

Takac, S., & Karakaya, A. (2009) Recovery of phenolic antioxidants from olive mill wastewater. Recent Patents in Chemical Engineering 2, 230–237.

Tekin, A.R, & Dalgiç, A.C. (2000) Biogas production from olive pomace. Resources, Conservation and Recycling 30, 301–313.

Terramocia, S., Bartocci, S., Taticchi, A., Di Giovanni, S., Pauselli, M., Mourvaki, E., Urbani, S., & Servili, M. (2013) Use of dried stoned olive pomace in the feeding of lactating buffaloes: effect on the quantity and quality of the milk produced. Asian-Australasian Journal of Animal Sciences 26, 971–980.

Tornberg, E., & Galanakis, C.M. (2008) Olive waste recovery. World Intellectual Property Organization, WO/2008/082343.

Torrecilla, J.S., Aragon, J.M., & Palancar, M.C. (2006) Improvement of fluidized-bed dryers for drying solid waste (olive pomace) in olive oil mills. European Journal of Lipid Science and Technology 108, 913–924.

Traveria, C.T. (1988) Process for obtaining squalene. Patent ES2004269.

Tsagaraki, E.V., Lazarides, H.N., & Petrotos, K. (2007) Olive mill wastewater treatment. In: Utilization of by-products and treatment of waste in the food industry, ed. V. Oreopoulou & W. Russ. New York: Springer Science, 133–157.

Turano, E., Curcio, S., De Paola, M.G., Calabro, V., & Iorio, G. (2002) An integrated centrifugation ultrafiltration system in the treatment of olive mill wastewater. Journal of Membrane Science 202, 519–531.

Yu, X., Zhu, P., Zhong, Q., Li, M., & Ma, H. (2014) Subcritical water extraction of antioxidant phenolic compounds from xiLan olive fruit dreg. Journal of Food Science and Technology 8, 5012–5020.
Vazquez, A., Maestro, R., & Graciani, E. (1974) Componentes fenolicos de la aceituna II. polifenoles del alpechfn. Grasas y Aceites 25, 341–345.
Velioglou, G.S., Curi, K., & Camlilar, S.R. (1992) Activated sludge treatability of olive oil-bearing wastewater. Water Research 26, 1415–1420.
Villanova, L., Fasiello, G., & Merendino, A. (2008) Process for the recovery of tyrosol and hydroxytyrosol from oil mill wastewaters and catalytic oxidation method in order to convert tyrosol in hydroxytyrosol. US Patent Application.
Vitolo, S., Petarca, L., & Bresci, B. (1999) Treatment of olive oil industry wastes. Bioresource Technology 67, 129–137.
Vivas, A., Moreno, B., Garcia-Rodriguez, S., & Benitez, E. (2009) Assessing the impact of composting and vermicomposting on bacterial community size and structure, and microbial functional diversity of an olive-mill waste. Bioresource Technology 100, 1319–1326.
Vlyssides, A.G., Loizidou, M., Gimouhopoulos, K., & Zorpas, A. (1998) Olive oil processing wastes production and their characteristics in relation to olive oil extraction methods. Fresenius Environmental Bulletin 7(5–6), 308–313.
Vlyssides, A.G., Loizides, M., & Karlis, P.K. (2004) Integrated strategic approach for reusing olive oil extraction by-products. Journal of Cleaner Production 12(6), 603–611.
Wilhelm, J., Mehdi, B., & Christinae, R.-E. (2003) Process for the isolation of antioxidants. Patent EP1310175 A.
Zaklis, D.P., Vavouraki, A.I., Kornaros, M.E., & Paraskeva, C.A. (2015) Purification of olive mill wastewater phenols through membrane filtration and resin adsorption/desorption, Journal of Hazardous Materials 285, 69–76.
Zumbe, A. (2004) Cured olive powder. Patent WO 042742 A1.

10 Olive oil quality and its relation to the functional bioactives and their properties

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10.1 Introduction

The quality of olive oil, produced internationally, is determined by several factors related to its origin and to the compositional changes that occur from the tree to the table. Such changes affect significantly the functional components of the oil.

In order to assess the quality and purity of olive oil, certain physical and chemical characteristics have been recommended (European Commission, 2003; International Oil Council, 2015).

Hydrolysis and oxidation are the most serious quality deteriorations of olive oil. Hydrolysis, also known as lipolysis, usually commences while the oil is still in the fruit, whereas oxidation proceeds mainly after obtaining the oil from the fruit and during its storage (Kiritsakis & Tsipeli, 1992; Kiritsakis, 1998).

10.2 Hydrolysis (lipolysis)

Hydrolysis causes release of fatty acids from the triacylglycerol molecule with consequent increase in total acidity (free fatty acids [FFAs]) and change in flavor. Changes in olive oil flavor may affect the absorption in the body of the antioxidants present in it. Factors affecting hydrolysis are moisture, temperature, enzymes, and microorganisms. Olive oil lipolysis is caused by microbials and enzymes.

10.2.1 Microbial lipolysis

Microorganisms, present in the olive fruit, liberate lipases that cause hydrolysis of the triacylglycerols (Kiritsakis & Markakis, 1984). Improper storage of the fruit favors the development of microorganisms and the hydrolysis of the oil.

10.2.2 Enzymatic lipolysis

Enzymatic lipolysis is caused by endogenous and microbial enzymes (lipases) present in the olive fruit. Endogenous lipase does not manifest its activity until the fruit starts turning purple. The optimum temperature for lipase action is 45 °C, and the optimum pH is 8.3. Fruit remaining on the tree for a long time or on the ground on collection nets, after natural fall, yield oil with high acidity and undesirable flavor. This is due to both native lipase and lipase of associated microorganisms. If the fruit is stored before processing, and especially if storage is improper (e.g., piling up in thick layers where the fruit is heated by its own respiratory

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activity), the combined effect of the endogenous and microbial lipases may result in an increase in acidity of the oil with resultant loss of oil quality and functionality. Olive fruit is a live, breathing organism and the transpiration releases heat, which raises the temperature and activates lipolytic enzymes. The presence of water facilitates lipolysis. Water dissolves the enzymes and favors microbial growth.

The degree of hydrolysis (acidity) is measured by titration of the liberated fatty acids, and the price of olive oil is mainly determined by its acidity. Acidity is not related to the bitter flavor (Kiritsakis, 1998; Bonastre *et al.*, 2004). Refined olive oils have very low acidity because they undergo neutralization.

Olive oil acidity is a determining parameter of olive quality, especially of extra virgin olive oil (EVOO), whose upper threshold is under pressure of being reduced to below 0.8% of FFAs. A recent study of Bustan *et al.* (2014) has shown that under regular environmental conditions, excessive oil acidity is strongly associated with certain factors, such as low fruit load (yield <20 kg tree^{-}1), advanced ripening index, high fruit N level, and fungus infestation. Harvest too late in the season is prone to fungal infections that may have adverse effects on oil quality.

10.3 Oxidation

Oxidation of olive oil may occur either in the dark (autoxidation) or in the light (photooxidation). The oxidation products have an unpleasant flavor and odor and may adversely affect the nutritional and functional value of the oil. Olive oil is resistant to autoxidation because of its low content of polyunsaturated fatty acids and presence of natural antioxidants. However, it is very sensitive to photooxidation, because of the presence of chlorophylls that act as photosensitizers (Kiritsakis & Dugan, 1985).

Research related to the problems of oxidative deterioration has been of interest due to the fact that such a process eliminates the functional compounds of the oil and can cause damage to cell membranes and DNA (Peter & Hakan, 1998); thus, it may be involved in the aging process (Yulan *et al.*, 1998), hypertension (Carla *et al.*, 1998), and cancer growth (Navarro *et al.*, 1999).

10.3.1 Autoxidation mechanism

The reaction of oxygen with unsaturated fatty acids constitutes the major means by which fatty substances undergo deterioration. Oxidation is frequently characterized as autoxidation because the rate of oxidation increases as the reaction proceeds. Unless mediated by other oxidants or enzyme systems, oxidation proceeds through a free-radical chain reaction mechanism involving the three classical stages of initiation, propagation, and termination.

1. *initiation*: formation of free radicals;
2. *propagation*: free-radical chain reaction, formation of hydroperoxides; and
3. *termination*: formation of non-radical products.

Hydroperoxides are the major initial reaction products, known as primary oxidation products. Dugan (1976) described the three autoxidation stages as the abstraction of hydrogen from an olefinic acid molecule (RH) to form a radical, $RH \rightarrow R\bullet + H\bullet$, the weakest bond, that is the abstraction of a hydrogen atom from diallyl methylene or allyl methylene group. At the *initiation* stage, the reactions proceed at a slow rate. The duration of the initiation stage varies among different fatty substances and depends on the degree of unsaturation and on the presence of natural antioxidants, which may exist in the oil. The activation energy required for this process originates from either high storage temperature or some other source.

Propagation follows the initiation stage at the time when the oil acquires a rancid flavor. The free radical (R•) formed at the initiation stage reacts with an oxygen molecule to produce a peroxyl radical (ROO•). The peroxyl radical reacts with another fatty acid molecule (RH) to produce hydroperoxides (ROOH) and new free radicals. The new radical, formed during autoxidation, contributes to the chain by reacting with another oxygen molecule. The oxidation process is now becoming more complicated since the peroxides formed, being unstable, are easily decomposed and form more free radicals that in turn participate in new reactions.

This chain reaction continues until either the unsaturated compound has been exhausted or the free radicals have inactivated each other.

Termination of oxidation is difficult since it is not likely for all free radicals formed to inactivate each other. The products so formed are, however, stable and usually do not break down.

The rate of oxidation depends on a number of factors, including the availability of oxygen and the presence of light and heat. Hydroperoxides formed in the propagation step are labile compounds and further decompose to produce a complex mixture of volatile compounds such as aldehydes, ketones, hydrocarbons, alcohols, and esters, which are responsible for the deterioration of olive oil flavor termed "oxidative rancidity" (Kiritsakis, 1998; Shahidi & Zhong, 2010, 2015).

The initiation reaction is a subject of great interest since it relates both to the site of attack and to the energy requirement. The energy requirement for radical production by hemolytic dissociation of a CH bond is about 80 kcal. The hydrogen α to the double bond is most labile because of the electron distribution at the double bond (Dugan, 1976). When oleic acid, which exists in high percentage in olive oil, is oxidized, abstraction of hydrogen during the initiation stage occurs from either of the carbons α to the double bond (Dugan, 1976). Thus, two radicals are possible, each of which can assume two resonance forms. Addition of oxygen at each radical site, followed by the addition of a hydrogen atom, results in hydroperoxides at four different positions. Formation of the four hydroperoxides in essentially equal amounts has been demonstrated when oleic acid of olive oil is oxidized. Saturated fatty acids such as palmitic and stearic acids, which are present in olive oil, are oxidized only when exposed to conditions of severe stress. The relative rates of oxidation of stearic/oleic/linoleic/linolenic acids are 1:100:1200:2500 (deMan, 1976; Shahidi & Zhong, 2010).

10.3.2 Formation and decomposition of hydroperoxides

During autoxidation of linoleic and other polyunsaturated fatty acids of olive oil, displacement of the double bonds and formation of conjugated hydroperoxides occur. The latter absorb light in the ultraviolet (UV) region at 232 nm.

Hydroperoxides, as stated previously, are unstable compounds, due to the weak bond energy of the oxygen–oxygen bond, and are readily decomposed by high-energy radiation, thermal energy, metal catalysis, or enzyme activity with the means depending on the system in which they exist. Hydroperoxides decompose to form additional radicals, which add to the chain process. This proliferation of radicals causes acceleration of oxidation without requiring new initiation reactions (Dugan, 1976).

When the concentration of hydroperoxides is low, decomposition may occur as a monomolecular decomposition.

$$\mathbf{ROOH \rightarrow RO\bullet + HO\bullet}$$

When the concentration of hydroperoxide is high, decomposition may occur as a bimolecular decomposition.

$$\mathbf{2\ ROOH \rightarrow RO\bullet + ROO\bullet + H_2O}$$

The RO• radicals can participate in the chain propagation stage, although the more energetic, ROO• radicals, predominate. Metallic prooxidants contribute to the formation of additional radicals by acting as hydroperoxide decomposers. A metal, capable of existing in two valence states, functions typically as:

$\mathbf{M^{+} + ROOH \rightarrow RO\bullet + HO^{-} + M^{2}}$ + The reduced metal ion is oxidized.
$\mathbf{M^{2+} + ROOH \rightarrow ROO\bullet + H^{+} + M^{+}}$ The oxidized metal ion is reduced.

$\mathbf{2\ ROOH \rightarrow RO\bullet + ROO\bullet + H_2O}$ Net reaction

In metal ions, such as Cu^{+} and Cu^{2+} or Fe^{2+} and Fe^{3+}, present in olive oil, the hydroperoxides decompose readily with the formation of both RO• and ROO• radicals as the metal ions undergo oxidation-reduction.

Peroxide decomposition products cause the main sensory deterioration of olive oil and deactivation, degradation of bioactives responsible for its functional activity. Odorants are formed in significant yields from 13-hydroperoxy-9,11-octadecadienoic acid (13-HPOD) and 9-hydroperoxy-10,12-octadecadienoic acid (9-HPOD). The 9-HPOD, however, was established as the more effective precursor of the odorants.

10.3.3 Off-flavor compounds formed during olive oil oxidation

During olive oil oxidation, various volatile compounds are formed (Snyder *et al.*, 1985), and it is difficult to determine which ones are responsible for the undesirable flavor and the loss of functional activity. Saturated carbonyl compounds such as pentanal, hexanal, octanal, and nonanal are the major compounds formed in oxidized olive oil. Camphene, myrcene, menthol, nerol, anethol, methyl acetate, and farnesol have been found in old olive oil. Solinas *et al.* (1988) observed a direct relation between perceived rancidity and 2-pentenal, hexanal, 2-heptenal, 2-octenal, octanal, and nonanal. 2-Pentenal and 2-heptenal were the main indicators of rancidity with detection thresholds of 0.5 and 1.5 ppm, respectively. Hexanal formed from linoleate hydroperoxides is the major volatile found in olive oil oxidized at 60 °C.

Gomes *et al.* (2012) studied the amount of oxidized triacylglycerols (ox-TAGs) and triacylglycerol oligopolymers (TAGPs), formed in olive oil during the refining process, and found that the refining process was responsible for the increase in the formation of oxidation products.

10.4 Prevention of olive oil autoxidation

Oil oxidation can be inhibited by adding antioxidants, which react with free radicals and hence break the chain reaction. Natural or synthetic antioxidants, such as phenolic antioxidants, function as free radical acceptors forming stable resonance hybrid compounds that will not propagate further oxidation. As a result, antioxidant free radicals are formed. The antioxidant radicals either annihilate each other or react with a peroxide free radical.

$$\underset{\text{Antioxidant}}{\mathbf{AH}} + \underset{\text{Free radical}}{\mathbf{ROO\bullet}} \rightarrow \underset{\text{Antioxidant radical}}{\mathbf{A\bullet}} + \underset{\text{Hydroperoxide}}{\mathbf{ROOH}}$$

The antioxidant radicals, as noted, react in two ways:

$$\mathbf{A\bullet + A\bullet \rightarrow AA}$$
$$\mathbf{ROO\bullet + A\bullet \rightarrow ROOA}$$

The antioxidant effect of the phenolic compounds in virgin olive oil can be significantly diminished if its initial peroxide value (PV) is too high. Several compounds such as tyrosol, hydroxytyrosol, phenolic acids (ferulic, syringic, caffeic, and *p*-coumaric), oleuropein aglycone, deacetoxyoleuropein aglycone, elenolic acid and derivatives, other secoiridoid compounds, and flavone aglycones (luteolin and apigenin) have been identified in olive oils obtained from different olive cultivars by high-performance liquid chromatography–mass spectrometry (HPLC-MS).

Chimi *et al.* (1991) studied the antioxidant effect of tyrosol, hydroxytyrosol, oleuropein, and caffeic acid, which were in the increasing order of tyrosol < caffeic acid < oleuropein < hydroxytyrosol. On the other hand, Cillard and Cillard (1987) determined the activity of different flavonoids in the order of myricetin > quercetin > rhamnetin > morin > diosmetin > naringenin > apigenin > catechin > 5,7-dihydroxy-3′,4′,5′-1-dimethoxyflavone > robinin > kaempferol > flavone.

Montedoro and Servili (1993) found that hydroxytyrosol, which was isolated from the wastewater of olive oil processing, exhibited a strong antioxidant activity. Polyphenols extracted from olive pomace inhibited the oxidation of olive oil when added to it. According to Uccella (2001), olive oil phenols (biophenols) (Figure 10.1) exhibit antioxidant effects in extra virgin olive oil.

The use of synthetic antioxidants is not permitted in virgin olive oil. Only α-tocopherol may be added to refined olive oil to replenish the lost natural tocopherol during the refining process.

hydroxytyrosilmalate

tyrosiloleate

Figure 10.1 Structures of esterified biophenols detected in olive fruit and in virgin olive oil.

10.5 Photooxidation

Photooxidation causes serious deterioration of olive oil, due to the presence of chlorophylls and pheophytins. These pigments act as prooxidants under light (Kiritsakis, 1998; Rahmani & Csallany, 1998) and as antioxidants in the dark (Psomiadou & Tsimidou, 2002). Thus, when olive oil is exposed to light, photooxidation occurs through the action of natural photosensitizers (i.e., chlorophylls and pheophytins), which react with triplet oxygen to form the excited state singlet oxygen. Singlet oxygen, then, forms a free radical from unsaturated fatty acids, leading to the production of hydroperoxides and eventually to carbonyl compounds resulting in the development of undesirable off flavors (Skibsted, 2000). Thus, protection from light is required for olive oil, which contains a significant amount of photosensitizers (Kiritsakis & Dugan, 1984; Khan & Shahidi, 1999). According to Pristouri *et al.* (2010), the relative effect of some factors affecting olive oil quality is in the order of light > container headspace > packaging material oxygen transmission rate, indicating the destructive contribution of the light on olive oil and the destruction of its functional compounds. It should also be noted that photooxidation is faster than autoxidation by at least three orders of magnitude.

10.5.1 Mechanism of photooxidation

Chlorophylls and pheophytins absorb in the visible or near-UV region. Radiations from these regions create electronically excited states. Chlorophylls and pheophytins exist in two states, *singlet* and *triplet*. Oxygen may also exist in a *singlet* or *triplet* state depending on the arrangement of the electrons in the outer orbitals of the molecule. Triplet (atmospheric) oxygen has three closely grouped energy states due to the two unpaired electrons. The singlet state of oxygen has paired electrons and no magnetic moment. Singlet oxygen is more active than triplet oxygen (Mistry & Min, 1992). The absorption of radiant energy is known as the primary photochemical process and results in an activated molecule.

During photosensitized oxidation, sensitizers (Sen) absorb visible or near-UV light and are converted to the excited singlet state. The excited singlet state of the sensitizer is then converted to its excited triplet state via intersystem crossing (Mistry & Min, 1992). The triplet state of the sensitizer can follow either Type I or Type II pathways. In the Type I pathway, $^3Sen^*$ reacts directly with the substrate (RH) and forms radicals by either hydrogen transfer or electron transfer. The radicals then react with triplet oxygen or other molecules to produce oxidized products. Another mechanism for photooxidation is the Type II mechanism, also known as singlet oxygen oxidation. Here, $^3Sen^*$ activates triplet oxygen to singlet oxygen ($^1O_2$) by triplet–triplet annihilation. This singlet oxygen then reacts with substrates to produce hydroperoxides. The Type II mechanism occurs very rapidly and accounts for almost all the photooxidation (Rawls & Van Santen, 1970; Mistry & Min, 1992).

Singlet oxygen accounts for approximately 80% of chlorophyll-induced photooxidation. Hydrogen atoms' abstraction from the photo-activated carbonyl group of chlorophyll can account for the remaining 20% of the observed photooxidation. Singlet oxygen is mainly responsible for photooxidation of olive oil containing chlorophyll (Kiritsakis & Dugan, 1985; Fakourelis *et al.*, 1987). Carlsson *et al.* (1976) reported that during exposure of chlorophyll to light, the following reaction occurs:

$$\mathbf{Chl + hv \rightarrow Chl^{*} \rightarrow {}^{3}O_2Chl + {}^{1}O_2}$$

Although it is generally accepted (Frankel *et al.*, 1979; Kiritsakis & Dugan, 1984) that singlet oxygen is involved during photooxidation, some photosensitized oxidation of lipids may involve the triplet oxygen state (Chan, 1977). Unlike autoxidation, photooxidation with triplet oxygen does not involve chain reactions. Photooxidation does not require an induction period, whereas autoxidation involves a long induction period (Logani & Davies, 1980).

10.5.2 Singlet oxygen quenchers

Photosensitized oxidation is not prevented by the antioxidants commonly used to inhibit autoxidation. Photooxidation may be prevented by singlet oxygen quenchers (Carlsson *et al.*, 1976), which exhibit the opposite effect of sensitizers. The quenchers decrease the rate of oxidation by quenching singlet oxygen, which is deactivated to the ground state.

There are two types of quenching mechanisms in singlet oxygen oxidation of oils: singlet oxygen quenching and triplet sensitizer quenching. Singlet oxygen quenchers deactivate singlet oxygen to the triplet ground state, or react with the singlet oxygen. Only the most efficient singlet oxygen quenchers can prevent singlet oxygen oxidation at a concentration of 0.01% by weight. Carotenoids are quenchers for either singlet oxygen or triplet sensitizers in singlet oxygen oxidation (Mistry & Min, 1992).

$$\begin{aligned} &\mathbf{{}^{1}Carotenoid + {}^{1}O_2} &&\rightarrow \mathbf{{}^{3}Carotenoid + {}^{3}O_2} \\ &\mathbf{{}^{1}Carotenoid + {}^{3}Sen^{*}} &&\rightarrow \mathbf{{}^{3}Carotenoid + Sen}\ \text{radiationless} \\ &\mathbf{{}^{3}Carotenoid} &&\rightarrow \mathbf{{}^{1}Carotenoid} \end{aligned}$$

Carotenoids with nine or more conjugated double bonds quench singlet oxygen. β-Carotene minimizes lipid oxidation of the oil under light storage by its light-filtering effect (Carlsson *et al.*, 1976; Fakourelis *et al.*, 1987).

10.5.3 Photooxidation of olive oil

The sensitivity of olive oil to light depends upon the intensity of the light source, the composition of the oil (chlorophylls, pheophytins, carotenes, etc.), the concentration of dissolved oxygen, the temperature, and the optical properties of the packaging material. Under the action of light, the four pigments (chlorophylls a and b and pheophytins a and b) present in olive oil develop an oxidizing effect, while in the dark they act as antioxidants (Kiritsakis & Dugan, 1985; Rahmani & Csallany, 1998), acting synergistically with phenolic antioxidants. In an autoxidizing dienoic system, the ratio of peroxide value to percentage of conjugated dienoic acids (PV to % CDA) remains fairly constant over a broad range of early oxidation, whereas it increases in a system in which singlet oxygen oxidation occurs, forming both conjugated and non-conjugated hydroperoxides (Kiritsakis & Dugan, 1985).

Olive oil stored in the dark was oxidized to a lesser extent than that exposed to diffused room light and even less than that exposed to direct sunlight (Figure 10.2). During photooxidation, bleaching of chlorophyll occurs via a rather complicated mechanism.

It is obvious that all factors causing either autoxidation or photooxidation of olive oil lead to loss of functional properties of the oil.

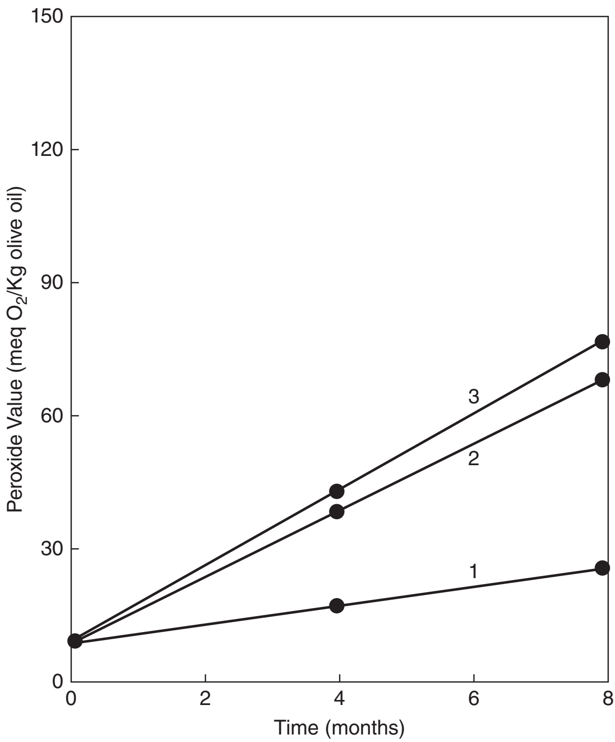

Figure 10.2 Effect of dark, direct sunlight and diffused room light on the photooxidation of olive oil. (1: dark; 2: diffused room light; 3: direct sunlight [4 hours per day and the rest in diffused light]).

10.6 Olive oil quality evaluation with methods other than the official

Fourier transform infrared spectroscopy (FTIR) has been employed by Gomez-Caravaca *et al.* (2013), who correlated the level of fly attack (*Bactocera oleae*) of olive fruit with FTIR spectra of the produced olive oils and observed differences in the regions of the fatty acids and phenolic compounds depending on the percentage of fly attack on the olive fruit. These authors suggested that FTIR methodology could be very useful for quality control of olives before the processing to obtain olive oil, avoiding organic solvents and non-lab-qualified workers. Velasco *et al.* (2014) tried to determine olive oil quality by analyzing olive fruit flesh. Fatty acids, tocopherols, phytosterols, and squalene were measured in olive fruit flesh and the olive oil so obtained, showing variability of oil quality characteristics. The results revealed that selection for the oil quality characteristics can be efficiently conducted through the analysis of olive fruit flesh (Velasco *et al.*, 2014).

Tarakowski *et al.* (2014) established a new protocol for quality assessment of olive oil, analyzing absorption and fluorescence, subjected in the high-pressure range and concluded that the measurement of kinetics of sample solidification processes could distinguish between different olive oil qualities.

Favati *et al.* (2013) studied blends of extra virgin olive oils (EVOOs) from different cultivars and EVOOs from monocultivars to estimate the bitterness intensity of the oils by measuring the total phenol content and compounds responsible for oil bitterness by measuring the absorbance at 225 nm. The oil bitterness intensity could be satisfactorily predicted by using the K225 values of oil samples according to the following equation:

$$\textbf{Bitterness Intensity} = \mathbf{14.055 \times K225 - 0.5787}$$

Figure 10.3 shows the relationship between K225 value and perceived bitterness intensity.

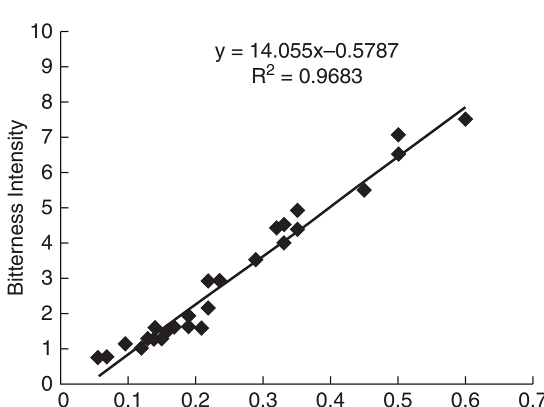

Figure 10.3 Relationship between K225 value and perceived bitterness intensity.

This model could be improved by the implementation of hedonic tests for sensory evaluation, in order to identify the maximum level of acceptability of oil bitterness.

Farhoosh (2014) denoted that monitoring of the primary or secondary oxidation products during heating of olive oils in a kinetic regime at high temperatures provides the stability index values that can be used to compare the oxidative stability of olive oils. Thus, olive oils showed higher temperature sensitivities compared to common vegetable oils, a fact that should be considered when using them in high-temperature processes. This is related to the fact that olive oils are not refined and hence may contain some organic constituents that decompose at temperatures higher than 180 °C (Kiritsakis, 1998). The kinetic studies demonstrated that the oxidative stability of olive oils is affected more by the indigenous antioxidant compounds (tocopherols and/or phenolic compounds) than by the fatty acid composition (Farhoosh & Hoseini-Yazdi, 2014).

The 3,4-DHPEA-EDA terpenic structure oxidation products are not present in fresh virgin olive oil (VOO), but they appear in the first oxidation stage of VOO during the autoxidation process, are increased according to the autoxidation state, and may be used as molecular markers of the early oxidation stage, indicating the degree of freshness of VOO.

Bonastre *et al.* (2004) proposed an on-line quality control analysis of olive oil, based on flow injection determination of total acidity, PV, and UV absorbance at K232 and K270, and obtained satisfactory results for olive oil qualities during storage. Di Maio *et al.* (2011), on the other hand, denoted that secoiridoid derivatives are the most important antioxidants of VOO, and their oxidation products could be used as molecular markers of VOO freshness. Terpenic oxidation products accumulated in VOO during the autoxidation process may be used as an early evaluation index of the VOO autoxidation state before the fatty acids oxidation.

Chlorophyll absorption and fluorescent peaks may be used as indicators of quality, while their absence indicates oxidation.

10.7 Behavior of olive oil during frying process

Olive oil is preferred as a salad oil, but it is also used for cooking and frying mainly in the Mediterranean countries. During frying (temperature >200 C °) of vegetable oils, a wide range of compounds such as hydroperoxides, alcohols, aldehydes, hydrocarbons, FFAs, esters, ketones, lactones, and furans, among others, were formed (Van de Voort, 1994). However, when olive oil, soybean oil, and rapeseed oil are heated at different temperatures, a lower number of the above compounds is formed in olive oil. Shahina *et al.* (2004) found that the oxidation rate of different oils after frying at 180 °C increased in the order of soybean > corn > olive. Solinas *et al.* (1988) denoted that olive oil is more stable during frying, and the amount of acrolein

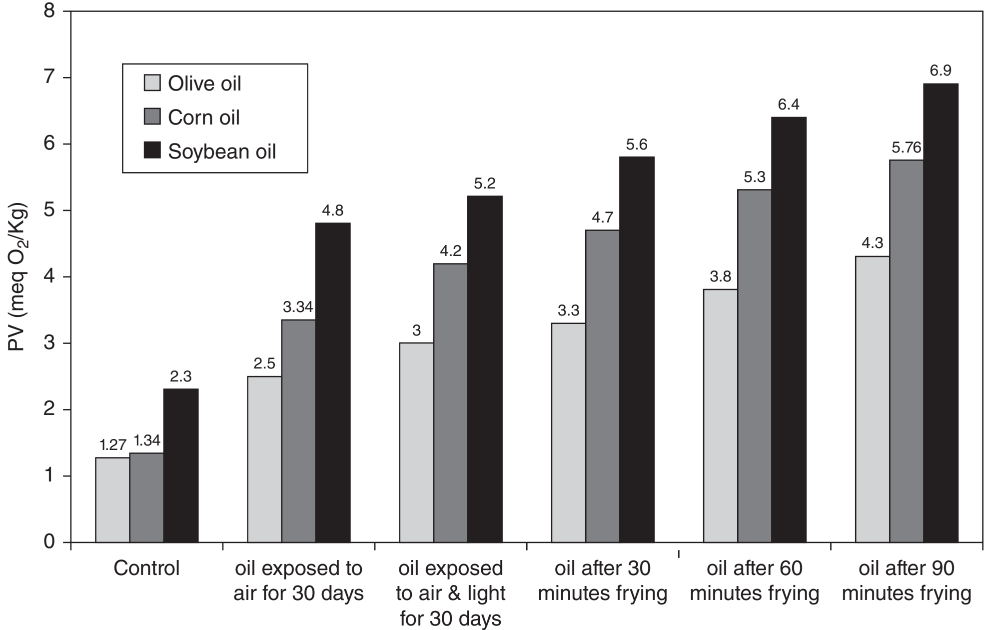

Figure 10.4 Effect of air, light, and deep frying on PV of olive oil, corn oil, and soybean oil.

formed is lower compared to that in peanut, soybean, sunflower, and cottonseed oils. Figure 10.4 shows the effect of air, light, and deep frying on the PV of olive oil, corn oil, and soybean oil.

According to Kiritsakis and Markakis (1989) and Gamel *et al.* (1999), olive oil during frying suffers the least *trans* isomerization among other vegetable oils. These fatty acids are metabolized differently than their *cis* isomers, with possible adverse health effects. They may cause disruption of biological membranes, damage to proteins and inactivation of enzymes, formation of age pigments in damaged membranes, damage to lungs, and cancer initiation. Oxidized olive oil loses its nutritional and functional value.

The linolenic acid content of olive oil is a relevant factor in increasing its off flavor and decreasing its functionality (Solinas *et al.*, 1988; Ledahudec & Pokorny, 1993). Olive oil shows great stability at high frying temperatures because it contains natural antioxidants, a high percentage of oleic acid, and a low percentage of linoleic acid. Genetic modification and breeding have been used to change the levels of fatty acids of other plant oils in order to approach the composition of olive oil, increasing its oleic content (Warner *et al.*, 1994). However, these achievements fall short of that of olive oil, which is rich in several functional phenolic components. Indeed, olive oil contains a large number of phenolics including phenyl-alcohols, such as 3,4-dihydroxyphenylethanol (3,4-DHPEA or hydroxytyrosol) and hydroxyphenylethanol (p-HPEA or tyrosol), as well as phenolic acids. Oleosidic forms of 3,4-DHPEA, in particular the dialdehydic form of elenolic acid linked to 3,4-DHPEA (3,4-DHPEA-EDA), an isomer of oleuropein aglycone (3,4-DHPEA-EA), and the dialdehydic form of elenolic acid linked to *p*-HPEA (*p*-HPEA-EDA) have been identified as the major secoiridoid compounds of VOO (Silva *et al.*, 2010). Thus, because of its specific composition, EVOO is particularly resistant to storage deterioration and hence is considered more suitable for frying than other vegetable oils (Fedeli, 1988).

10.8 Off flavors of olive oil

Olive oil readily absorbs extraneous flavors. The presence of smoke in the olive oil mill is responsible for any undesirable flavor and odor in the oil. Fruits damaged by the olive fruit fly also acquire an unpleasant

taste. The presence of iron contributes to an off flavor in the oil. All these undesirable flavors reduce the absorption of antioxidant compounds by the body and result in the loss of functional properties of the oil.

10.9 Factors affecting the quality of olive oil and its functional activity

The quality of olive oil is subject to deterioration. Several factors such as oxygen, heat, light, and transition affect the oxidation of olive oil.

10.9.1 Oxygen

Oxygen comes in contact with oil at the interface of the fatty phase with air (or with water, which may contain dissolved air). Air may also be dissolved in the oil itself. The amount of air in the oil depends on the duration and vigor of the contact of air with the oil during fruit processing in the olive oil mill and storage of the oil. The amount of oxygen dissolved in olive oil is about 2.5% by volume.

10.9.2 Temperature

Temperature affects significantly the rate of oxidation of olive oil. The ideal temperature to avoid oxidation of olive oil is less than 10 °C.

10.9.3 Metals

Olive oil contains trace amounts of heavy metals. These metals originate from the soil of the olive orchard and from the processing and storage equipment (tanks, pipes, drums, etc.). Among metals found in olive oil, iron appears to be most involved in its oxidation. Transition metals react rapidly with hydroperoxides by donating an electron to form radicals. Nawar (1985) demonstrated that metal ions can activate molecular oxygen to form singlet oxygen and peroxyl radicals from an unsaturated acyl lipid. The relationship between olive oil stability and iron concentration of the oil is:

$$\mathbf{S = K \times Fe^{\alpha}}$$

where K and α are constants; Fe is iron concentration in mg/kg (ppm); and S is stability by the AOM (active oxygen method).

The rate of absorption of oxygen, under accelerated oxidation conditions, is a function of the amount of metals (Ca, Na, Co, Ni, Fe, Cu, and Mg) present in olive oil. Removal of metals by cation exchange increases resistance of the oil to oxidation.

10.9.4 Free fatty acids

Frega *et al.* (1999) found higher oxidation rates for FFAs than for their methyl esters, and this was attributed to the catalytic effect of the carboxyl groups on the formation of free radicals. Thus, olive oil with acidity is more oxidized than oil with lower hydrolytic deterioration (Kiritsakis, 1998). The effect of antioxidants is also considerably decreased in the presence of FFAs.

10.9.5 Light and pigments

Among other factors promoting the oxidation of olive oil and causing quality deterioration, the most serious are light and pigments, mainly chlorophylls and pheophytins, which function as photosensitizers. The process involving light and relevant pigments has already been discussed.

Generally, the factors affecting the final quality of olive oil and its functional activity can be grouped as those acting during the formation of oil in the fruit, methods of harvesting, storage of the fruit, fruit processing, and storage of the resultant olive oil.

The quality characteristics of the oil are dictated by factors that start in the orchard and are affected by genetic (cultivar), climatic, and environmental factors (Kiralan *et al*., 2012; Longobardi *et al*., 2012; Hassine *et al*., 2014, 2015). The physical environment in which the orchard is located can also influence the quality of the oil (Mousa *et al.*, 1996). Olive oil is a product of the plant metabolism and is therefore heavily influenced by the olive cultivar (Kiritsakis, 1998). Cultivar affects the polyphenol, sterol, and mainly stigmasterol and Δ-5-avenasterol contents of the oil, aroma, and the functional compounds present. Certain cultivars produce oil of more desirable sensory qualities than others. Oils from some cultivars lose their sharp, bitter flavor soon after they are obtained.

Dry climates with a lot of sunshine produce oil of good flavor. The duration of sunlight, the temperature, the rainfall, and the relative humidity have also a great effect on the functional composition of olive oil. In southern environments, olive oil is more saturated than in northern areas. The environment also affects the taste and the aroma characteristics of olive oil. Hilly ground normally produces finer oils than flat land. Dry years are associated with particularly sharp, bitter oil.

Drained and calciferous soils yield oil of better sensory characteristics (Kanavouras *et al.*, 2005) compared to wet and clay soils. Nitrogen is considered essential for lipogenesis. Potassium is responsible for enzyme activity that leads to the synthesis of amino acids and phenolic acids.

It is well accepted that water availability is very important for oil quality. It facilitates fruit ripening. However, a decrease of phenolic compounds is observed with tree irrigation (Dhifi *et al.*, 2004; Berenguer *et al.*, 2006; Baccouri *et al.*, 2007; Bedbabis *et al.*, 2010; Arslan *et al.*, 2013). According to Chartzoulakis *et al.* (2004), significant decrease of total phenol contents appeared during irrigation of olive orchards with wastewater. This is probably the result of K+ supply to the olive orchard by wastewater, which causes probably a sharp increase of K+ concentrations in the fruits, resulting in a decrease of total phenols.

Arslan *et al.* (2013) determined significantly higher phenolic content in olive oils from areas with lower rainfall levels, which shows the effect of rain on the phenolic concentration. The main phenols were hydroxytyrosol, tyrosol, their acetates, most of the secoiridoids (3,4-DHPEA-EDA,p-HPEA-EDA and 3,4-DHPEA-EA), and lignans ((+)-pinoresinol, hydroxypinoresinol, and acetoxypinoresinol). Angerosa *et al.* (1996) and Tovar *et al.* (2002), however, show results that do not agree with these findings and report that irrigation increases the activity of phenylalanine ammonia lyase (PAL), the key enzyme in phenol biosynthesis (Baccouri *et al.*, 2007), and therefore the increase of phenolic compounds. The altitude of the olive orchard also seems to affect the phenol content of olive oil (Osman *et al.*, 1994; Yasser *et al.*, 1996; Kiritsakis, 1998).

Pruning results in a concentration of fruiting, in branches exposed to abundant sunlight, and a more uniform maturation of the olive fruit with good-quality olive oil that is rich in functional compounds (Kiritsakis, 1998). Generally, agronomical practices may affect olive fruit composition that may be difficult to detect unless a broad-spectrum analysis is used (Tamendjari *et al.*, 2009; Gómez-Caravaca *et al.*, 2008, 2013; Rosati *et al.*, 2014).

Insect (*Bactocera oleae* or *Dacus oleae*, Gmelin) attacks cause a decrease of secoiridoid derivatives, and this may be related to the increase of the polyphenol oxidase activity due to the entrance of oxygen from the exit hole, made by the insect larvae, which enhances phenolic oxidation (Gómez-Caravaca *et al.*, 2008; El Riachy *et al.*, 2011). Isobutyl alcohol and 2-penten-1-ol increase linearly with the percentage of olive fruit infested by *Bactocera oleae*, and this information can be used for determining the degree of olive fruit infestation.

Generally, olive fruit fly (*Bactrocera oleae*), is the most serious insect pest of the olive fruit, causing serious qualitative deteriorations in olive oil (Neuenschwander & Michelakis, 1979; Economopoulos *et al.*, 1982; Kapatos & Fletcher, 1984). This deterioration eliminates the functional constituents, mainly the polyphenols of olive oil.

Both autoxidation and enzymatic lipid oxidation are likely to occur in the fruit during the time from oil formation to fruit collection (Kiritsakis & Markakis, 1984). However, enzymatic lipid oxidation is limited since the lipoxidase enzymes, located in the endosperm, are inhibited by the natural antioxidants (polyphenols) present in the flesh.

Collection (harvest) techniques greatly affect both quality and quantity of the oil (Kiritsakis & Markakis, 1987; Metzidakis *et al.*, 1995). Processing of olive fruit and the conditions employed during processing (Kiritsakis *et al.*, 1985) affect the quality of olive oil, its composition, and its functionality.

10.10 Effect of storage on quality and functional constituents of olive oil

Storage of olive oil may affect its quality and functionality (Kiritsakis & Dugan, 1984). However, the presence of hydrophilic phenols in EVOO has a beneficial effect on its shelf life during storage (Montedoro *et al.*, 1993; Shahidi & Wanasundara, 1997; Cornwell & Ma, 2008; Servili *et al.*, 2009; Fabiani *et al.*, 2011; Frankel, 2011). Secoiridoids and hydroxytyrosol (3,4-DHPEA) are the strongest natural antioxidant phenolics present in VOO (Brenes *et al.*, 2001; Servili *et al.*, 2007; Lercker & Cerretani, 2009; Di Maio *et al.*, 2011).

According to Bosque-Sendra *et al.* (2011), when olive oil is kept in the dark and at low temperature (0–8 °C), its acidity, PV, K-values, and total wax content remain unchanged for at least 24 months. Storage under frozen conditions could help in better maintaining of nutraceutical and beneficial properties of EVOOs in comparison with storage at room temperature. Mulinacci *et al.* (2013) studied the effects of long-term storage of EVOO at −23 °C and observed insignificant differences in the aromatic profile until 12 months of storage.

For olive oil storage in large quantities, stainless-steel tanks must be used. The tanks should have conical bottoms that allow drainage of sedimented particles in order to avoid their fermentation. Occasionally, clay containers and metallic drums are used for olive oil storage. Tanks or any other containers used for olive oil storage must be constructed of materials that are impermeable to oil, are inert, protect olive oil from light and air, and maintain constant temperature, preferably near 10 °C so that the oil quality and its functional constituents are preserved.

10.11 Conclusion

In order to obtain superior quality virgin olive oil (VOO) with the most desired characteristics (low acidity, negligible oxidation, and best sensory and functional characteristics), the following are suggested (Kiritsakis, 1998; Fregapane & Salvador, 2013).

- Protect the olive fruit from any kind of infestation while it is still on the tree.
- Collect it at the optimum maturity stage with minimum damage, and store it for the shortest possible time under favorable conditions (cool place) before processing.
- Use only healthy and properly ripened olive fruit. The characteristics and the quality of the olive fruit entering the olive oil mill are crucial.
- Apply integrated agriculture and traceability. To increase phenolic content of the oil, one needs water stress of the tree, less ripened olives, and strong crushing.
- Use stainless-steel equipment for processing, avoiding high temperature and excessive exposure to air.
- Use natural phenolic antioxidants, present in olive leaves (Kiritsakis *et al.*, 2010), wastewater, and olive pomace.
- Store and pack the oil in proper containers at low temperatures under minimum contact with air and exposure to light (pack under vacuum or inert gas).

References

Angerosa, F., Di Giacinto, L., Basti, C., & Serraiocco, A. (1996) Influenza della variabile "ambiente" sulla composizione degli oli vergini di oliva. Rivista Italiana delle Sostanze Grasse 73, 461–467.

Arslan, D., Karabekir, Y., & Schreiner, M. (2013) Variations of phenolic compounds, fatty acids and some qualitative characteristics of Sarıulak olive oil as induced by growing area. Food Research International 54, 1897–1906.

Bedbabis, S., Ben Rouina, B., & Boukhris, M. (2010) The effect of waste water irrigation on the extra virgin olive oil quality from the Tunisian cultivar Chemlali. Scientia Horticulturae 125, 556–561.

Bonastre, A., Ors, R., & Peris, M. (2004) Advanced automation of a flow injection analysis system for quality control of olive oil through the use of a distributed expert system. Analytica Chimica Acta 506, 189–195.

Bosque-Sendra, P., Espinosa, M., Cuadros-Rodriguez, L., Gonzalez-Casado, A., Rodriguez-Garcia, E.P., & Garcia-Toledo, H. (2011) Stability for olive oil control materials. Food Chemistry 125, 1418–1422.

Bustan, A., Keremb, Z., Yermiyahua, U., Ben-Gala, A., Lichter, A., Droby, S., Zchori-Fein, E., Orbachd, D., Zipori, & I., Daga, A. (2014) Preharvest circumstances leading to elevated oil acidity in 'Barnea' olives. Scientia Horticulturae 176, 11–21.

Chan, H.W.S. (1977) Photo-sensitized oxidation of unsaturated fatty acid methyl esters. The identification of different pathways. Journal of the American Oils Chemists' Society 54, 100–102.

Chartzoulakis, K.S., Psarras, G., Vemmos, S., & Loupassaki, M. (2004) Effects of salinity and potassium supplement on photosynthesis, water relations and Na, Cl, K and carbohydrate concentration of two olive cultivars. Agriculture Research 27, 75–84.

Commission Regulation (EEC) No. 2568/91 (1991). On the characteristics of olive oil and olive-residue oil on the relevant methods of analysis. Official Journal of European Communication L248/1-83.

Dhifi, W., Hamrouni, I.,Ayachi, S.,Chahed, T., Saidani, M., & Marzouk, B. (2004) Biochemical characterization of some Tunisian olive oils. Food Chemistry 11, 287–296

Di Maio, I., Esposto, S., Taticchi, A., Selvaggini, R., Veneziani, G., & Urbani, S. (2011) HPLC-ESI-MS investigation of tyrosol and hydroxytyrosol oxidation products in virgin olive oil. Food Chemistry 125, 21–28.

Dugan, L.R. (1976) Lipids. In: Principles of food science. Part I. Food chemistry, ed. O.R. Fennema. Marcel Dekker Inc., New York, 140–203.

Economopoulos, A.P., Haniotakis, G.E., Michelakis, S., Tsiropoulos, G.J., Zervas, G.A., Tsitsiris, J.A., Manoukas, A.G., Kiritsakis, A., & Kinigakis, P. (1982) Population studies on the olive fruit fly, *Dacus ofeae* (Gmel.) (Dipt., Tephritidae) in Western Crete. Zeitschrift fur Angewandte Entomologie 93, 463–476.

El Riachy, M., Priego-Capote, F., León, L., Rallo, L., & Luque de Castro, M. D. (2011) Hydrophilic antioxidants of virgin olive oil. Part 2: biosynthesis and biotransformation of phenolic compounds in virgin olive oil as affected by agronomic and processing factors. European Journal of Lipid Science and Technology 113, 692–707.

European Commission. (2003) European Commission Regulation No. 2568/91, 1991. European Commission Directorate General for Agriculture, Brussels.

Fakourelis, N., Lee, E.C., & Min, D.B. (1987) Effects of chlorophyll and β-carotene on the oxidation stability of olive oil. Journal of Food Science 52, 234–235.

Farhoosh, R., & Seyedeh, H.Y. (2014) Evolution of oxidative values during kinetic studies on olive oil oxidation in the Rancimat test. Journal of the American Oils Chemists' Society 91, 281–293.

Favati, F., Condelli N., Galgano, F., & Caruso, M.C. (2013) Extra virgin olive oil bitterness evaluation by sensory and chemical analyses. Food Chemistry 139, 949–954

Fedeli, E. (1988) The behaviour of olive oil during cooking and frying. In: Frying of food, principles, changes, new approaches, ed. G. Varela, A.E. Bender, & I.D. Morton. Ellis Horwood Press, Chichester.

Frankel, E.N. (2011) Nutritional and biological properties of extra virgin olive oil. Journal of Agricultural and Food Chemistry 59, 785–792.

Frankel, E.N., Neff, W.E., & Bessler, T.R. (1979) Analysis of autoxidized fats by gas chromatography mass spectrometry. V. Photosensitized oxidation. Lipids 14, 961–967.

Frega, N., Mozzon, M., & Lercker, G. (1999) Effects of free fatty acids on oxidative stability of vegetable oil. Journal of the American Oils Chemists' Society 76, 325–329.

Fregapane, G., & Salvador, M.D. (2013) Production of superior quality extra virgin olive oil modulating the content and profile of its minor components. Food Research International 54, 1907–1914.

Gamel, T. H., Kiritsakis, A., & Petrakis, C. (1999) Effect of phenolic extracts on trans fatty acid formation during frying. Grasas y Aceites 50, 421–425.

Gomes, T., Caponio, F., Durante, V., Summo, C., & Paradiso, V.M. (2012) The amounts of oxidized and oligopolymeric triacylglycerols in refined olive oil as a function of crude oil oxidative level. LWT – Food Science and Technology 45, 186–190.

Gómez Caravaca, A. M., Cerretani, L., Bendini, A., Segura-Carretero, A., Fernández- Gutiérrez, A., Del Carlo, M., Compagnone, D., & Cichelli, A. (2008) Effects of fly attack (Bactrocera oleae) on the phenolic profile and selected chemical parameters of olive oil. Journal of Agricultural and Food Chemistry 56, 4577–4583.

Gómez Caravaca, A.M., Maggio, R.M., Verardo, V., Cichelli, A., & Cerretani, L. (2013) Fourier transform infrared spectroscopy-partial least squares (FTIR-PLS) coupled procedure application for the evaluation of fly attack on olive oil quality. LWT – Food Science and Technology 50, 153–159.

Hassine, K.B., Riachy, M.E., Taamalli A, Malouche D., Ayadi, M., Talmoudi, K., Aouini, M., Jlassi, Y., Benincasa, C., Romano, E., Perri, E., Kiritsakis, A., Hamdi, M., Grati Kammoun, N., & Hammami, M. (2014) Consumer classification of Chemlali and Arbequina olive oil cultivars according to their cultivar, geographical origin, and processing system. European Journal of Lipid Science and Technology 116, 812–824.

Hassine, K.B., Taamalli, A., Slama, M.B., Khouloud, T., Kiritsakis, A., Benincasa, C., Perri, E., Malouche, D., Hammami, M., Bornaz, S., & Grati Kammoun, N. (2015) Characterization and preference mapping of

autochthonous and introduced olive oil cultivars in Tunisia. European Journal of Lipid Science and Technology 117, 112–121.

International Oil Council (IOC). (2015). COI/T.15/NC No 3 /Rev.8. IOC, Madrid, Spain.

Kanavouras, A., Kiritsakis, A., & Hernandez, R. (2005) Comparative study on volatile analysis of extra virgin olive oil by dynamic headspace and solid phase micro-extraction. Food Chemistry 90, 69–79.

Kapatos, E.T., & Fletcher, B.S. (1984) The phenology of the olive fruit fly, *Dacus oleae* (Gmelin) (Diptera, Tephritidae), in Corfu. Zeitschrift fur Angewandte Entomologie 97, 360–370.

Khan, M.A., & Shahidi, F. (1999) Rapid oxidation of commercial extra virgin olive oil stored under fluorescent light. Journal of Food Lipids 6, 331–339.

Kiralan, M., Ozkan, G., Koyluoglu, F., Ugurlu, H.A., Bayrak, A., & Kiritsakis, A. (2012) Effect of cultivation area and climatic conditions on volatiles of virgin olive oil. European Journal of Lipid Science and Technology 114, 552–557.

Kiritsakis, A.K. (1998). Olive oil: From the tree to the table, 2nd ed. Food and Nutrition Press, Trumbull, CT, 155–189.

Kiritsakis, A.K., & Dugan L.R. (1985) Studies in photooxidation of olive oil. Journal of the American Oils Chemists' Society 62, 892–896.

Kiritsakis, A.K., & Markakis, P. (1984) Effect of olive collection regimen on olive oil quality. Journal of the Science of Food and Agriculture 35, 677–678.

Kiritsakis, A.K., & Markakis, P. (1987) Olive oil: a review. Advances in Food Research 31, 453–482.

Kiritsakis, A.K., & Markakis, P. (1989) Trans isomerization of certain vegetable oils during frying. In: Flavors and off-flavors, Proceedings of the 6th international flavor conference, Rethymnon, Crete, Greece, ed. G. Charalambous. Elsevier Science, Amsterdam.

Kiritsakis, A.K., & Tsipeli, A. (1992) Hydrolysis and Oxidation of olive oil during the time that olive fruit remain on the tree. Rivista Italiana delle Sostanze Grasse 69, 453–456.

Kiritsakis, K.A., Kontominas, M.G., Kontogiorgis, C., Hadjipavlou-Litina, D., Moustakas, A., & Kiritsakis, A.K. (2010) Composition and antioxidant activity of olive leaf extracts from Greek olive cultivars. Journal of the American Oils Chemists' Society 87, 369–376.

Ledahudec, J., & Pokorny, J. (1993) Effect of nonvolatile oxidation products on sensory characteristics of frying oils. Die Nahrung 37, 97–98.

Longobardi, F., Ventrella, A., Casiello, G., Sacco, D., Tasioula-Margari, M., Kiritsakis, A.K., & Kontominas, M.G. (2012) Characterisation of the geographical origin of Western Greek virgin olive oils based on instrumental and multivariate statistical analysis. Food Chemistry 133, 169–175.

Logani, M.K., & Davies, R.E. (1980) Lipid oxidation: Biological effects and antioxidants a review. Lipids 15(6), 485–495.

Montedoro, G.F., Servili, M., Baldioli, M., Selvaggini, R., Miniati, E., & Maccchioni A. (1993) Simple and hydrolyzable compounds in virgin olive oil: spectroscopic characterizations of the secoiridoid derivatives. Journal of Agriculture and Food Chemistry 41, 2228–2234.

Mousa, Y.M., Gerasopoulos, D., Metzidakis, I., & Kiritsakis, A. (1996) Effect of altitude on fruit and oil quality characteristics of 'Mastoides' olives. Journal of the Science of Food and Agriculture 71, 345–350.

Mulinacci, N., Ieri, F., Ignesti, G., Romani, A., Michelozzi, M., Creti, D., Innocenti, M., & Calamai, L. (2013) The freezing process helps to preserve the quality of extra virgin olive oil over time: a case study up to 18 months. Food Research International 54, 2008–2015.

Nawar, W.W. (1985). Lipids in food chemistry, 2nd ed., ed. O.R. Fennema. Marcel Dekker Inc., New York, 139–244.

Neuenschwander, P., & Michelakis, S. (1979) McPhail trap captures of *Dacus oleae* (Gmel.) (Diptera: Tephritidae) in comparison to the fly density by sondage technique in Crete, Greece. Bulletin Societe Entomologique Suisse 52, 343–357.

Osman, M., Metzidakis, I., Gerasopoulos, D., & Kiritsakis, A.K. (1994) Qualitative changes in olive oil collected from trees grown at two altitudes. Rivista Italiana delle Sostanze Grasse 71, 187–190.

Pristouri, G., Badeka, A., & Kontominas, M.G. (2010) Effect of packaging material headspace, oxygen and light transmission, temperature and storage time on quality characteristics of extra virgin olive oil. Food Control 21, 412–418.

Psomiadou, E., & Tsimidou, M. (2002) Stability of virgin olive oil. 1. Autoxidation studies. Journal of Agricultural and Food Chemistry 50, 716–721.

Rahmani, M., & Csallany, A. (1998) Role of minor constituents in the photoxidation of virgin olive oil. Journal of the American Oils Chemists' Society 75, 837–843.

Rawls, H.R., & Van Santen, P.J. (1970) A possible role for singlet oxygen in the initiation of fatty acid autoxidation. Journal of the American Oils Chemists' Society 47, 121–125.

Servili, M., Taticchi, A., Esposto, S., Urbani, S., Selvaggini, R., & Montedoro, G.F. (2007) Effect of olive stoning on the volatile and phenolic composition of virgin olive oil. Journal of Agricultural and Food Chemistry 55, 7028–7035.

Servili, M., Esposto, S., Fabiani, R., Urbani, S., Taticchi, A., Mariucci, F., Selvaggini, R., & Montedoro G.F. (2009) Phenolic compounds in olive oil: Antioxidant, health and organoleptic activities according to their chemical structure. Inflammopharmacology 17, 76–84.

Shahidi, F., & Zhong, Y. (2010) Lipid oxidation and improving oxidative stability. Chemical Society Reviews 39, 4067–4079.

Shahidi, F., & Zhong, Y. (2015) Measurement of antioxidant activity. Journal of Functional Foods. doi:10.1016/j.jff.2015.01.047.

Silva, L., Garcia, B., & Paiva-Martins, F. (2010) Oxidative stability of olive oil and its polyphenolic compounds after boiling vegetable process. LWT – Food Science and Technology 43, 1336–1344.

Snyder, J.M., Frankel, E.N., & Selke, E. (1985) Capillary gas chromatographic analyses of headspace volatiles from vegetable oils. Journal of the American Oils Chemists' Society 62, 1675–1679.

Solinas, M., Angerosa, F., & Marsilio, V. (1988) Imagine su alcuni componenti dell'aroma degli oli vergini di oliva in relazione all varieta delle olive. Rivista Italiana delle Sostanze Grasse 65, 361–368.

Tamendjari, A., Angerosa, F., Mettouchi, S., & Bellal, M.M. (2009) The effect of fly attack (*Bactrocera oleae*) on the quality and phenolic content of Chemlal olive oil. Grasas y Aceites 60, 507–513.

Tarakowski, R., Malanowski, A., Kosciesza, R., & Siegoczynski, R.M. (2014) VIS spectroscopy and pressure induced phase transitions – chasing the olive oils quality. Journal of Food Engineering 122, 28–32.

Tovar, M., Romero, M., Alegre, S., Girona, J., & Motilva, M. (2002) Composition and organoleptic characteristics of oil from Arbequina olive (*Olea europaea* L.) trees under deficit irrigation. Journal of the Science of Food and Agriculture 82, 1755–1763.

Uccella, N. (2001) Olive biophenols: biomolecular characterization, distribution and phytoalexin histochemical localization in the drupes. Trends in Food Science & Technology 11, 315–327.

Van de Voort, F.R., Ismail, A.A., Sedman, J., & Emo, G. (1994) Monitoring the oxidation of edible oils by Fourier transform infrared spectroscopy. Journal of the American Oils Chemists' Society 71, 243–253.

Velasco, L., Ivaro Fernandez-Cuesta, A., Raul De la Rosa, M., Ruiz-Mendez, V., & Lorenzo, L. (2014) Selection for some olive oil quality components through the analysis of fruit flesh. Journal of the American Oils Chemists' Society 91, 1731–1736.

Warner, K., Orr, P., Parrott, L., & Glynn, M. (1994) Effects of frying oil composition on potato chip stability. Journal of the American Oils Chemists' Society 71, 1117–1121.

11 Optical nondestructive UV-Vis-NIR-MIR spectroscopic tools and chemometrics in the monitoring of olive oil functional compounds

Vasiliki Lagouri, Vasiliki Manti, and Thanasis Gimisis

11.1 Introduction: functional compounds in olive oil

Olive oil is a high-quality natural product typical of the Mediterranean area. Virgin olive oil (VOO) is obtained from the olive fruits of *Olea europaea* L. with only mechanical means, and has nutritional and sensory characteristics that make it a unique and basic ingredient of the Mediterranean diet. These highly nutritional and sensory properties of olive oil are attributed mainly to the presence of minor functional components that other seed oils often lack, such as phenolic compounds, tocopherols, chlorophylls, and carotenoids (Kiritsakis, 1990; Cicerale *et al.*, 2009).

The phenolic composition of olive oil and its concentration depend mainly on the cultivar and geographic origin as well as the degree of ripening of the olive fruit. Phenolic compounds are important antioxidants in biological systems and act as radical scavengers or chain breakers, thus decreasing the overall rate of lipid oxidation. In particular, phenols with catechol moieties known as o-diphenols are effective antioxidants. Tyrosol, hydroxytyrosol, simple phenolic acids, and esterified derivatives of tyrosol and hydroxytyrosol are among the most representative phenolic compounds present in olive oil (Baldioli *et al.*, 1996; Bendini *et al.*, 2007a, 2007b; Cerretani *et al.*, 2009).

Because of their antioxidant activity, phenolic compounds are responsible for the stability of the olive oil, which is a very important quality factor of olive oil storage. Moreover, phenols are the main contributors to the typical taste of VOO (bitter and pungent attributes), and may also contribute to the prevention of several human diseases due to their positive effects on certain physiological parameters, such as protection of human low-density lipoproteins from oxidation, inhibition of lipoxygenase activity, and protection of peroxide-induced cytotoxicity in erythrocytes (Boskou *et al.*, 2006).

Concerning tocopherols, the major constituent in olive oils is α-tocopherol, while β- and γ-tocopherols are only present in minor amounts. In virgin olive oils, they compete with polyphenols at the early stages of oxidation, and their contribution to virgin olive oil stability is considered to be of minor importance compared to that of phenolic compounds. Tocopherols also inhibit photooxidation by reacting with singlet oxygen and increase the oxidative stability of oils during storage in the presence of light (Yamauchi & Matsushita, 1977; Kamal-Eldin *et al.*, 1996).

Chlorophyll pigments are responsible for the greenish color of virgin olive oil. Among chlorophylls, pheophytin a, is found in high amounts in olive oils. The major "yellow" pigments of virgin olive oils are due to lutein and β-carotene. Chlorophylls and carotenoids play an important role in the oxidative stability of olive oil during storage, due to their antioxidant nature in the dark and prooxidant activity in the light (Kiritsakis & Dugan, 1985; Psomiadou & Tsimidou, 2001). Carotenoids, together with phenols and tocopherols, provide oxidative stability to olive oils and have a synergistic antioxidant and anticarcinogenic action. The potential

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health benefits of a diet rich in carotenoids have been indicated in several studies reporting their antioxidant and anticancer activity, and their capacity to prevent cardiovascular and other degenerative diseases (Van Poppel & Goldbohm, 1995; Kritchevsky, 1999; Landrum & Bone, 2001).

Mono- and diunsaturated fatty acids, oleic and linoleic acids, respectively, can be considered as functional compounds although they are major lipid components of olive oil. They play a significant role in human health by controlling the cholesterol level and lowering the risk of coronary heart diseases and cancers (Huang & Sumpio, 2008; Viola, 2009). Another functional compound in olive oil, the triterpene squalene, is a major constituent of the unsaponifiable matter of olive oil (with a concentration of up to 40% by weight) and a key intermediate in cholesterol synthesis that has shown a moderate antioxidant activity at low temperatures in the dark (Kiritsakis, 1998; Psomiadou & Tsimidou, 1999). As a result, the content of functional compounds in olive oil, especially in extra virgin olive oil, is highly related to its quality and positive sensory properties. Different instrumental and analytical techniques, both colorimetric and separation (mainly high-performance liquid chromatography coupled to diode array and mass detectors [HPLC-DAD/MS] and gas chromatography coupled with flame ionization and mass detectors [GC-FID/MS]), have been applied so far to determine the content of functional compounds in olive oil. However, most of these methods are time-consuming, environmentally nonfriendly, and expensive, and they require highly qualified staff.

11.2 An introduction to UV-Vis-NIR-MIR spectroscopy in olive oil analysis

Spectroscopic methods such as absorption spectroscopy in ultraviolet (UV), visible (Vis), near-infrared (NIR), or mid-infrared (MIR) or their combinations have been used for nondestructive, fast, real-time, off/on-line monitoring of olive oil quality parameters, and for detection and determination of olive oil adulterants as well as geographic origin (Wesley *et al*., 1995; Downey *et al*., 2003; Tapp *et al*., 2003; Mailer, 2004; Marquez *et al*., 2005; Szlyk *et al*., 2005; Manley & Eberle, 2006; Armenta *et al*., 2007; Galtier *et al*., 2007; Mignani *et al*., 2008; Mignani *et al*., 2011; Casale *et al*., 2012; Lin *et al*., 2012; Garcia *et al*., 2013; Inarejos *et al*., 2013; Sanchez *et al*., 2013; Casale & Simonetti, 2014). These methods have also been used in the industrial sector to analyze the quality of olive fruit with respect to oil content, moisture, color, and fatty acid content (Leon *et al.*, 2004; Barros *et al.*, 2009).

However, statistical analysis of spectral measurements is often not easy and requires expertise. The mathematical and statistical models created might not be general and need to be adjusted to new conditions and oil samples. In the following sections, several applications of UV-Vis-NIR or/and MIR spectroscopy in off/on-line monitoring of olive oil functional compounds and oxidation status are considered in combination with appropriately used spectral preprocessing and data analysis (chemometrics). In the last section, some literature details are considered; they are summarized in Table 11.1.

11.3 Spectroscopic regions with interest for olive oil analysis

The UV-Vis-NIR and MIR spectroscopic methods have the advantages of (a) minimal or no sample preparation and (b) the ability of a very simple and rapid simultaneous analysis of several constituents. They constitute one of the most promising and useful on/in-line detection methods for olive oil quality control. The regions of the infrared spectrum that are used in olive oil analysis include MIR and NIR. MIR spectra present well-resolved bands exhibiting absorbances of varying intensity in the range of 4000 to 400 cm^{-1} (2500–25000 nm) originating from the fundamental vibrations. NIR spectroscopy is based on the absorption of electromagnetic radiation in the range of 14000 to 4000 cm^{-1} (700–2500 nm) and, compared to MIR spectra, presents less well-resolved or overlapped bands corresponding to overtones and combinations of fundamental vibrations.

The NIR main spectroscopic regions (Figure 11.1a) that can be observed include (a) the region of 9000–8000 cm^{-1} (1111–1250 nm), which can be ascribed to the second overtone of the C—H stretching vibration modes of methyl, methylene, and ethylene groups of fatty acids and triacylglycerols; (b) the region between

Table 11.1 UV-Vis-NIR-MIR spectroscopy and chemometrics in olive oil analysis.

Subject	Dataset	Data analysis	Spectra region	Results	References
α-, β-, γ- tocopherols (α-, β-, γ- TOHs), o-diphenols, hydroxytyrosol (HTyr) and tyrosol (Tyr) derivatives, total phenol (TP), oleuropein score (OIS)	97 PDO VOOs of Spain	First, second derivative and MSC, PLS	α-, β-, γ-TOHs: 1333 nm-1640 nm, Htyr derivatives: 1333–1470 nm, Tyr derivatives: 800–1334 nm and 1639–2175 nm TP: 1333–1471 nm OIS: 18345–2175 nm	α-TOH: r^2 = 0.71, RPD = 1.42 TP: r^2 = 0.94, RPD = 2.98 OIS: r^2 = 0.92, RPD = 2.62 Htyr derivatives: r^2 = 0.81, RPD = 1.73 Tyr derivatives: r^2 = 0.76, RPD = 1.53 o-diphenols: r^2 = 0.81, RPD = 1.53	Inarejos-Garcia *et al.* (2013)
Total phenols (TP), chlorophylls, oleic acid, linoleic acid, induction time	216 Australian olive oils	SNV and detrend and first derivative, PLS	400–2500 nm	TP: r^2 = 0.89, r^2cv = 0.8233 SEC = 47.66 SECV = 58.67 Chlorophylls: r^2 = 0.98, r^2cv = 0.9685 SEC = 0.42, SECV = 0.51 Induction time: r^2 = 0.88, r^2cv = 0.83 SEC = 0.84, SECV = 0.97 Oleic acid: r^2 = 0.99, r^2cv = 0.9876 SEC = 0.38, SECV = 0.47 Linoleic acid: r^2 = 1.0, r^2cv = 0.9958 SEC = 0.17, SECV = 0.23	Mailer (2004)
Carotenoids and chlorophylls on-line process	183 Spanish VOOs	Initial smoothing combined with first derivative treatment, PLS	750–2.500 nm wavelength selection: 450–1000 nm and 1800–2300 nm	r^2 = 0.970 and SEP = 0.66 for carotene totals r^2 = 0.986 and SEP = 0.96 for chlorophyll totals	Marquez (2003)

(*continued*)

Table 11.1 (*Continued*)

Subject	Dataset	Data analysis	Spectra region	Results	References
Total chlorophylls and carotenoids	258 Spanish VOOs, varieties 'Picual', 'Arbequina', and 'Manzanilla'	transmittance data transformed to absorbance and mean normalized, Savitzsky Golay first and second derivatives, MLR, PLS	Total chlorophylls: 670–686 nm Total carotenoids: 350–2500 nm	total chlorophylls: $r^2 = 0.97$, SEC = 2.63, RPD = 5.76 total carotenoids: $r^2 = 0.95$, SEC = 1.74, RPD = 3.86	Cayuela *et al.* (2014)
Oleic and linoleic acid, chlorophyll and carotenoids, total phenols (TP), thiobarbituric acid reactive substances (TBRAS) values	14 South African EVOO	MSC, different normalization and derivative treatments, PCA, PLS	978–2500 nm	r^2 values for Buchi and PerkinElmer spectra, respectively: TP: 0.34, 0.21 Linoleic acid: 0.88, 0.90 Oleic acid: 0.56, 0.53 TBARS value: 0.24, 0.40 SEP for Buchi and PerkinElmer spectra, respectively: total phenols: 82.10, 89.66 Linoleic acid: 0.83%, 0.83% Oleic acid: 1.47%, 1.53% TBARS value: 0.0042, 0.0038	Manley and Eberle (2006)
Oleic acid, linoleic acid, squalene	125 VOOs from 5 France RDO	PLS	Oleic and linoleic acid: 1330–2200 nm Squalene: 1000–2200 nm	Oleic acid: $r^2 = 0.97$, $Q^2 = 0.96$ Linoleic acid: $r^2 = 0.98$, $Q^2 = 0.97$ REP < 5% Squalene: $r^2 = 0.96$, $Q^2 = 0.96$ REP <8%	Galtier *et al.* (2007)

Oleic acid and linoleic acid	20 Sicilian EVOOs	PLS	200–1700 nm Oleic acid and linoleic acid: 1333–2222 nm	Oleic acid $r^2 = 0.9986$, linoleic acid $r^2 = 0.9553$	Mignani *et al.* (2008a)
Oleic acid and linoleic acid	57 Italian EVOOs	First derivatives (Savitzky-Golay), SNV, PLS	UV-Vis (190–1100 nm), NIR (1000–2500 nm) MIR (2500–14286 nm)	Oleic acid RMECV = 0.80 Linoleic acid RMECV = 0.315	Casale *et al.* (2012)
Oxidation stability index (OSI)	278 Spanish VOO samples	Frst and second Savitzky–Golay derivatives, maximum normalization, PLS	350–2500 nm	Model M1 $r^2 = 0.93$ SEC = 6.07 (10.9%), V1 RPD = 3.30 Model M2 $r^2 = 0.94$ and SEC = 5.64 (10.0%), V2 RPD = 3.02	Sanchez *et al.* (2013)
Total phenolic content (TP), o-diphenols, flavonoids, antioxidant activity (ABTS)	EVOOs of three cultivars from Portugal (cv 'Cobrança, 'Galega', and 'Picual')	PCA, PLS	500 to 3000 $cm–^1$ or 3333–20000 nm	TP: $r^2 = 0.94$ $r^2cv = 0.91$ RMSEC = 0.02 RMSECV = 0.02 o-diphenols: $r^2 = 0.99$ $r^2cv = 0.99$ RMSEC = 0.01 RMSECV = 0.02 flavonoids: $r^2 = 0.99$ $r^2cv = 0.98$ RMSEC = 0.02 (2.93%) RMSECV = 0.03 (4.40%) ABTS: $r^2 = 0.93$ $r^2cv = 0.88$ RMSEC = 0.04 RMSECV = 0.05	Gouvinhas *et al.* (2015)
Total phenol (TP), antioxidant activity (ABTS)	47 VOO and 7 olive oil (OO) from Italy	PLS	4000–700 cm^{-1} or 2500–14286 nm TP: 3610–816 cm^{-1} or 2770–12200 ABTS: 3707–1105 cm^{-1} or 2700–9000 nm	TP $r^2 = 0.74$ ABTS $r^2 = 0.67$	Cerretani *et al.* (2010)

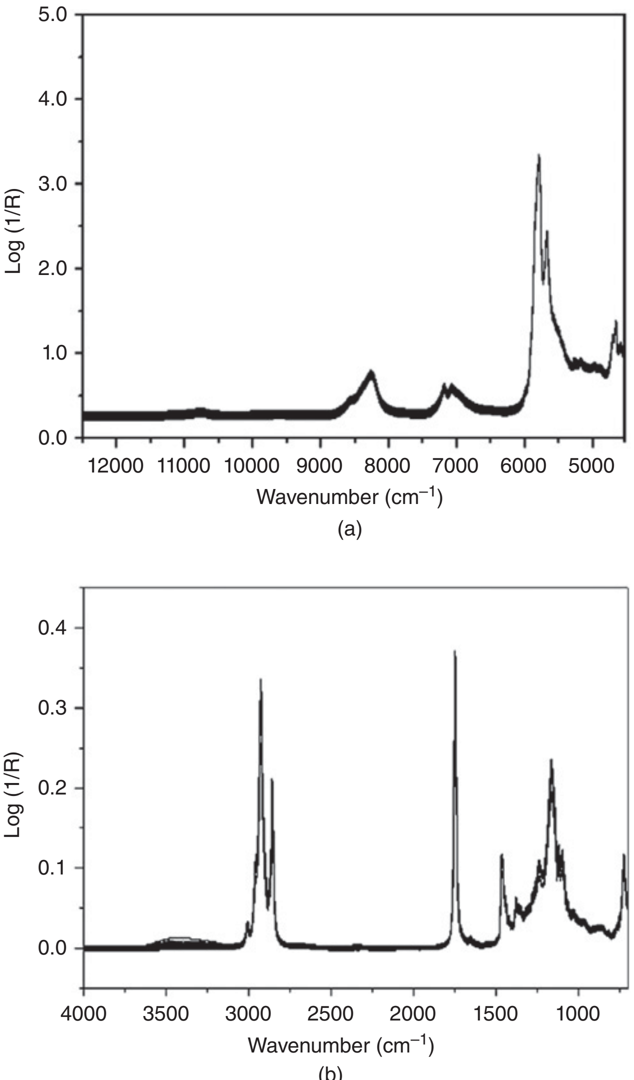

Figure 11.1 (a) NIR spectra of monovarietal extra virgin olive oils. (b) MIR spectra of monovarietal extra virgin olive oils. *Source*: Sinelli *et al.* (2010). Reproduced with permission of Elsevier.

7500 cm^{-1} (1333 nm) and 6150 cm^{-1} (1626 nm), which can be attributed to the first overtone of the O—H stretching vibrations, whereas in (c), the absorptions located around 6000–5700 cm^{-1} (1666–1754 nm) correspond to the first overtone of the C—H stretching vibration modes of methyl, methylene, and ethylene groups; and (d) the next region bands between 5350 cm^{-1} (1869 nm) and 4550 cm^{-1} (2197 nm) resulting from combinations of fundamentals of the C—H stretching vibration and of bands corresponding to water molecules. Finally, (e) the 4370–4260 cm^{-1} (2288–2347 nm) region can be ascribed to the C—H stretching combination of methyl and methylene groups (Galtier *et al.*, 2007).

The representative olive oil MIR spectrum in the 4000–900 cm^{-1} (2500–11111 nm) region (Figure 11.1b), shows several characteristic bands related to lipid functional groups. The 3100–2800 cm^{-1} (3226–3571 nm) spectral region has the appearance of the signals assigned to C—H stretching mode from methylene and methyl groups of fatty acid and triacylglycerols. The low-intensity peak near 3100 cm^{-1} may be explained

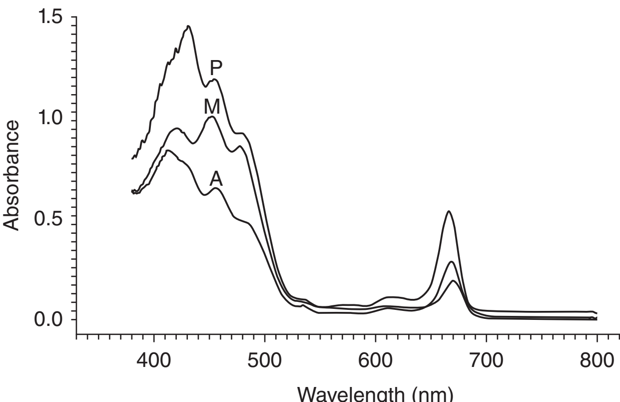

Figure 11.2 Example of olive oil visible spectrum from 'Picual' (P), 'Manzanilla' (M), and 'Arbequina' (A) varieties. *Source*: Cayuela *et al.* (2014). Reproduced with permission of Springer.

by the CH=CH elongation, and the signals of weak absorption around 2800 cm^{-1} are the result of the presence of secondary oxidation products, such as aldehydes and ketones. At 1800–1700 cm^{-1} (5556–5882 nm), the C=O stretching mode is found. The very strong band located at 1743 cm^{-1} can be ascribed to the triacylglycerol C=O ester group, and a shoulder found around 1710 cm^{-1} is characteristic of the presence of free fatty acids (carboxylic n−C=O). The C—H deformation is detected between 1400 cm^{-1} (7143 nm) and 900 cm^{-1} (11111 nm), a spectroscopic region that is also known as the fingerprint region.

Concerning the olive oil visible spectra, as shown in Figure 11.2, an initial peak appears close to 420 nm. This area corresponds to the absorption by olive oil of dark blue colored light, which could mainly be due to carotenoids. A second peak near 460 nm, also corresponding to the absorption of blue light, is characteristic of carotenoids. Finally, a third peak is observed approximately at 670 nm, coinciding with the chlorophyll absorption.

The infrared spectra, especially the NIR, are difficult to interpret without the use of further mathematical and statistical methods, which fall in the area of chemometrics.

11.4 The basics of chemometrics

In the case of spectroscopic measurements, we could define chemometrics as the mathematical processing of chemical analysis and spectra by statistics. In this method, the spectroscopic data are calibrated against reference analytical data from a database of samples representing the best variability in the population. The basic steps of a chemometric analysis include the following:

- The creation of a library of representative spectra and relative analytical data to which the spectrum of a test sample may be compared.
- A data dimensionality reduction, which usually leads to a scatter plot where samples are clustered based on the similarity of their spectra.
- A more specific analytical tool according to the type of the variable that has to be predicted (quantitative or qualitative).
- A "calibration method" is created from which the constituent of interest may be calculated by means of a linear combination of spectroscopic data. The calibration equation has associated statistics that define the closeness of the actual and predicted values.
- A "validation procedure" is then applied for testing the effectiveness of the calibration method. Although the data dimensionality reduction is usually capable of identifying similarities, the correlation to quality indicators always needs further steps of calibration and validation (Mignani *et al.*, 2012).

Given the nature and complexity of the spectroscopic and analytical datasets involved, many multivariate chemometric techniques have been proposed (Mark & Workman, 2007). One of the most popular techniques is the principal component analysis (PCA) (Jackson, 2003). This technique linearly combines the spectroscopic data characterizing each oil sample to produce new variables. The coefficients giving the weight of each variable in the linear combination are called loadings. The new variables are called principal components (PCs). A typical approach for modeling is regression analysis. The training of the model can be understood as a "calibration." The training data should be subjected to detection of residuals, outliers, and nonlinearity. The outliers should be excluded and nonlinearity corrected, if present.

The most often used regression models are multiple linear regression (MLR), principal component regression (PCR), and partial least squares (PLS). Both PLS and PCR calculate one component at a time using regression. A residual is then calculated, and the calculation of the next component is based on this residual. The obtained results need to be evaluated by using a test set. The predicted values are calculated using test values from the optical measurements and parameters obtained from the training phase. The difference between actual and predicted values in the test set can be evaluated using the root mean squared error of prediction and the coefficient of determination.

There are a few fundamental parameters for assessing the accuracy of the fit and the model performance: (a) the estimation of the expected error, calculated as root mean square error of calibration (RMSEC) or standard error of calibration (SEC) and root mean square error of prediction (RMSEP and RMSECV) or standard error of prediction (SEP), evaluated in the calibration and the validation sets. (b) The systematic averaged deviation between the reference and predicted values (bias). (c) The bias-corrected mean error of prediction of the validation (RPD, or residual prediction deviation), which is a qualitative measure for assessing the validation results. (d) The determination coefficient (r^2) is the squared correlation coefficients between predicted and reference values. The fit is better when the value is closer to 1 ($r^2 = 1$).

11.5 Spectral preprocessing methods

The NIR spectra of an olive oil sample can be affected by particles, turbidity, and variations in the optical path length. To avoid or decrease these interferences, mathematical pretreatments are applied to the spectra to improve the model development. The most current data pretreatments are normalization methods such as standard normal variate (SNV) and multiplicative scatter correction (MSC), derivative methods, and orthogonal signal correction (OSC).

In a study by Manley and Eberle (2006), the FT-NIR spectra taken from two different instruments were first pretreated with MSC and then examined in terms of spectral differences due to oxidation by means of PCA. MSC and different normalization and derivative treatments were also applied in order to optimize the performance of PLS regression models for oleic acid, linoleic acid, chlorophylls, carotenoid, total phenols, and thiobarbituric acid reactive substances (TBRAS) values.

In a study by Sanchez (2013), mean and maxima normalization of absorbance spectral data and first and second Savitzky-Golay derivative treatments were tested for oxidative stability index (OSI) models development. The absorbance data maxima normalization provided the best outcomes for OSI models. Normalization and derivatization of NIR spectra by the Savitsky-Golay method were also performed for the PLS determination of α-tocopherol in olive oils after extraction with ethanol (Szlyk *et al.*, 2005).

An initial smoothing technique combined with the first derivative treatment was used by Marquez (2003) to correct the signal of the Vis-NIR spectra in order to monitor the on-line levels of chlorophyll and carotene in olive oil during processing. Inarejos-Garcia *et al.* (2013) showed the influence of wavelength selection and data preprocessing on the monitoring of phenolic compounds. In the latter work, first and second derivative treatments and MSC were applied in the spectral data to generate the most successful PLS models. The derivative profiles generally improve the resolution of overlapping peaks and provide a correction for baseline shifts. Mailer (2004) applied scatter correction SNV and detrend and first derivative treatments to the spectra for determination of polyphenols, chlorophylls, oleic and linoleic acid, and the induction time, while a limited spectral range from 400 to 2250 nm (the region above 2250 nm appeared highly saturated) improved the correlation for polyphenols.

It can be concluded by the studies discussed here that the proper choice of preprocessing is difficult to assess prior to model evaluation, and frequently, it is necessary to test different pretreatments in order to select the most suitable one.

11.6 UV-Vis-NIR-MIR spectroscopy and chemometrics in monitoring olive oil functional compounds

The olive oil polar phenol fraction is a complex mixture of phenolic compounds with varying chemical structures and significant biological activities. There are compounds in olive oil such as oleocanthal (*p*-HPEA-EDA), oleacein (3,4-DHPEA-EDA), and their aglycons, present as tyrosol (*p*-HPEA) and hydroxy-tyrosol (3,4-DHPEA) derivatives, which are highly important and related to specific health claims (Bianco *et al.*, 1999. Bianco & Uccella, 2000; Andreadou *et al.*, 2006). Because of the technical difficulties in the quantitative determination of these ingredients, the application of alternative methods, such as UV-Vis-NIR spectroscopy, would be of high importance.

For this purpose, a NIR technique combined with chemometrics was applied by Inarejos-Garcia *et al.* (2013) to evaluate the functional components of monocultivar virgin olive oils (VOOs) produced in the PDO (protected denomination of origin) 'Montes de Toledo' geographic area of Spain. The determined phenolic components were: α-, β-, γ- tocopherols (α-, β-, γ-TOHs), hydroxytyrosol (Htyr) derivatives such as the dialdehydic form of elenolic acid linked to Htyr (3,4-DHPEA-EDA) and the aldehydic form of elenolic acid linked to Htyr (3,4-DHPEA-EA), and tyrosol (Tyr) derivatives such as the dialdehydic form of elenolic acid linked to Tyr (p-HPEA-EDA) and the aldehydic form of elenolic acid linked to Tyr (p-HPEA-EA). The same NIR technique was also used to evaluate the total phenolic content (TP) and oleuropein score (OIS) correlated with the bitter taste of VOO. For this reason, PLS chemometric analyses of the NIR spectra were correlated with analytical (i.e., phenolics by HPLC, TP by Folin-Ciocalteau reagent, and Oleuropein Score (OIS) by the measurement at 225 nm of VOO polar extracts) parameters to generate calibration and validation models.

As shown in Figure 11.3, the spectral range used for preprocessing of the α-tocopherol (α-TOH) was 7502–6098 cm^{-1} (1333–1640 nm), where the first overtone of the O—H stretching vibrations associated with alcohol are present. The most appropriate spectral region employed for preprocessing of the Htyr derivatives

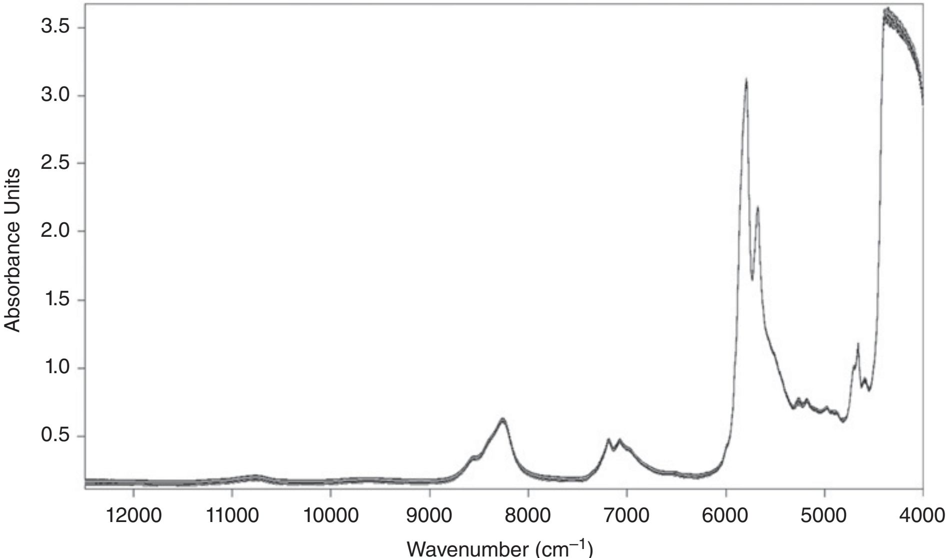

Figure 11.3 NIR spectra of the PDO monovarietal VOO 'Cornicabra' sample set acquired in the region 12500–4000 cm^{-1}. *Source*: Inarejos-Garcia *et al.* (2013). Reproduced with permission of Elsevier.

cross-validation and test set validation models was 7502–6800 cm^{-1} (1333–1470 nm), related to the OH stretch first overtone, as in the case of tocopherols (Sinelli *et al.*, 2010). Important frequency ranges for Tyr derivatives used were 12493–7498 cm^{-1} (800–1334 nm) and 6102–4598 cm^{-1} (1639–2175 nm) related to the OH stretch overtones and OH–deformation combination of hydroxyl groups. Multivariate PLS cross-validation models for TP content by Folin-Ciocalteau and OIS were obtained through preprocessing of the spectral data in the frequency ranges 7502–6800 cm^{-1} (1333–1470 nm) and 5450–4598 cm^{-1} (1839–2175 nm), as in the case of HTyr and Tyr secoiridoids.

The complex phenolic compounds analyzed by HPLC showed good prediction models (r^2 values over 0.70). Htyr derivatives and o-diphenols showed very similar determination coefficients (r^2 = 0.81) but a higher residual prediction deviation (RPD) in the case of Htyr derivatives (RPD = 1.73). Among tocopherols, the best PLS validation model was found for α-tocopherol (r^2 = 0.71, RPD = 1.42), which is the main isomer in VOO.

The best cross-calibration models were obtained for total phenolic content determined with the Folin-Ciocalteau reagent (r^2 = 0.94, RPD = 2.98) and OIS (r^2 = 0.92; RPD = 2.62), as shown in Figure 11.4a–b and Figure 11.5a–b.

NIR spectroscopy and partial least-squares regression were also used for determination of α-TOH in olive oils after extraction with ethanol (Szlyk *et al.*, 2005). The SEC and SEP (with the lowest SEC = 0.17% and SEP = 0.20%) were calculated for evaluation of the calibration models. The chemometric calibration model was prepared in spectral region 6500–4500 cm^{-1} (1538–2222 nm) (overtones and combination bands of CH aromatic, CH_3, CH_2, and C=C groups vibrations) for standard α-TOH solutions (0.54–53.54 mg/mL). A PerkinElmer Spectrum 2000 FTIR spectrometer was used at 8 cm^{-1} resolution, spectra range 4000–10000 cm^{-1}, and 2 mm glass cells. The calibration was represented graphically (Figure 11.6a) by plotting the theoretical concentration of the reference α-TOH samples (used for the calibration model) versus the predicted values by the model based on the NIR spectra.

The proposed NIR method is relatively simple, precise, accurate, and convenient for the determination of α-TOH in olive oils after direct extraction. The NIR measurements are generally rapid (ca. 1–2 min), but extraction of tocopherol (60 min, ethanol) from oil samples is required. The mean concentrations of α-TOH in studied oils determined by the proposed NIR method agreed with those obtained by the standard HPLC method (Figure 11.6b). It is noteworthy that the proposed NIR method does not require toxic solutions and reagents. Moreover, the cost of the instrumentation is considerably lower than in the case of the standard HPLC method.

Mailer (2004) used Vis-NIR spectroscopy between 400 and 2500 nm in olive oil samples from throughout the Australian olive-growing areas in order to provide PLS calibration models to determine the content of polyphenols, chlorophylls, and the fatty acids oleic and linoleic acid. High correlation coefficients squared (r^2) were found for the calibration models of oleic acid (0.99), linoleic acid (1.00), and chlorophyll (0.98) with high levels of accuracy (low errors of cross-validations). The calibration models for polyphenols (0.89) showed lower prediction values. All of the parameters measured were sufficiently accurate for routine screening of olive oil, but the limitation of this study is that no test set was used.

The interaction between natural pigments and visible and near-infrared radiation could provide useful correlations for determining olive oil chlorophylls and carotenoids. Vis-NIR spectroscopy (750–2500 nm) was used for the simultaneous quantification of carotenoids and chlorophylls in Spanish VOOs using PLS analysis (Marquez, 2003). In the Vis-NIR transmittance spectra of VOO samples with different contents of chlorophyll and carotenoid pigments, spectral differences were observed in the wavelength ranges 450–1000 nm and 1800–2300 nm. For this study, 183 VOOs were used to develop PLS models: 151 samples for calibration and 32 for validation to monitor on-line levels of these compounds during VOO processing in olive oil mills. The validation set gave r^2 = 0.970 and SEP 0.66 mg kg^{-1} for carotene totals and r^2 = 0.986 and SEP = 0.96 mg kg^{-1} for chlorophyll totals, indicating the feasibility of using Vis-NIR measurements for the quantitative prediction of carotenoid and chlorophyll pigments in VOO.

The determination of total chlorophylls and total carotenoids in olive oil by using only Vis and the study of the contributions of visible and NIR regions in predictive models for these olive oil compounds represent an interesting approach, since the simplicity of the Vis spectroscopic technique may have a significant impact on reducing the cost of the instrumentation used (Cayuela *et al.*, 2014). For this reason, the proposed technique has been compared with the determination of these pigments by near-infrared spectroscopy (NIRS) and Vis

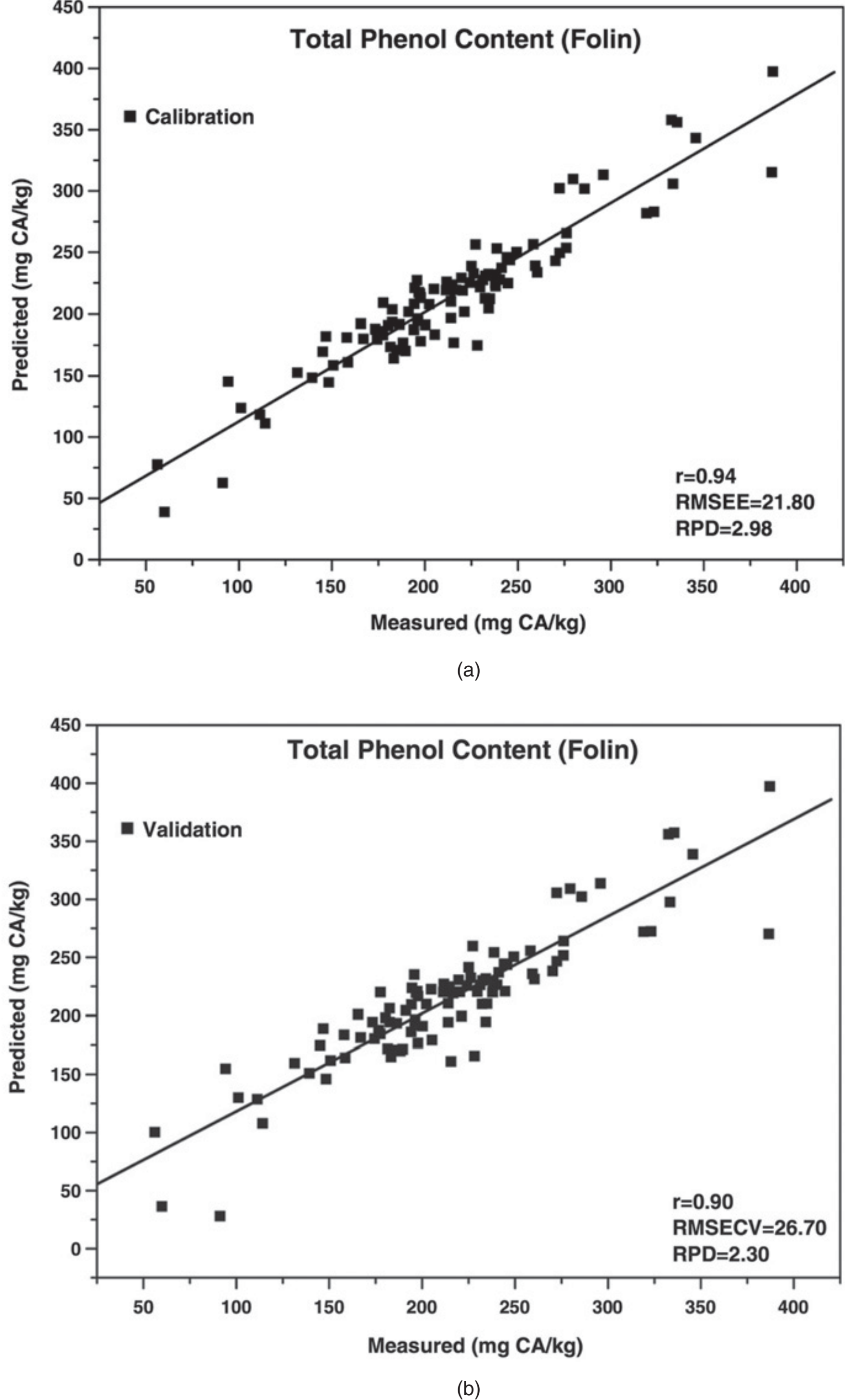

Figure 11.4 Partial least squares (PLS) model for total phenol content by Folin-Ciocalteau (mg/kg of caffeic acid): (a) calibration, (b) validation. RMSEE, Root mean square error of estimation; RMSECV, root mean square error of cross validation; r, multiple coefficient of determination; RPD, residual prediction deviation. *Source*: Inarejos-Garcia *et al.* (2013). Reproduced with permission of Elsevier.

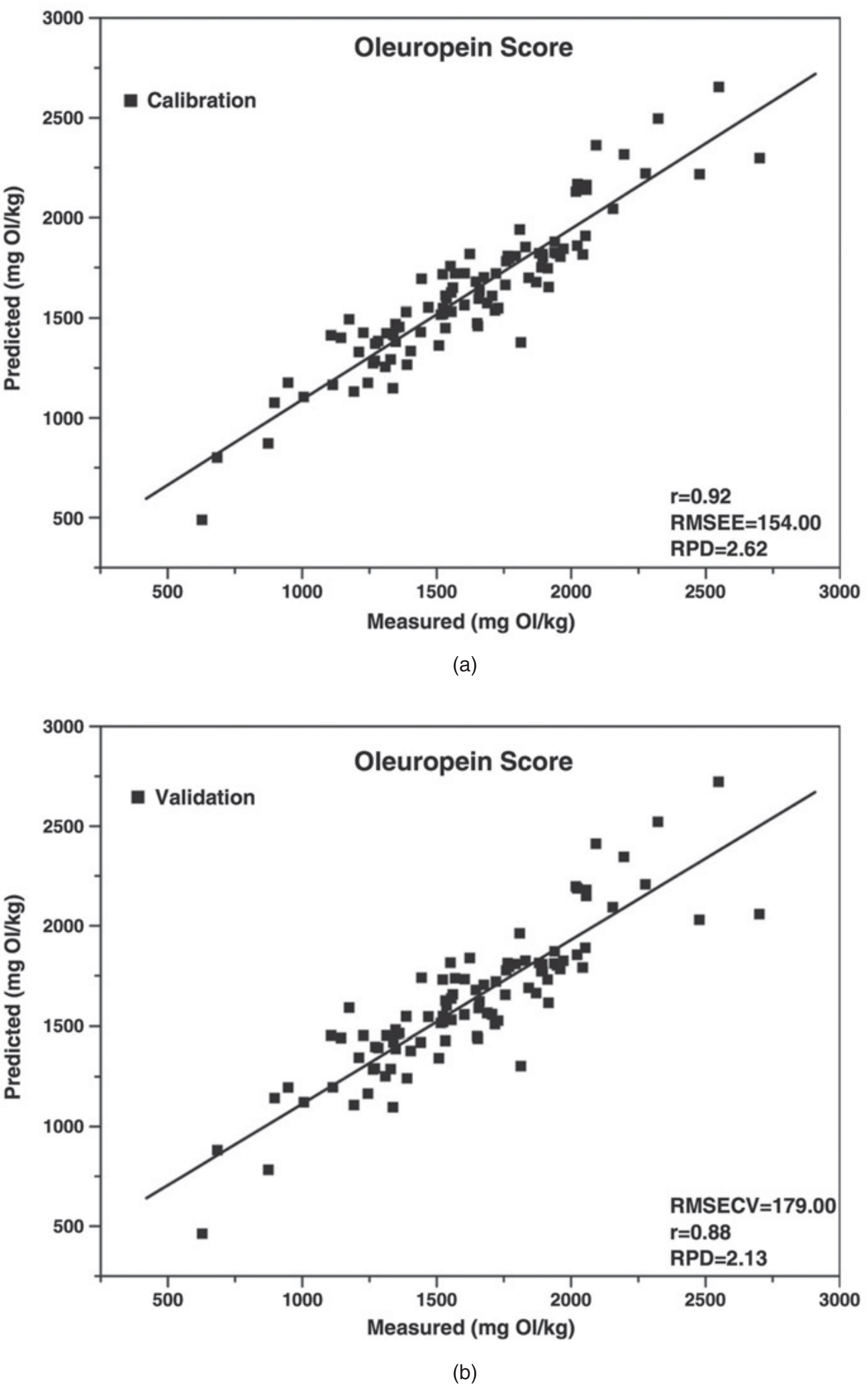

Figure 11.5 Partial least squares (PLS) model of oleuropein score (mg/kg of oleuropein): (a) calibration, (b) validation. RMSEE, Root mean square error of estimation; RMSECV, root mean square error of cross validation; r, multiple coefficient of determination; RPD, residual prediction deviation. *Source*: Inarejos-Garcia *et al.* (2013). Reproduced with permission of Elsevier.

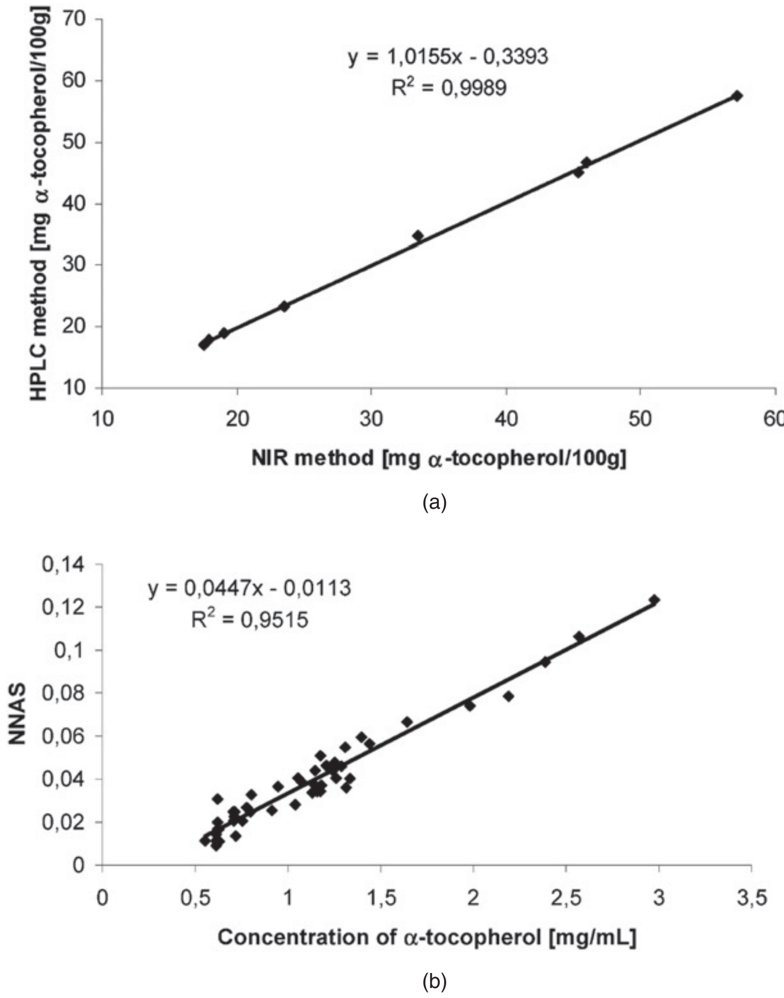

Figure 11.6 (a) Full cross-validation model plotting the theoretical concentration of reference α-tocopherol solutions vs. the predicted values using PLS-NIR regression. (b) Correlation between HPLC and PLS-NIR methods for the determination of α-tocopherol in oil samples. *Source*: Szlyk *et al.* (2005). Reproduced with permission of American Chemical Society.

together with NIRS. The reference methods used were the determination of the extinction coefficient K_{670} for total chlorophylls and K_{470} for total carotenoids.

The optimization of the calibration for total chlorophylls was set by using MLR for the wavelengths 670–686 nm exclusively in the Vis area. Its satisfactory performance was proven from the model coefficients SEC (2.63) and $r^2 = 0.97$, and the residual predictive deviation (RPD = 5.76) from the external validation (Figure 11.7a). For the total carotenoids, the best Vis calibration was fit by using a window of 465–475 nm and PLS, which provided an RPD of 3.68. However, the model built using the entire spectrum Vis-NIR available (350–2500 nm) was slightly better for this last pigment, showing an RPD of 3.86 (Figure 11.7b). This study showed that the proposed Vis technique can be advantageous for the determination of total chlorophylls in olive oils, while it is also suitable for determining total carotenoids.

Another interesting approach has been described by Manley and Eberle (2006), in South African extra virgin olive oils (EVOOs) using two FT-NIR instruments at different resolutions and path lengths. These authors compared the PLS regression models for quality parameters including the functional compounds

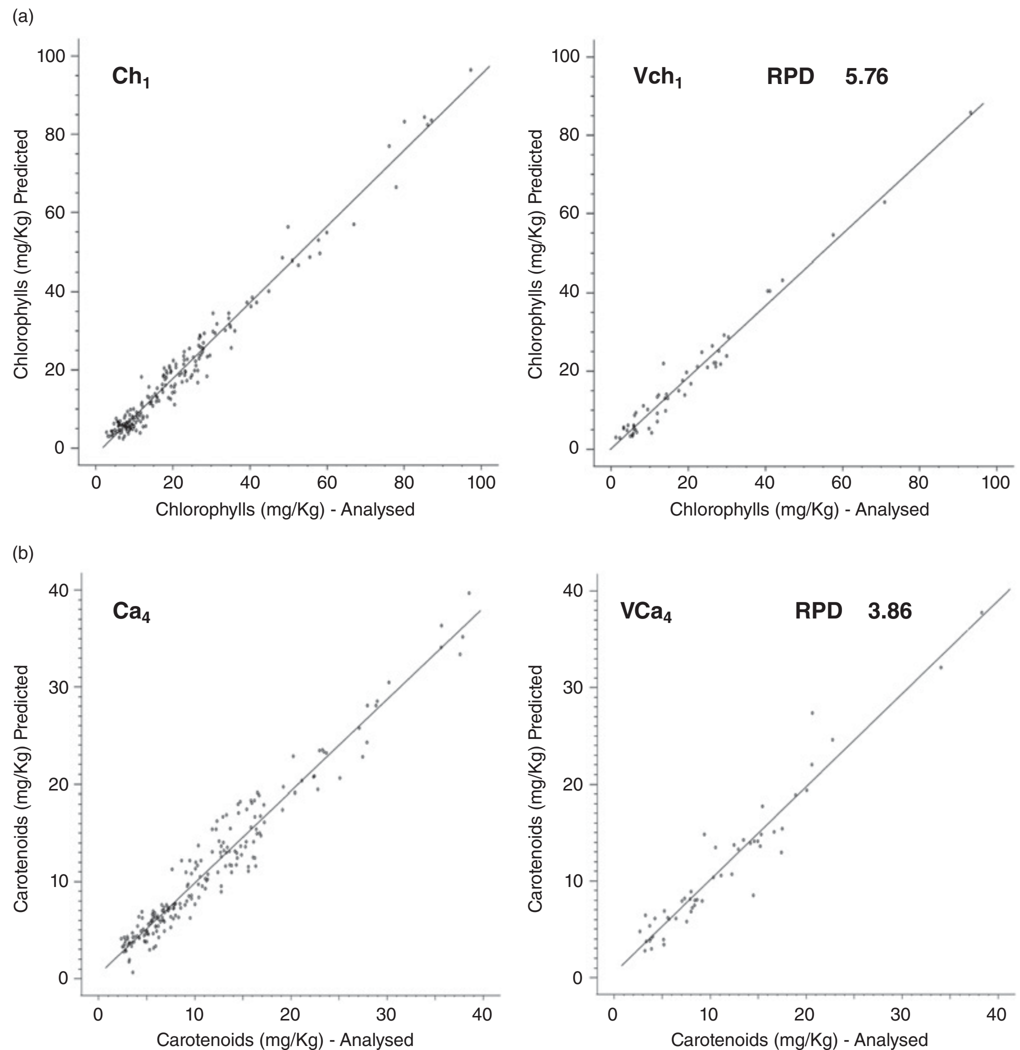

Figure 11.7 (a) Predictive model Vis for total chlorophylls (Ch1) and external validation (Vch1). (b) Predictive model Vis/NIRS for total carotenoids (Ca4) and external validation (Vca4). *Source*: Cayuela *et al.* (2014). Reproduced with permission of Springer.

oleic and linoleic acids, the chlorophyll and carotenoid pigments, the total phenol content, as well as the TBARS values.

For this purpose, they recorded spectra by using two FT-NIR instruments – a PerkinElmer IdentiCheck spectrophotometer (9091–4000 cm^{-1}) (1100–2500 nm) in transmittance mode at different resolutions of 64, 32, 16, and 8 cm^{-1} at each of two path lengths (0.2 and 0.5 mm), and a Büchi NIRLab N-200 instrument (10224–4000 cm^{-1}) in transflectance mode at a fixed resolution of 8 cm^{-1} and a path length of 0.6 mm – in order to determine the most appropriate instrument settings and sample presentation modes.

The comparison of the PLS regression models developed from PerkinElmer spectra revealed that spectra recorded at the lowest resolution of 64 cm^{-1} produced equally accurate models when compared to higher resolution spectra and that the two path lengths (0.2 and 0.5 mm) resulted in no significant differences. The 0.2 mm and 64 cm^{-1} PE PLS regression models were compared to those developed from Büchi spectra. Significant differences ($p < 0.05$), using the spectra data without pretreatment, were observed for the PLS model's oleic acid and TBARS values, which performed better when developed from PE spectra.

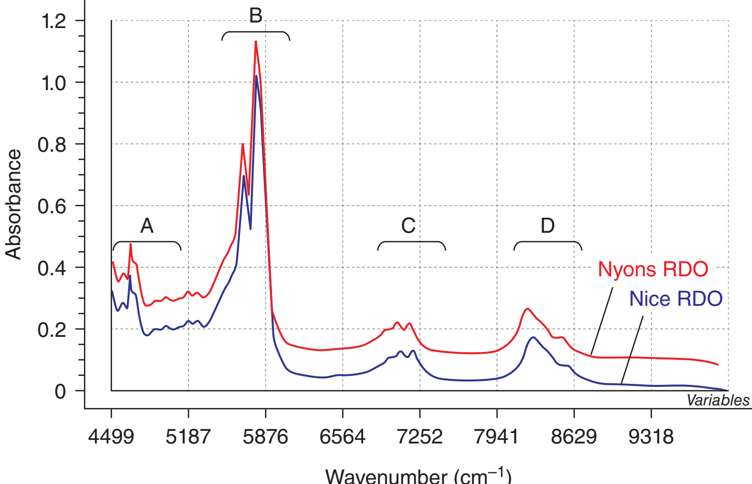

Figure 11.8 Near-infrared spectra of two RDO virgin olive oil samples. (A) Combination of the —CH stretching vibration with other vibrational modes. (B) First overtone of —CH stretching vibration (methyl, methylene, and ethylene groups). (C) Combination of the —CH stretching vibration. (D) Second overtone of —CH stretching vibration (methyl, methylene, and ethylene groups). *Source*: Galtier *et al.* (2007). Reproduced with permission of Elsevier.

The prediction results of the regression models developed in this study were not as successful as those reported by Mailer (2004). This can be explained by the fact that the regression models were not fully optimized as the samples in the calibration and validation sets had to be kept identical to allow comparisons between the two sample presentation modes. A tendency for spectra recorded on a Büchi FT-NIR spectrophotometer to produce slightly weaker PLS regression models for linoleic acid, chlorophylls, and carotenoids might suggest that transmittance spectra are more appropriate for olive oil applications than transflectance spectra.

Chemometric treatment of NIR spectra was taken directly from French VOO samples (registered designations of origin, or RDOs) in 2 mm path length quartz cells, and were assessed for the quantification of oleic and linoleic acids and also the triterpene squalene (Galtier *et al.*, 2007). Typical spectra of two RDO VOO samples are presented in Figure 11.8.

The best prediction results for oleic and linoleic acids were obtained using either raw spectral data in the 4500–7500 cm^{-1} (1333–2222 nm) range or in the case of squalene prediction using the full spectrum 4500–10000 cm^{-1} (1000–2222 nm). The reference data on fatty acid composition were obtained by chromatographic methods. For the determinations of oleic acid ($r^2 = 0.97$, $Q^2 = 0.96$) and linoleic acid ($r^2 = 0.98$, $Q^2 = 0.97$), very good results were obtained with REP lower than 5%. Due to the lower concentration of squalene (<0.1), the results obtained with REP lower than 8% were satisfactory.

Mignani *et al.* (2008a) used a commercially available spectrophotometer (cuvettes with 10 mm optical path) and transmission measurements in the UV-Vis-NIR (200–2800 nm) spectral range to quantify the content of oleic and linoleic acids for 20 Sicilian EVOOs. PLS was applied to predict the identified fatty acids, using cross-validation to assess the optimal number of PLS factors. Several spectral ranges have been investigated for each component in order to determine the best prediction range of each particular compound. The optimum spectral ranges were both in the NIR region for oleic acid and linoleic acids 1333–2222 nm. The prediction ability was measured in terms of the square correlation coefficient, r^2 (oleic acid $r^2 = 0.9986$; linoleic acid $r^2 = 0.9553$).

In a similar study by Mignani *et al.* (2008b), a fiber-optic-based device for UV-Vis-NIR (200–1700 nm spectral range) absorption spectroscopy was developed, in order to obtain the hyper-spectral optical signature of olive oil. This was done not only for authentication purposes but also to correlate the spectral data with the content of fatty acids, among them oleic and linoleic acids, which are important nutritional factors.

To find the degree of correlation between the spectral and chemical data, the spectral data were processed by means of PLS analysis. Both UV-Vis and NIR bands were tested as predictor matrices, and then the most convenient one was chosen. The set of fatty acids was quantified by conventional analytical techniques. A good correlation ($r^2 = 0.91$) was found in the content of oleic acid, the main monounsaturated fatty acid of olive oil (average content 74.4%, SEC value 2.5%).

From this study, it can be concluded that through fiber-optic probes, it is possible to design low-cost small spectroscopic devices capable of operating on-line, in one or more sections of the production process, to obtain timely information on functional compounds of olive oil.

A case of UV-Vis-NIR-MIR spectroscopy and chemometrics in monitoring olive oil functional compounds was published by Casale *et al.* (2012). In this study, UV-Vis (190–1100 nm), NIR (10000–4000 cm^{-1}) (1000–2500 nm) (in quartz cuvettes with a path length of 5 mm), and MIR (4000–700 cm^{-1}) (2500–14286 nm) spectra were used separately and combined in 57 Italian extra virgin olive oils (Figure 11.9), and PLS regression was applied in order to predict the content of oleic and linoleic acids.

The models developed with the MIR and UV-Vis data were not sufficient to predict the amount of oleic and linoleic acids. In the UV-Vis region, there are no absorptions characteristic of the two studied fatty acids, while in the MIR region the absorptions arising from methyl, methylene, and carboxylic groups do not represent selective peaks for oleic and linoleic acids. In this case, combining information from the above three spectroscopic techniques did not improve the models.

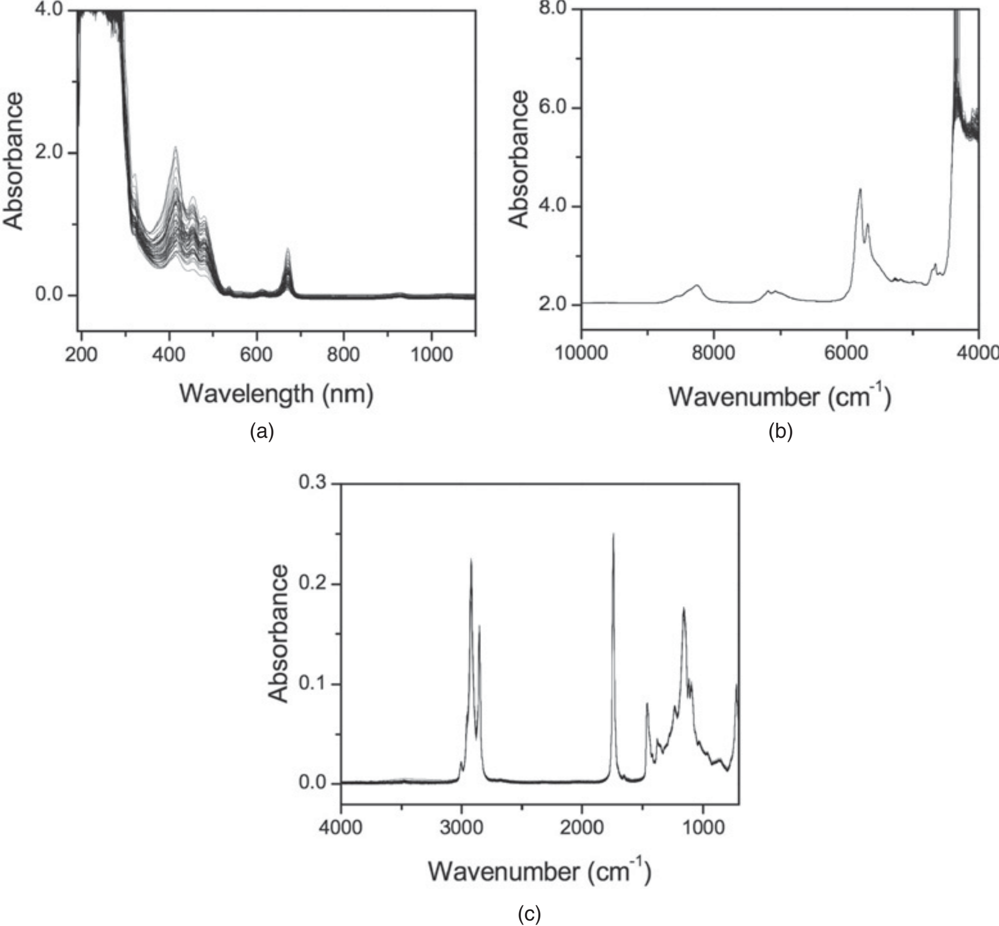

Figure 11.9 Raw (a) UV-Vis, (b) NIR and (c) MIR spectra. *Source*: Casale *et al.* (2012). Reproduced with permission of Elsevier.

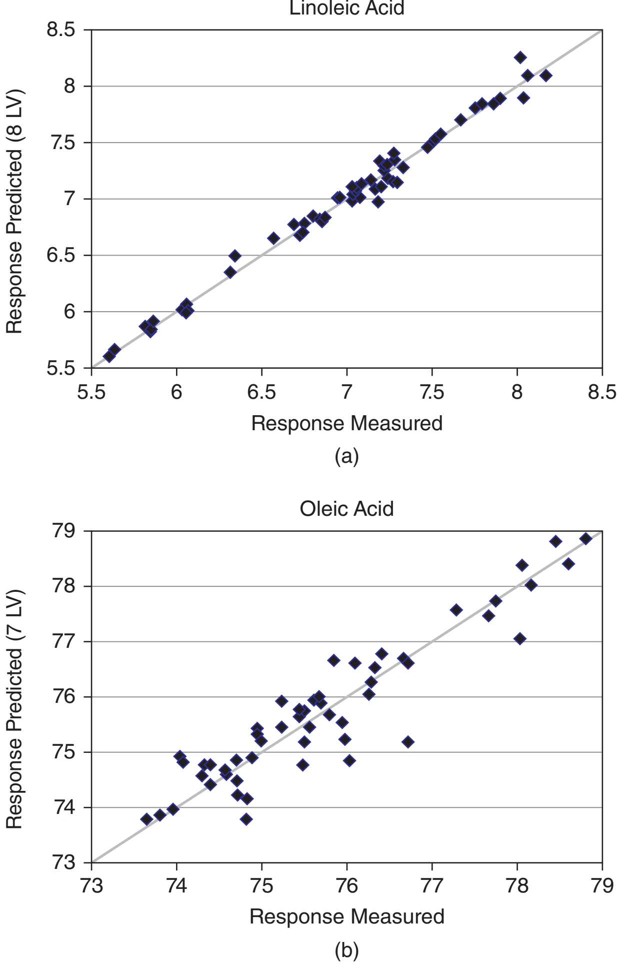

Figure 11.10 Predicted versus measured response (amount of (A) linoleic and (B) oleic acids) for the NIR spectra. *Source*: Casale *et al.* (2012). Reproduced with permission of Elsevier.

The results obtained by NIR data alone were equivalent to or even better than those obtained using all the data jointly. In particular, as far as the test set results are concerned, the best regression models, for both oleic and linoleic acids, were obtained using the whole NIR spectra: the predicted versus the measured response (amount of linoleic and oleic acids) are plotted in Figure 11.10. NIR spectroscopy appeared as the most powerful spectroscopic technique to predict the content of oleic and linoleic acids in olive oil samples.

11.7 UV-Vis-NIR-MIR spectroscopy and chemometrics in monitoring olive oil oxidation

Lipid oxidation has been recognized as the major problem affecting edible oils, as it is the cause of important deteriorative changes in their chemical, sensory, and nutritional properties. Oxidation normally proceeds slowly at the initial stage and then is followed by a sudden rise in the oxidation rate, once the antioxidant content of the oil has been depleted. The time period that marks this change in the oxidation rate is called the induction period or induction time. Oil quality is concerned with the present state of oil, while oil stability is related to its resistance to future changes. Due to the evolution of oxidative degradation, the level of

oxidation at early stages of the process gives poor information on the later oil behavior, and for this reason the evaluation of an oil's stability toward oxidation is considered even more important than the extent to which the oil is oxidized at a given time.

Virgin olive oil, one of the few oils that can be consumed without any chemical treatment, has a high resistance to oxidative deterioration mainly due to two reasons: first, since its fatty acid composition is characterized by a high monounsaturated–polyunsaturated fatty acid ratio; and second, since it contains a pool of minor compounds of powerful antioxidant activity among which polyphenols stand out (Kiritsakis, 1990). Most of these compounds are eliminated or drastically reduced during the refining process and, consequently, are present in much lower amounts in edible refined oils than in virgin oils.

The olive oil stability (OSI) measurement is necessary in order to gain a reasonable estimate of the product shelf life. Fatty acid composition is fairly constant, which explains why olive oil stability is more affected and better correlated by its minor compound content, which have a great influence on its stability. Thus, the OSI, which is the same as induction time or induction period, is useful as a check of the antioxidant role of the olive oil minor components. The prediction of oxidative stability during oil storage is commonly carried out using several methods such as the Schaal oven test, active oxygen, OSI, or oxygen uptake. All of these methods are time-consuming and expensive.

Although the literature on olive oil oxidative stability analysis by NIR is very scarce, an interesting study has been described by Manley and Eberle (2006) in South African EVOOs under oxidation conditions, using two FT-NIR instruments.

The FT-NIR spectra of an EVOO at low (week 0), medium (week 6), and high (week 10) degrees of oxidation, as recorded on both the PE and Büchi spectrophotometers, are shown in Figure 11.11. Olive oil spectra are characterized by strong absorptions at 8333 cm^{-1} (1200 nm) (CH_2 second overtone), 5767 and 5666 cm^{-1} (1734 and 1765 nm) (CH_2 first overtones), as well as 4329 and 4264 cm^{-1} (2310 and 2345 nm) (CH_2 stretch bend combinations). It appears from the spectra that absorption across the whole spectrum increases slightly as the oil becomes oxidized.

A PCA model was developed for clustering olive oils at different oxidation levels. The score plots of PCs 1 and 2, constructed for both instruments, are shown in Figure 11.12.

The PCA score plot (PC1 vs. PC2) of the MSC pretreated spectra collected on the Büchi spectrophotometer illustrates the separation of spectra into clusters of olive oil samples oxidized for 0–3 weeks and 4–10 weeks, respectively. The two clusters formed due to different degrees of oxidation are separated along the

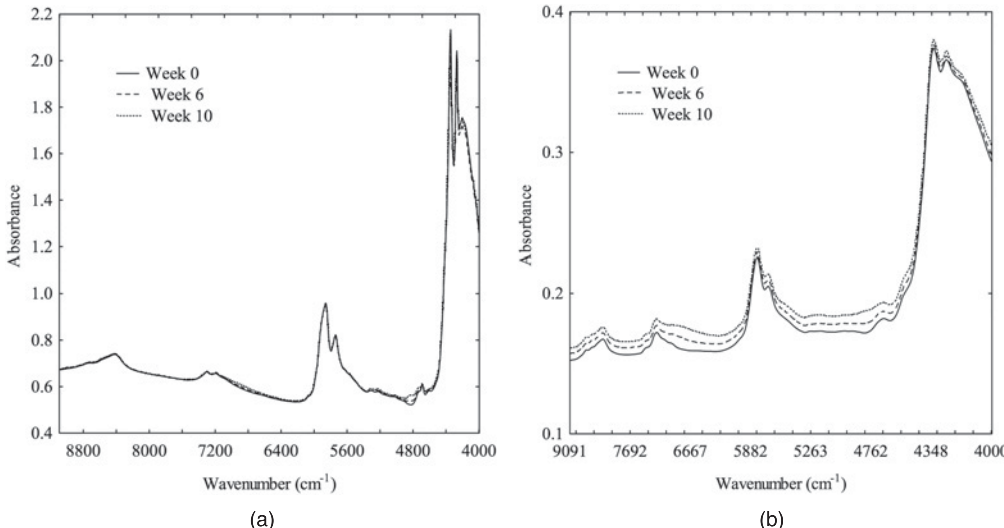

Figure 11.11 Spectra of an extra virgin olive oil at low (week 0), medium (week 6), and high (week 10) degrees of oxidation, as recorded on a (a) Buchi NIRLab200 spectrophotometer with a path length of 0.6 mm and resolution of 8 cm^{-1} and a (b) PerkinElmer Identi Check spectrophotometer with a path length of 0.2 mm and resolution of 64 cm^{-1}. *Source*: Manley and Eberle (2006). Reproduced with permission of IM Publications.

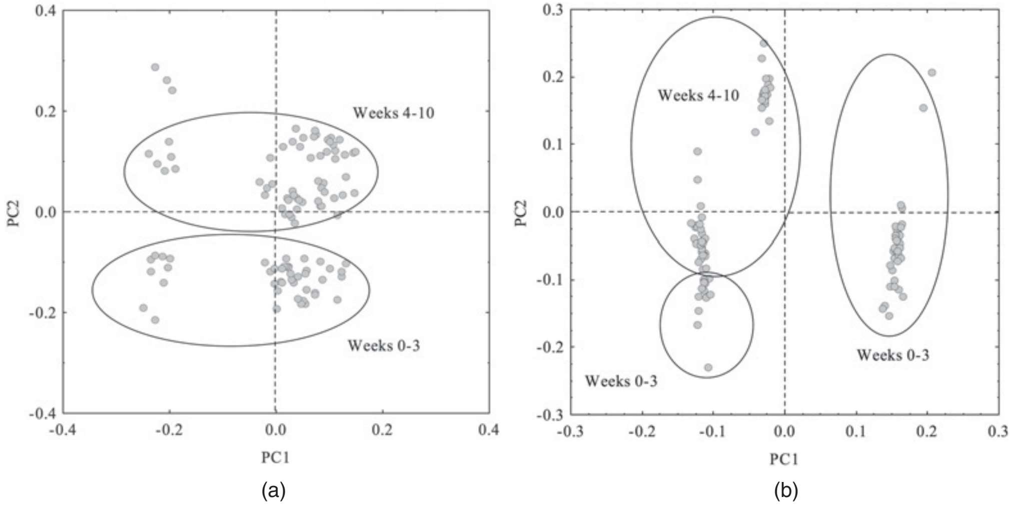

Figure 11.12 PCA score plots (PC1 vs. PC2) of (a) Buchi (8 cm^{-1} resolution, 0.6 mm path length) and (b) PerkinElmer spectra (64 cm^{-1} resolution, 0.2 mm path length). *Source*: Manley and Eberle (2006). Reproduced with permission of IM Publications.

PC2 axis. The PCA score plot (PC1 vs. PC2) of the spectra recorded on the PE spectrophotometer reveals that these spectra are separated along the PC1 axis rather than the PC2 axis, even though a group of samples oxidized for 0–3 weeks was only distinguished from the 4–10-week samples along the PC2 axis.

A similar study reported the rapid evaluation of olive oil oxidative stability, measuring induction time by NIR reflectance (Mailer, 2004). In this study, Vis-NIR spectroscopy was used between 400 and 2500 nm in olive oil samples from the entire Australian olive-growing areas in order to provide PLS calibration models to determine the induction time as a measure of the olive oil oxidative stability. It is important to note that in this study, calibration models for induction time did not include a procedure for calibration assessment using validation, with samples different from those used to develop the calibrations.

UV-Vis-MIR and PLS spectroscopic techniques have also been used to study the thermal degradation of olive oil at 180 °C (Maggio *et al.*, 2011). In a related study, Roman *et al.* (2012) used FT-NIR for the same purpose, developing a quality control for olive oil employed in frying. It is noteworthy that near-infrared emission spectroscopy (NIRES) has also been reported as an alternative method to measure the oxidative stability of edible oils at frying temperatures (Gonzaga & Pasquini, 2006; Gonzaga *et al.*, 2007), in order to monitor oil thermal degradation, as in the two previous studies. In the latter studies, oxidative stability was determined by means of the emission band at 2900 nm and its increase and broadening during heating time.

The possibility of measuring olive oil resistance to oxidation under storage conditions by NIR transmittance has been described by Sanchez *et al.* (2013). In this study, visible and near-infrared (Vis/NIRS) spectroscopy (350–2500 nm spectral range and 5 mm path length) and multivariate models have been used in order to estimate the oxidative stability of VOO. The OSI method using a Rancimat instrument was applied as the reference analysis for all olive oil samples.

The calibration sets used for the development of OSI models were formed excluding external validation sets from the total of the olive oil samples. Two multivariate calibration models (M1 and M2) and validation models (V1 and V2) were independently developed for OSI prediction. PLS models were obtained for all of the parameters studied from the whole spectrum acquired (350–2500 nm). Model fitness was assessed independently from the model validation procedure by its SEC and proximity between r^2 and r^2 cv. Model performance was assessed mainly according to the RPD from the external validation exercises, referred above, which is the most consensual statistic for assessing a model's predictive accuracy. The r^2 of the simple linear regression between the analyzed and predicted values in this external validation exercise and the RMSEP were also considered.

The visible spectrum can be highlighted for its contribution in both models, as well as the NIR spectral windows corresponding to 1300–1700 and 1800–2250 nm. The OSI models corresponding to spectral data from absorbance treated with maximum normalization provided the best fits. The model M1 provided $r^2 = 0.93$ and SEC = 6.07 (10.9%), whereas M2 calibration statistics were $r^2 = 0.94$ and SEC = 5.64 (10.0%). Mailer (2004) reported, in a previous study, a correlation coefficient of 0.83 from the cross-validation procedure, in a calibration from NIR reflectance for measuring induction time, with SEC = 0.84 (19.2%), using VOO, having an OSI mean lower than that used in the present work and, hence, the smallest value of SEC. Despite the fact that this correlation value was not very high, what must be noted is this assay's lack of assessing models on the basis of external validation sets. The OSI model performance was satisfactory according to these data, as evidenced by the RPD values of 3.30 and 3.02 from M1 and M2, respectively. The above data demonstrate the feasibility of estimating OSI through Vis/NIR spectroscopy for measuring the oxidative stability of olive oils. The quality of the models' statistics and their positive evaluation could mean that this technique is an advantageous alternative to other methods of analysis of oxidative stability of olive oils.

11.8 FTIR spectroscopy and chemometrics in monitoring olive oil functional compounds and antioxidant activity

The determination of total phenolic compounds, including o-diphenols and flavonoids, and antioxidant activity by colorimetric methods involves pretreatment of olive oil samples and consequently the destruction of the sample. Furthermore, these analyses are time-consuming and require large amounts of reagents and solvents, which are quite expensive and often toxic. Fourier-transform infrared spectroscopy (FTIR) is a rapid, direct, and simple-to-perform analytical technique that is nondestructive by nature and does not require any sample preparation, particularly when used in conjunction with attenuated total reflectance (ATR).

The application of FTIR in the study of olive oils has increased recently, mainly to evaluate the composition of fatty acids (Inarejos-Carcía *et al.*, 2013), oxidized fatty acids (Lerma-García *et al.* 2011), peroxide value (Bendini *et al.*, 2007a, 2007b), acidity (Lerma-García *et al.*, 2011), adulterations (Rohman & Che Man, 2012), sensory characteristics, phenolic and volatile compounds (Lerma-García *et al.*, 2011), freshness (Sinelli *et al.*, 2007), and authenticity (Lerma-Garcia *et al.*, 2010). Other authors described the use of FTIR-ATR for the simultaneous quantification of fatty acid composition, peroxide value, and free acidity (Maggio *et al.*, 2009). FTIR-ATR-PLS is a useful tool to determine some analytical parameters (water content, phenolic content, flavonoids, and antioxidant activity) in olive oils (Cerretani *et al.*, 2010; Gouvinhas *et al.*, 2015).

Chemometric PLS analysis of extra virgin olive oil FTIR spectra from three cultivars (cv. 'Cobrançosa', 'Galega', and 'Picual') with different maturation stages were combined with analytical parameters, determined only by colorimetric methods, to develop calibration and validation models in order to be able to estimate total phenolic content, o-diphenols, flavonoids, and antioxidant activity during the ripening process (Gouvinhas *et al.*, 2015). FTIR-ATR measurements were performed by pipetting a small drop (1 μl) of olive oil on top of the ATR baseplate, which was kept at 30 °C. All infrared spectra were recorded from 500 to 3000 cm^{-1} (3333–20000 nm). These spectra were subtracted against a background air spectrum.

Figure 11.13 shows an example of the measured spectra collected from 500 to 3000 cm^{-1} (3333–20000 nm). Although the spectra present mainly features related with the fatty acids content, namely triacylglycerols formed by trans fatty acids, these spectra also present fingerprint regions, where the differences allow to distinguish the samples and can be related to the phenolic content of the olive oil samples, and antioxidant activity, by chemometric procedures.

For o-diphenols and flavonoids, the slope r^2 and the RMSE, indicators of the quality of fit, were higher than 0.98 and lower than 0.03, respectively. The phenolic content showed the lowest multiple coefficient of determination in the calibration model ($r^2 = 0.94$), as well as in the cross-validation ($r^2 = 0.91$). The RMSE values were lowest and the same for calibration and cross-validation (RMSE = 0.02). For the quantification of antioxidant activity, the slope r^2 and the RMSE were 0.93 and 0.04, respectively, which decreased when data were cross-validated ($r^2 = 0.86$ and RMSECV = 0.05).

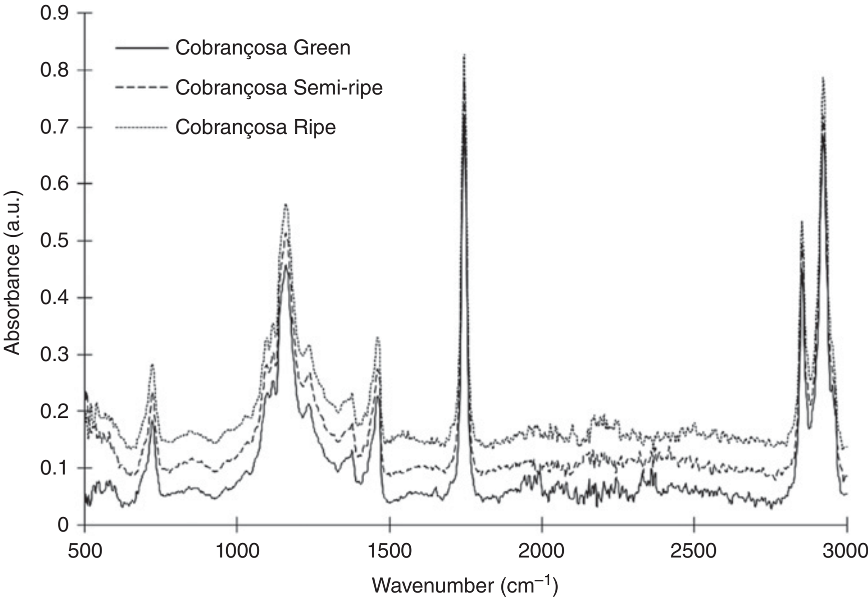

Figure 11.13 FTIR-ATR spectra collected for Cobrancosa olive oils samples in three maturation stages. *Source*: Gouvinhas *et al.* (2015). Reproduced with permission of Elsevier.

Figure 11.14 reflects the accuracy and the performance of the models, which correlate the actual values of (a) total phenolics, (b) o-diphenols, (c) flavonoids, and (d) antioxidant activity with estimated values obtained from FTIR spectra.

Another FTIR-ATR spectroscopic method was applied to the determination of total phenol amount (TP) and antioxidant activity (ABTS) of virgin olive oil (VOO) and other olive oils (Cerretani *et al.* 2010). Spectra were acquired in the range of 4000–700 cm^{-1} (2500–14286 nm). For each sample (2 mL of oil uniformly spread throughout the crystal surface), the absorbance spectrum was collected against a background, obtained with a dry and empty ATR cell. Calibration models were constructed using PLS regression. Oil samples with TPC from 46 to 877 mg gallic acid equivalents/kg oil and with ABTS from 0 to 5.7 mmol Trolox/kg oil were considered for chemometric analysis.

The spectral range selection permitted an increase of the r^2 value for TP (full spectra: 0.74, 3610–816 cm^{-1} or 2770–12200 nm: 0.87), while no increase in the ABTS evaluation was observed for r^2 (full spectra: 0.67, 3707–1105 cm^{-1} or 2700–9000 nm: 0.63). The lower value of r^2 for ABTS prediction could be explained considering that this test is based on a kinetic measure showing a higher variation.

The FTIR-ATR method provided results that were comparable to conventional procedures. This approach represents an easy and convenient means for monitoring olive oil functional compounds with the advantage of ease of operation, speed, no sample pretreatment, and no solvent use. The data obtained in this method are comparable to those procured by using the official reference method. Therefore, the technique can be considered as an alternative to the standard procedure for routine analysis or control at-the-line-of-production processes.

11.9 The use of UV-Vis-NIR-MIR spectroscopy in olive oil industry and trade

The olive oil industry of the 21st century has moved away from the traditional processing and the regional Mediterranean trade of the past. The globalization of the olive culture and trade imposed not only international standards for the final products but also regulations on the quality management of the production

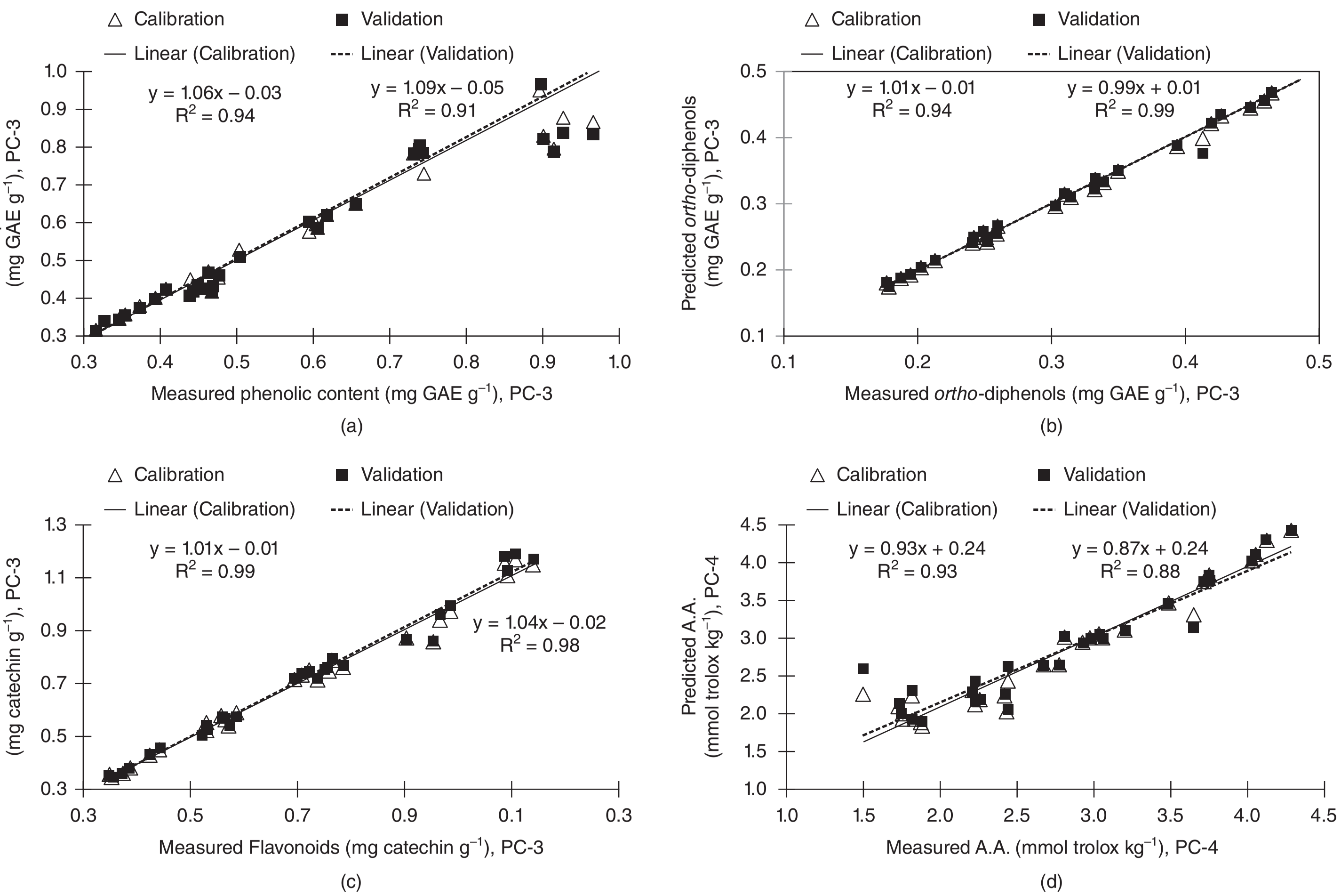

Figure 11.14 Calibration models for the relationship between measured values and FTIR predicted values of (a) phenolic content, (b) o-diphenols, (c) flavonoids, and (d) antioxidant activity (AA). *Source*: Gouvinhas *et al.* (2015). Reproduced with permission of Elsevier.

process, from the tree to the consumer. The profile and concentration of the functional compounds (antioxidants and natural pigments) in the olive fruit and oil are directly related to the cultivar and provenance, to the sensory characteristics of table olives and oil, to the health benefits, as well as to the degree of oxidation and shelf life; in other words they are related to the overall olive and olive oil quality, food safety, and consumer satisfaction (Mignani *et al*., 2006; Bouaziz *et al*., 2010; Frankel *et al*., 2013).

In order to comply with the regulations and the trade demands, monitoring of the antioxidants and lipids at critical control points of the production chain is essential for the olive oil quality. It should be rapid, efficient, and validated in compliance with the official reference methods (IOC, 2009a, 2009b, 2015).

Spectroscopic techniques have several advantages: they are quick, simple, nondestructive, very sensitive, and selective. They can also be hyphenated with separation techniques (i.e., chromatography). Tothill (2003), offers a comprehensive review of rapid instrumentation for food analysis, while in Aliander *et al.* (2013) and Nawraska *et al.* (2013) the reader can find the principles of spectroscopic techniques for food applications. Ozaki *et al.* (2006) and Abbas *et al.* (2012) compiled detailed chapters, particularly on infrared spectroscopic techniques. In general, in-line analysis is the one performed directly on the production line, on-line at a bypass, at-line on samples taken out of the bulk but still in the production site, and off-line when the analysis is performed in the laboratory. The designated instrumentation could be roughly divided into portable spectrometers, benchtop spectrometers that are connected to a fiber-optic probe placed at the point of interest; spectroscopic cameras for hyperspectral imaging; and analyzer spectrophotometers calibrated to provide direct readings in the units of interest.

Besides the speed and convenience, real-time analysis of the antioxidants is taken into consideration because of the intrinsic instability of the compounds and their reactivity with oxygen. Handling of samples, time delays, and exposure to oxygen easily alter their composition, while in-line or on-line measurements are performed with minimal sample changes. But, more importantly, benchtop analyzers or portable spectrometers with applications for the analysis of antioxidants have the major advantage that they can provide reliable results quickly and without the need of highly skilled analysts.

The very first assay widely used in trade for the quality screening of olive oil was based on nondestructive ultraviolet fluorimetry (Frehse, 1925). In 2012, the EC 432/2012 regulation established the health benefits of the phenolics in olive oil defining a required minimum concentration (5 mg in 20 g of olive oil). This triggered the IOC to seek new methodologies to be used in trade standards and focused the scientific efforts in developing a generally approved analysis for the quantification and identification of these compounds. The colorimetric determination, after extraction and addition of molybdate reagent (IOC, 2009a), is the one still in action. The chromatographic method for biophenols (IOC, 2009b) is based on ultraviolet detection at 280 nm, after the separation of the compounds by reverse phase chromatography.

Besides the reference methods, many efforts have been made for the development of alternative assays and screening probes with a good precision and accuracy to satisfy the regulative requirements. The assays should ensure good correlation with the reference methods. A useful comparison of the official methods is published by Garcia *et al.* (2013) along with an alternative colorimetric method specific for o-diphenols. Another colorimetric assay, based on oxidation of gold nanoparticles, has been recently published (Della Pella, 2015) with a good correlation with the official method. Fluorescence spectroscopy has also some promising possibilities for phenol quantification (Papoti & Tsimidou, 2009; Sikorska *et al.*, 2012).

The interest of the olive oil industry toward antioxidants is rather recent, and although many researchers investigated and published new methodologies and instrumentation for the direct measurement of functional compounds, only a few have been yet made commercially available. The Oxitester, a spectrophotometric analyzer, developed by an Italian company (CDR) is one of the longest marketed instruments in this area. It combines the analysis of phenols (stability index) with free fatty acids, K_{270}, and peroxides with a colorimetric assay (Kamvissis *et al.*, 2008).

Portable spectrophotometers are now available for screening color and composition with internal calibrations for oils and olives. Navspec-plus, an instrument marketed by Forston Labs (USA), operating in the region of 350–980 nm and combined with a fluorimeter, provides applications on oil pigments, phenols, and oxidation coefficients. The user can quickly screen the quality of olive oil samples.

Several NIR or FT-IR analyzers are designated for olive oil applications. In the Spectrastar XL of Unity Scientific, quantification of olive oil phenols is included in the calibration parameters. The analyzers are nowadays robust, user-friendly, and resistant to an industrial environment. Many manufacturers of IR olive analyzers (FOSS, Bruker) provide the software and support to the user to build calibrations for additional

analytes. A complete review of the NIR spectroscopy and chemometrics in the olive oil industry is given by Armenta *et al.* (2010).

Imaging spectrometers that correlate the olive fruit maturity, color, and shape to the quality of the olive oil have been developed for the optimization of the harvest time (Guzmán *et al.*, 2015) as well as for the improvement of the milling process (Puerto *et al.*, 2015). An interesting application of an NIR-AOTF (Acousto Optically Tunable Filter) device in diffuse reflectance mode (1100–2300 nm) is targeted to the phenol content and maturity index when measuring the ripening of olives with a portable spectrometer (Bellincontro *et al.*, 2012).

Jimenez Marquez *et al.* (2005) presented an on-line use of an NIR probe for the process control with the phenol content expressed as bitterness index. Mignani *et al.* (2008) described a fiber-optic sensor for the UV-Vis-NIR fingerprinting of olive oils of various origins. The spectral characteristics are directly correlated to the functional compounds. Recently, Grossi *et al.* (2015) announced an opto-electronic sensor that can directly measure antioxidants and oxidation level of oils in tanks and on-line by measuring the optical density of an emulsion.

Table 11.1 summarizes a number of important applications of UV-Vis-NIR-MIR spectroscopy and chemometrics for the analysis of functional components of olive oil that were described in detail in this chapter. These recent developments in the field of chemometrics have illustrated that UV-Vis-NIR-MIR spectroscopy combined with chemometrics has emerged as a nondestructive, fast, and real-time analytical tool of olive oil functional compounds, in the olive oil sector.

11.10 Conclusion

The challenges for the future will be the development and application of low-cost portable spectroscopic devices that can be used directly in one or more stages of the olive oil production process to obtain timely information on functional compounds of olive oil and oxidative stability of olive oil. Estimations of these parameters are very important for the characterization of olive oil, especially EVOO, as a high-quality natural product with beneficial health effects.

Acknowledgments

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References

Abbas O., Dardenne P., & Baeten V. (2012) Near infra-red, mid infra-red and Raman spectroscopy. In: Chemical analysis of food: techniques and applications, ed. Y. Pico. Elsevier, Amsterdam.

Aliander J., Bochko V., Martinkauppi B., Saranwong S., & Mantere T. (2013) A review of optical nondestructive visual and near-infrared methods for food quality and safety. International Journal of Spectroscopy 1–37.

Andreadou I., Iliodromitis E., Mikros E., Constantinou M., Agalias A., Magiatis, P., Skaltsounis A.L., Kamber E., Tsantili-Kakoulidou A., & Kremastinos D. (2006) The olive constituent oleuropein exhibits anti-ischemic, antioxidative, and hypolipidemic effects in anesthetized rabbits. Journal of Nutrition 136, 2213–2219.

Armenta S., Garrigues S., & De la Guardia M. (2007) Determination of edible oil parameters by near infrared spectrometry. Analytica Chimica Acta 596, 330–337.

Armenta S., Moros J., Garrigues S., & de la Guardia M. (2010) The use of near-infrared spectrometry in the olive oil industry. Critical Reviews in Food Science and Nutrition 50, 567–582.

Baldioli M., Servili M., Perretti G., & Montedoro G.F. (1996) Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. Journal of the American Oils Chemists' Society 73, 1589–1593.
Barros A., Nunes A., Martins J., & Delgadillo I. (2009) Determination of oil and water in olive and olive pomace by NIR and multivariate analysis. Sensing and Instrumentation for Food Quality and Safety 3, 180–186.
Bellincontro A., Taticchi A., Servili M., Esposto S., Farinelli D., & Mencarelli F. (2012) Feasible application of a portable NIR-AOTF tool for on-field prediction of phenolic compounds during the ripening of olives for oil production. Journal of Agriculture and Food Chemistry 60, 2665–2673.
Bendini A., Cerretani L., Carrasco-Pancorbo A., Gómez-Caravaca A.M., Segura-Carratero A., Fernández-Gutiérrez A., & Lercker G. (2007a) Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. Molecules 12, 1679–1719.
Bendini A., Cerretani L., Virgilio F.D., Belloni P., Bonoli-Carbognin M., & Lercker G.I. (2007b) Preliminary evaluation of the application of the FTIR spectroscopy to control the geographic origin and quality of virgin olive oils. Journal of Food Quality 30, 424–437.
Bianco A.D., Muzzalupo I., Piperno A., Romeo G., & Uccella N. (1999) Bioactive derivatives of oleuropein from olive fruits. Journal of Agriculture and Food Chemistry 47, 3531–3534.
Bianco A.D., & Uccella N. (2000) Biophenolic components of olives. Food Research International 33, 475–485.
Bouaziz M., Feki I., Ayadi M., Jemai H., & Sayadi S. (2010) Stability of refined olive oil and olive-pomace oil added by phenolic compounds from olive leaves. European Journal of Lipid Science and Technology 112, 894–905.
Boskou D., Tsimidou M., & Blekas G. (2006) Olive oil composition. In: Olive oil: chemistry and technology, ed. D. Boskou. AOCS Press, Champaign, IL.
Casale M., Oliveri P., Casolino C., Sinelli N., Zunina P., Armanino, C., Forina M., & Lanteria S. (2012) Characterisation of PDO olive oil Chianti Classico by non-selective (UV-visible, NIR and MIR spectroscopy) and selective (fatty acid composition) analytical techniques. Analytica Chimica Acta 712, 56–63.
Casale M., & Simonetti R. (2014) Review: near infrared spectroscopy for analysing olive oils. Journal of Near-Infrared Spectroscopy 22, 59–68.
Cayuela J.A., Yousfi K., Martınez M.C., & Garcıa J.M. (2014) Rapid determination of olive oil chlorophylls and carotenoids by using visible spectroscopy. Journal of the American Oils Chemists' Society 91, 1677–1684.
Cerretani L., Toschi T.G., & Bendini A. (2009) Phenolic fraction of virgin olive oil: an overview on identified compounds and analytical methods for their determination. Functional Plant Science and Biotechnology 3 (SI1), 69–80.
Cerretani L., Giuliani A., Maggio R.M., Bendini A., Toschi T.G., & Cichelli A. (2010) Rapid FTIR determination of water, phenolics and antioxidant activity of olive oil. European Journal of Lipid Science and Technology 112, 1150–1157.
Cicerale S., Conlan X.A., Sinclair A.J., & Keast R.S.J. (2009) Chemistry and health of olive oil phenolics. Critical Reviews in Food Science and Nutrition 49, 218–236.
Downey G., McIntyre P., & Davies A.N. (2003) Geographic classification of extra virgin olive oils from the Eastern Mediterranean by chemometric analysis of visible and near-infrared spectroscopic data. Applied Spectroscopy 57, 158–163.
Frankel E., Bakhouche A., Lozano-Sánchez J., Segura-Carretero A., & Fernández-Gutiérrez A. (2013) Literature review on production process to obtain extra virgin olive oil enriched in bioactive compound: potential use of byproducts as alternative sources of polyphenols. Journal of Agriculture and Food Chemistry 61, 5179–5188.
Frehse R. (1925) Application of Wood's light to the examination of olive oil and turpentine. Analyst 50, 361–362.
Galtier O., Dupuy N., Le Dréau Y., Ollivier D., Pinatel C., Kister J., & Artaud J. (2007) Geographic origins and compositions of virgin olive oils determined by chemometric analysis of NIR spectra. Analytica Chimica Acta 595, 136–144.
Garcia B., Coelho J., Costa M., Pinto J., & Paiva-Martins F. (2013) A simple method for the determination of bioactive antioxidants in virgin olive oils. Journal of the Science of Food and Agriculture 93, 1727–1732.
Gonzaga F., & Pasquini C. (2006) A new method for determination of the oxidative stability of edible oils at frying temperatures using near infrared emission spectroscopy. Analytica Chimica Acta 570, 129–135.
Gonzaga F., Pasquini C., Christianne E.C., Rodrigues C.E., & Meirelles A.J. (2007) Comparison of near-infrared emission spectroscopy and the Rancimat method for the determination of oxidative stability. European Journal of Lipid Science and Technology 109, 61–65.
Gouvinhas I., de Almeida J.M.M.M., Carvalho T., Machado N., & Barros, A.I.R.N.A. (2015) Discrimination and characterisation of extra virgin olive oils from three cultivars in different maturation stages using Fourier transform infrared spectroscopy in tandem with chemometrics. Food Chemistry 174, 226–232.
Grossi M., Di Lecce G., Arru M., Toschi T.G., & Riccò B. (2015) An opto-electronic system for in-situ determination of peroxide value and total phenol content in olive oil. Journal of Food Engineering 146, 1–7.

Guzmán E., Baeten, V., Pierna J.A., & García-Mesa J.A. (2015) Determination of the olive maturity index of intact fruits using image analysis. Journal of Food Science and Technology 52, 1462–1470.
Huang C.L., & Sumpio B.E. (2008) Olive oil, the Mediterranean diet, and cardiovascular health. Journal of the American College of Surgeons 207, 407–416.
Inarejos-Garcia A.M., Gomez-Alonso S., Fregapane G., & Salvador M.D. (2013) Evaluation of minor components, sensory characteristics and quality of virgin olive oil by near infrared (NIR) spectroscopy. Food Research International 50, 250–258.
International Olive Council (IOC) (2009a) DECISION NO DEC-17/97-V/2009a: Identification and quantification of the phenolics in olive oil. IOC, Madrid, Spain.
International Olive Council (IOC) (2009b) COI/T.20/Doc No 29, November 2009b Determination of biophenols in olive oil by HPLC. IOC, Madrid, Spain.
International Olive Council (IOC) (2015) COI/T.20/ Doc. No 19/Rev. 3 February 2015 Spectrophotometric investigation in the ultraviolet. IOC, Madrid, Spain.
Jackson J.E. (2003) A user's guide to principal components. J. Wiley & Sons Inc., Hoboken, NJ.
Kamal-Eldin A., & Appelqvist L.A. (1996) The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 31, 671–701.
Kamvissis V.N., Barbounis E.G., Megoulas N.C., & Koupparis M.A. (2008) A novel photometric method for evaluation of the oxidative stability of virgin olive oils. Journal of AOAC International 91, 794–801.
Kiritsakis A.K. (1990) Olive oil. AOCS Press, Champaign, IL.
Kiritsakis, A. (1998) Olive oil: from the tree to the table, 2nd ed. Food and Nutrition Press, Trumbull, CT.
Kiritsakis, A., & Dugan, L.R. (1985) Studies in photooxidation of olive oil. Journal of the American Oils Chemists' Society 62, 892–896.
Kritchevsky S.B. (1999) β-Carotene, carotenoids and the prevention of coronary heart disease. Journal of Nutrition 129, 5–8.
Landrum J.T., & Bone R. (2001) Lutein, zeaxanthin, and the macular pigment. Archives of Biochemistry and Biophysics 85, 28–40.
Leon L., Garrido V., & Downey G. (2004) Parent and harvest year effects on near infrared reflectance spectroscopic analysis of olive fruit traits. Journal of Agriculture and Food Chemistry 52, 4957–4962.
Lerma-García M.J., Ramis-Ramos G., Herrero-Martínez J.M., & Simó-Alfonso E.F. (2010) Authentication of extra virgin olive oils by Fourier-transform infrared. Food Chemistry 118, 78–83.
Lerma-García M.J., Simó-Alfonso E.F., Bendini A., & Cerretani L. (2011) Rapid evaluation of oxidized fatty acid concentration in virgin olive oil using Fourier-transform infrared spectroscopy and multiple linear regression. Food Chemistry 124, 679–684.
Lin P., Chen Y., & He Y. (2012) Identification of geographical origin of olive oil using visible and near-infrared spectroscopy technique combined with chemometrics. Food and Bioprocess Technology 5, 235–241.
Maggio R., Kaufman T.S., Karlo M.D., Cerretani L., Bendini A., Cichelli A., & Compagnone D. (2009) Monitoring of fatty acid composition in virgin olive oil by FT-IR spectroscopy coupled with partial least squares. Food Chemistry 114, 1549–1554.
Maggio R. M., Valli E., Bendini A., Gómez-Caravaca A.M., Gallina T., & Cerretani, L. (2011) A spectroscopic and chemometric study of virgin olive oils subjected to thermal stress. Food Chemistry 127, 216–221.
Mailer R.J. (2004) Rapid evaluation of olive oil quality by NIR reflectance spectroscopy. Journal of the American Oils Chemists' Society 81, 823–827.
Manley M., & Eberle K. (2006) Comparison of Fourier transform near infrared spectroscopy partial least square regression models for South African extra virgin olive oil using spectra collected on two spectrophotometers at different resolutions and path lengths. Journal of Near-Infrared Spectroscopy 14, 111–126.
Mark H., & Workman J. (2007) Chemometrics in spectroscopy. Academic Press, London.
Marquez A.J. (2003) Monitoring carotenoid and chlorophyll pigments in virgin olive oil by visible-near infrared transmittance spectroscopy: on line application. Journal of Near-Infrared Spectroscopy 11, 219–226.
Marquez A.J., Molina Diaz A., & Pascual Reguera M.I. (2005) Using optical NIR sensor for on-line virgin olive oils characterization. Sensors and Actuators B 107, 64–68.
Mignani A.G., Ciaccheri L., Mencaglia A.A., Paolesse R., Di Natale C., Del Nobile A., Benedetto R., & Mentana A. (2006) Quality monitoring of extra-virgin olive oil using an optical sensor. In: Optical sensing II, ed. B. Culshaw, A.G. Mignani, H. Bartelt, & L.R. Jaroszewicz. Proceedings of SPIE 6189, 61892F.
Mignani A.G., Beatriz García-Allende P., Ciaccheri L., Conde, O.M., Cimato A., Attilio C. & Tura D. (2008a) Comparative analysis of quality parameters of Italian extra virgin olive oils according to their region of origin. In: Optical sensors, ed. F. Berghmans, A.G. Mignani, A. Cutolo, P.P. Meyrueis, & T.P. Pearsall. Proceedings of SPIE 7003, 700326.
Mignani A.G., Ciaccheri L., Cucci A., Mencaglia A., Cimato A., Attilio C., Ottevaere H., Thienpont H., Paolesse R., Mastroianni M., Monti D., Gerevini M., Buonocore G., Nobile M.A., Mentana A., Grimaldi M.F., Dall'Asta

C., Faccini A., Galaverna G., & Dossena A. (2008b). EAT-by-LIGHT: fiber-optic and micro-optic devices for food quality and safety assessment. IEE Sensors Journal 8, 1342–1352.

Mignani A.G., Ciaccheri L., Ottevaere H., Thienpont H., Conte L., Marega M., Cichelli A., Attilio C., & Cimato, A. (2011) Visible and near-infrared absorption spectroscopy by an integrating sphere and optical fibers for quantifying and discriminating the adulteration of extra virgin olive oil from Tuscany. Analytical and Bioanalytical Chemistry 399, 1315–1324.

Mignani A.G., Ciaccheri L., Mencaglia A.A., & Cimato A. (2012) In Olive oil – constituents, quality, health properties and bioconversions, ed. D. Boskou. InTech Europe, Rijeka, Croatia, 50–53.

Mignani A.G., Ciaccheri L., Cucci C., Mencaglia A.A., Cimato A. Attilio A., Ottevaere H., Thienpont H., Paolesse R., Mastroianni M., Monti D., Gerevini G., Buonocore G., Del Nobile M.A., Mentana A., Grimaldi M.F., Dall Asta D., Faccini A., & Galaverna G.D. (2013) A. EAT-by-LIGHT fiber-optic: and micro-optic devices for food quality and safety assessment. Sensors Journal IEEE, 1342–1354.

Nawrocka A., & Lamorska J. (2013) Determination of food quality by using spectroscopic methods. In: Advances in agrophysical research, ed. S. Grundas & A. Stepniewski. Open Access Books, Rijeka, Croatia, 347–367.

Ozaki Y., McClure W.F., & Christy A.A. (Eds.) (2006) A near-infrared spectroscopy in food science and technology. J. Wiley & Sons. Inc., Hoboken, NJ.

Papoti V.T., & Tsimidou M.Z. (2009) Looking through the qualities of a fluorimetric assay for the total phenol content estimation in virgin olive oil, olive fruit or leaf polar extract. Food Chemistry 112, 246–252.

Psomiadou E., & Tsimidou M. (1999) On the role of squalene in olive oil stability. Journal of Agriculture and Food Chemistry 47, 4025–4032.

Psomiadou E., & Tsimidou, M. (2001) Pigments in Greek virgin olive oils: occurrence and levels. Journal of the Science of Food and Agriculture 81, 640–647.

Puerto D.A., Gila D.M., García J.G., & Ortega J.G. (2015) Sorting olive batches for the milling process using. Image Processing Sensors 15, 15738–15754.

Román I.P., Grané N., Prats S., & Martín M.L. (2012) Kinetic study of olive oil degradation monitored by Fourier transform infrared spectrometry: application to oil characterization. Journal of Agriculture and Food Chemistry 60, 11800–11810.

Rohman A., & Che Man, Y.B. (2012) Quantification and classification of corn and sunflower oils as adulterants in olive oil using chemometrics and FTIR spectra. Scientific World Journal, 1–6.

Sánchez J.A.C., Moreda W., & García J.M.J. (2013) Rapid determination of olive oil oxidative stability and its major quality parameters using Vis/NIR transmittance spectroscopy. Journal of Agriculture and Food Chemistry 61, 8056–8062.

Sikorska E, Khmelinskii I., & Sikorski M. (2012) Analysis of olive oils by fluorescence spectroscopy: methods and applications. In: Olive oil – constituents, quality, health properties and bioconversions, ed. D. Boskou. InTech, Rijeka, Croatia.

Sinelli N., Cosio M.S., Gigliotti C., & Casiraghi E. (2007) Preliminary study on application of mid infrared spectroscopy for the evaluation of the virgin olive oil "freshness." Analytica Chimica Acta 598, 128–134.

Sinelli N., Casale M., Di Egidio V., Oliveri P., Bassi D., Tura D., & Casiraghi E. (2010) Varietal discrimination of extra virgin olive oils by near and mid infrared spectroscopy (NIR, MIR). Food Research International 43, 2126–2131.

Szlyk E., Szydlowska-Czerniak A., & Kowalczyk-Marzek A. (2005) NIR spectroscopy and partial least-squares regression for determination of natural α-tocopherol in vegetable oils. Journal of Agriculture and Food Chemistry 53, 6980–6987.

Tapp H.S., Defernez M., & Kemsley, E.K. (2003) FTIR spectroscopy and multivariate analysis can distinguish the geographic origin of extra virgin olive oils. Journal of Agriculture and Food Chemistry 51, 6110–6115.

Tothill I. (Ed.) (2003) Rapid and on-line instrumentation for food quality assurance. CRC Publications, Cambridge.

Van Poppel G., & Goldbohm R.A. (1995) Epidemiologic evidence for beta-carotene and cancer prevention. American Journal of Clinical Nutrition 62, 1393S–1402S.

Viola P., & Viola M. (2009) Virgin olive oil as a fundamental nutritional component and skin protector. Clinics in Dermatology 27, 159–165.

Wesley I.J., Barnes R.J., & McGill A.E.J. (1995) Measurement of adulteration of olive oils by near-infrared spectroscopy. Journal of the American Oils Chemists' Society 72, 289–292.

Yamauchi R., & Matsushita S. (1977) Quenching effect of tocopherols on methyl linoleate photoxidation and their oxidation products. Agricultural and Biological Chemistry 41, 1425–1430.

12 Oxidative stability and the role of minor and functional components of olive oil

Giuseppe Fregapane and María Desamparados Salvador

12.1 Introduction

Lipid oxidation occurs slowly at room temperature; nevertheless, it is the main cause of quality deterioration of olive oil and determines its shelf life. The main components of olive oil are triacylglycerols, the unsaturated fatty acids of which are susceptible to autoxidation; however, it also contains minor compounds with antioxidant activities that provide resistance to oxidative degradation. Furthermore, the quality of olive oil, mainly its sensory characteristics and nutritional value, is directly related to its content in minor components, mainly phenolics and volatile compounds, and therefore to their stability during the market period.

In this chapter, the influence and the behavior of olive oil natural antioxidants and minor functional components with regard to the oxidative stability and quality of the oil during storage and shelf life are reported and discussed.

12.2 Olive oil oxidative stability

The stability of olive oil (OO) usually ranges from 9 to more than 18 months; and hence, mainly because of the long time (12–24 months) that such studies require, only a limited number of articles deal with the study of the evolution of the quality parameters and shelf life of OO, either during storage in oil mill tanks (Di Giovacchino *et al.*, 2002), in glass or plastic bottles (Leonardis de & Macciola, 1998; Cinquanta *et al.*, 2001; Gutiérrez & Fernandez, 2002; Okogeri & Tasioula-Margari, 2002; Gómez-Alonso *et al.*, 2007; Silva et al., 2015), or under commercial display conditions (Pagliarini *et al.*, 2000; Zanoni *et al.*, 2005; Fregapane *et al.*, 2013). Results from these studies have contributed to a better understanding of the autoxidation process; however, they are not conclusive, as of yet, and the prediction of OO shelf life and the exact role and behavior of its natural antioxidants is still a prime research goal.

A study by Fregapane *et al.* (2013) on the stability of commercial Spanish virgin olive oils (VOO) and OO under normal market conditions (closed bottle at room temperature) showed that only a slight increase in the values of the main oxidation indices (peroxide value [PV], and UV characteristics K_{232} and K_{270}; established in European regulation 2568/91 and later amendments; EU, 2013) was observed after one year in the marketplace, showing the expected low oxidation rate and therefore high stability of the oils. Indeed, with a low monthly increase in the PV (between 0.017 and 0.33 meq/kg/month) as well as in the free acidity (0.003–0.016%/month), an index of hydrolysis was found.

The most basic and fundamental concepts related to OO stability during storage are summarized in Table 12.1.

In order to be able to measure significant differences in the OO oxidation rate, as well as in the stability of its minor components, it is generally necessary to accelerate the autoxidation process by increasing the temperature or the availability of oxygen, and therefore several studies are carried out in open bottle (OB),

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Table 12.1 Key points on olive oil oxidative stability during storage.

Olive oil oxidative stability

- Lipid oxidation occurs slowly at room temperature; nevertheless, it is the main cause of olive oil quality deterioration.
- The stability or shelf life of olive oil under appropriate storage conditions usually ranges from 9 to over 18 months.
- The quality and oxidation indices, PV, K_{232}, and K_{270}, increase linearly during storage (Figure 12.1) at room temperature (25 °C).
- Relevant positive effects of minor components of olive oil on oxidative stability are observed (Figure 12.1).
- The shelf life of olive oil is considerably extended at reduced storage temperature (i.e., at 15 °C).
- K_{232} is often the first oxidation index to exceed the upper limit for commercial-grade olive oil.
- Oxidation rate during storage at room temperature is not clearly related to the initial oxidation level of the oil nor to the initial concentration of its natural antioxidants.

Accelerated oxidative assays and shelf life prediction

- A good relationship exists between the oxidative stability by accelerated oxidation tests and the initial content of phenolic compounds (Figure 12.2).
- Accelerated assays have a drawback in that the oxidation process takes place under drastic conditions, and therefore its mechanism is different from that under normal storage conditions.
- No correlation was found between the shelf life at room temperature and Rancimat oxidative stability.

Stability of olive oil minor components

- Increase in simple phenolics (hydroxytyrosol and tyrosol) is observed during storage due to the degradation of their complex derivatives (secoiridoids) and may be used as freshness or aging indices of olive oil during its shelf life.
- The degradation of secoiridoid phenolics during storage fits a pseudo-first-order kinetics (Figure 12.3).
- A linear relationship exists between phenolic degradation rate and its initial concentrations. Therefore, the effect of olive oil cultivar on the stability of phenolic compounds may be explained by their different initial concentrations.
- The effect of storage conditions on the stability of phenolic compounds is of great interest with regard to overall oil quality and the health claim of olive oil polyphenols.

Antioxidant capacity of olive oil functional components

- Hydroxytyrosol possesses a strong antioxidant effect (Figure 12.4), similar to its secoiridoid derivatives.
- Prooxidant behavior of α-tocopherol is observed at room temperature.

to avoid the reduction in the oxidation rate due to the oxygen limitation in closed bottle (CB). As expected, oxidation rates are higher in OB compared to CB conditions. For instance, almost three times higher PV, based on the oxidation rate in OB (1.073 meq O_2/kg/week) compared to CB (0.385 meq O_2/kg/week), was found in VOO stored at 25 °C (Krichene *et al.*, 2015).

Another research paper examined the evolution of major and minor components and oxidation indices of several samples of VOO that significantly differ in their initial contents of natural antioxidants, during 21 months of storage at 25 °C in darkness in open amber glass bottles (inner diameter: 4.2 cm; surface area exposed to the atmosphere: 13.85 cm^2; Gómez-Alonso *et al.*, 2007). It also looked at the relationship between VOO initial composition and the time required to reach the upper limits established by European regulations for the OO quality parameters. The European regulation 2568/91 and subsequent amendments (EU, 2013) have set maximum values for PV, K_{232}, and K_{270} for VOO to qualify for the extra virgin category at 20 meq/kg, 2.5, and 0.22, respectively; exceeding these limits produces a depreciation of the commercial grade of the VOO. At room temperature, the quality and oxidation indices, PV, K_{232}, and K_{270}, increased linearly during a storage period of almost two years under OB conditions studied, as shown by the regression coefficients found (R^2> 0.95; Gómez-Alonso *et al.*, 2007). Based on these findings, it should therefore be possible to extrapolate and predict the shelf life of OO from results recorded over a relatively short period of storage (i.e., a matter of weeks).

In order to determine whether the oxidation rate during storage is related to the initial oxidation level of the oil, the correlation between the rate of increase of PV and its initial value in the oil was examined.

However, the results showed that there was no statistically significant correlation between these parameters ($R^2 = 0.357$, p = 0.156). Moreover, the evolution of PV during storage showed no clear correlation with the initial Rancimat induction period (IP) of these oils ($p = 0.905$), as also reported by Cinquanta *et al.* (2001), with their phenolic ($p = 0.422$) or α-tocopherol ($p = 0.303$) contents. For example, VOO with very different phenolic compound levels exhibited very similar oxidation rates (Gómez-Alonso *et al.*, 2007): 0.086 and 0.094 meq/kg/week for oils containing 3.88 and 1.08 mmol/kg of total phenolics, respectively.

The behavior observed for the K_{232} index is very similar to that found for PV, both indices of primary oxidation products, in that it followed a pseudo-zero-order kinetic ($R^2 < 0.95$) and there was no simple correlation between the evolution of this index and the initial K_{232} values, Rancimat IP, or natural antioxidant content. As expected, the rate of increase of these two indices, K_{232} and PV, correlated directly ($R^2 = 0.764$; $p < 0.05$). However, unlike PV, K_{232} was often the first index to exceed the upper limit established by the European regulations for VOO, and therefore it should be used to follow the progress of the oxidation and to establish or predict the shelf life of OO (Gómez-Alonso *et al.*, 2007).

The storage conditions of bottled or tank-stored VOO, as well as all the agronomical and technological variables of the processing stages, are particularly relevant for preserving the highly valued organoleptic quality of this product. All the efforts made in the olive grove and in the oil mill to produce VOO with good sensory characteristics can be undermined if improper storage conditions are used. The oxidation process during storage is known and involves changes to both the major and minor components of VOO. To slow down the oxidation rate during storage, certain factors such as the presence of oxygen and traces of metals, exposure to light, as well as storage temperature and time must be kept under control (Frankel, 2005).

It is therefore of primary interest to know more about the variables that affect the oxidative stability and consequently the shelf life of the OO. This would make it possible to delay the oxidation reaction and increase the stability of the oil and its functional components.

12.2.1 Effect of oxygen availability on oil stability

As already mentioned, reduction in the partial pressure of the oxygen reduces the oxidation rate. Indeed, OO tanks in oil mills and the commercial products are stored in closed containers, with the addition in some cases of an inert gas (nitrogen or argon), since it is established that under these limited oxygen availability conditions the oil oxidation is reduced (Di Giovacchino *et al.*, 2002; Pristouri *et al.*, 2010; Mancebo-Campos *et al.*, 2014).

12.2.2 Effect of oil composition: fatty acids and natural antioxidants

Edible vegetable oils mainly contain triacylglycerols (TAGs), the unsaturated fatty acids (UFAs) of which are susceptible to autoxidation, and the degree of their unsaturation greatly decreases their stability (Frankel, 2005). The high monounsaturated-to-polyunsaturated fatty acid ratio, which is typical of OO, is one of the main reasons for better stability of OO compared to other vegetable edible oils. However, this ratio varies widely according to olive cultivar.

The relative rate of oxidation of oleate, linoleate, and linolenate is reported to be 1, 40–50, and 100, respectively (Frankel, 2005), and it is suggested that the oxidative susceptibility of the oil shall be evaluated by means of a formula that includes the contents of these fatty acids (Cert *et al.*, 1996). Nevertheless, edible vegetable oils also contain minor compounds with antioxidant activities, mainly α-tocopherol and phenolic compounds, that provide resistance to oxidative degradation (Vázquez-Roncero *et al.*, 1975; Blekas *et al.*, 2002; Servili & Montedoro, 2002; Mateos *et al.*, 2005; Lee *et al.*, 2007), although they are increasing the complexity of the oxidation process, thus making prediction and mathematical modeling more difficult. VOO is characterized by a high natural content of minor compounds with antioxidant activity, consisting mainly of tocopherols and phenolic compounds, which greatly improve its stability during the market period (Velasco & Dobarganes, 2002).

Although α-tocopherol is considered to be the most relevant antioxidant in vegetable oils, as well as in the protection of the lipid structures *in vivo*, several researchers have reported a lower antioxidant activity than

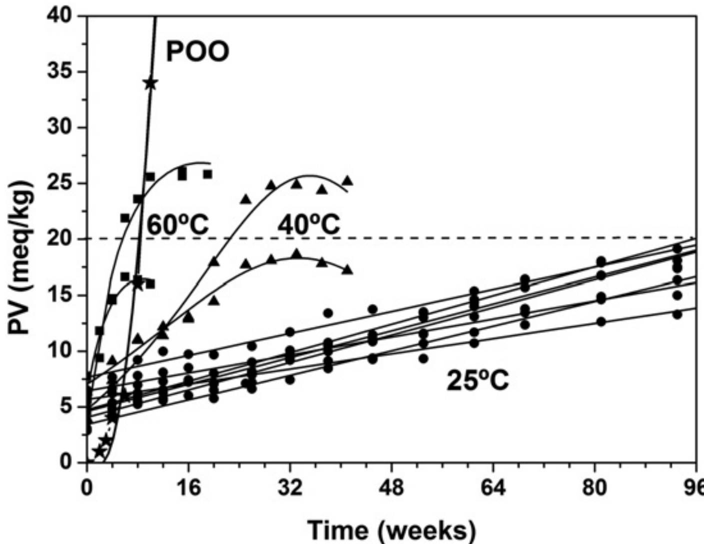

Figure 12.1 Evolution of oxidation (PV) during storage at different temperatures (25–60 °C) for virgin and purified olive oil (POO).

the simple phenol hydroxytyrosol (Le Tutour & Guedon, 1992; Baldioli *et al.*, 1996; Mancebo-Campos *et al.*, 2014). This may be explained by the "antioxidant polar paradox," which states that hydrophilic antioxidants are often less effective in oil-in-water emulsions than lipophilic antioxidants, whereas lipophilic antioxidants are less effective in bulk oils than hydrophilic antioxidants (Porter *et al.*, 1989; Frankel *et al.*, 1994). Limitations of the polar paradox have recently been pointed out by Zhong and Shahidi (2012); the effect of concentration on validity of the polar paradox theory was investigated using polar and nonpolar representative antioxidants in bulk oil, and a model on antioxidant behavior in response to their polarity is proposed.

The relationship between the content of phenolic compounds in VOO and its oxidative stability measured by accelerated assays has been well known for a long time (Vázquez-Roncero *et al.*, 1973; Gutiérrez *et al.*, 1977; Gutfinger, 1981; Montedoro *et al.*, 1992; Baldioli *et al.*, 1996; Litridou *et al.*, 1997; Salvador *et al.*, 1999, 2001). In the case of hydroxytyrosol and its secoiridoid derivatives, which show a similar antioxidant activity (Baldioli *et al.*, 1996; Carrasco-Pancorbo *et al.*, 2005; Krichene *et al.*, 2010, 2015), the formation of a stable radical has been proposed to explain their antioxidant activity (Visioli & Galli, 1998). In contrast, tyrosol and its derivatives show a very low or no antioxidant activity (Baldioli *et al.*, 1996; Carrasco-Pancorbo *et al.*, 2005; Krichene *et al.*, 2015), due to the absence of an electron donor group that has a high transition energy and a limitation in the formation of the phenoxyl radical.

The significant effect of minor OO compounds on oxidative stability is clearly shown in Figure 12.1, in which the time required to reach the same oxidation level (PV = 20, upper limit established by European regulations) in purified olive oil (POO) was less than seven weeks, whereas it was between one year 10 months and three years for the very stable and phenolic-rich OO variety studied (cv. 'Cornicabra'; Gómez-Alonso *et al.*, 2007). The relationship between the content and profile of natural antioxidants and the oxidative stability of OO is going to be developed and discussed throughout this chapter.

The main factors that affect OO composition, namely, fatty acid profile and natural antioxidant content, are mainly genetically related to the olive cultivar (Gómez-Rico *et al.*, 2008); the effects of environmental and technological factors are generally less important. The effect of the olive cultivar is noteworthy, but as previously reported (Mateos *et al.*, 2001), no clear relationship between composition (mainly, the fatty acid profile and natural antioxidant content) and initial oxidation rate could be established.

12.2.3 Effect of storage temperature

Storage temperature is one of the most important variables that affect the stability of OO; its effect on the progress of the oxidation is depicted in Figure 12.1. As already mentioned, the oxidation indices PV, K_{232}, and K_{270} increase linearly during storage at 25 °C (Gómez-Alonso *et al.*, 2007). Maximum values for PV, K_{232}, and K_{270} for VOO to qualify for the extra virgin category of 20 meq/kg, 2.5, and 0.22, respectively, are set by European regulation 2568/91 and its following amendments (EU, 2013). On the contrary, at higher temperatures (40, 50, and 60 °C), a stationary phase is observed after a few weeks of storage (Figure 12.1).

During the early storage, before reaching the plateau, PV and K_{232} follow an apparent pseudo-zero-order kinetics ($R^2 > 0.951$) at all temperatures (25–60 °C) and for all OO studied (Calligaris *et al.*, 2006; Gómez-Alonso *et al.*, 2007). It is worth noting that the behavior of the PV was very similar in all oils, regardless of differences in initial antioxidant content, except at the highest temperature, 60 °C; in this case, the stationary phase was reached later in samples with higher initial antioxidant content.

The time required to reach the upper limit (TRUL) represents the storage time needed to lose the maximum VOO commercial category due to oxidation. The upper limit for PV is not reached by any OO studied at 25 °C, nor by several oils at 40 °C or even at 50 or 60 °C. In all cases, stabilization occurred below or slightly above this limit (Figure 12.1). The fact that this limiting value for peroxides was not reached after 21 months of storage in open bottles at 25 °C or even under more drastic conditions of storage temperature (40, 50, and 60 °C) could mean that it is not a good reference value to assess the quality of OO, especially when it has been stored for a certain period of time. It is known that peroxides decompose at advanced oxidation stages (Frankel, 2005); while the PV decreases, oxidation and hence quality depreciation carry on.

It is very relevant to establish the relationship between storage temperature and OO oxidation rate since oxidative stability may in principle be defined in terms of a rate constant (k), defining lipid matrix degradation or oxidation product formation, and temperature (T), which is one of the most important factors affecting oxidation in Accelerated Shelf-Life Testing (ASLT). Regression analyses employing an Ln k versus 1/T equation indicate that the temperature dependence of oxidation indices (PV, K_{232}, and K_{270}) or UFA rates are well described by the linear Arrhenius model between 25 and 60 °C ($0.960 < R^2 < 0.999$, $p < 0.05$) (Mancebo-Campos *et al.*, 2008).

The apparent activation energy (Ea) calculated from the increase in primary oxidation products indices or UFA degradation data was comparable for all samples; mean values were 64.8 ± 2.8 kJ/mol for PV, 69.6 ± 4.9 kJ/mol for K_{232}, and 67.6 ± 5.4 kJ/mol for UFAs. The similarity of these values supports the assumption that degradation of UFAs leads to peroxide and conjugated dienes formation. A higher activation energy (mean 77.1 ± 4.4 kJ/mol) was calculated for the secondary oxidation products index K_{270}. As regards oxidizing substrates, the variation between samples was higher in the case of oleic acid (76.3 ± 22.9 kJ/mol). The experimental values obtained in this study (Mancebo-Campos *et al.*, 2008) were consistent with earlier data that report Ea values for lipid oxidation from 24 to 240 kJ/mol (Frankel, 1993, 2005; Tan *et al.*, 2001). It is worth noting that the activation energy for the formation of primary and secondary oxidation products was twice the activation energy for PV and K_{270} determined in purified olive oil (32.1 and 42.2 kJ/mol, respectively; Gómez-Alonso *et al.*, 2004). The action of antioxidants as peroxyl radical scavengers delays the propagation phase of lipid oxidation, so that more energy would be needed for the formation and decomposition of hydroperoxides.

Another useful way of expressing the oxidation reaction rate temperature dependence is the Q_{10} factor, defined as the ratio of the shelf life at two storage temperatures differing by 10 °C. According to the Ln k versus T equation, plots resulted in linear correlations ($0.948 < r^2 < 0.998$, $p < 0.05$) for the PV, K_{232}, and K_{270} indices (Mancebo-Campos *et al.*, 2008). Based on these results on the negative effect of increase in the storage temperature on OO stability, it is of great interest to study the beneficial effect of a lower storage temperature on the shelf life of OO and its functional components. Recent research showed that the initial oxidation rate indeed decreased almost five times when the storage temperature was decreased from 1.542 meq O_2/kg/week at 25 °C down to 0.313 meq O_2/kg/week at 15 °C (Krichene *et al.*, 2015). As a consequence, the shelf life of OO considerably increased at a reduced storage temperature. For example, the shelf life of a monocultivar OO in CB (based on PV = 20) increased from 48 weeks at 25 °C to 57 weeks at 15 °C, due to a reduction of 22% in its oxidation rate, from 0.229 meq O_2/kg/week to 0.188 meq O_2/kg/week. The increased shelf life of another VOO variety in CB (at PV = 20) was even greater: from 26 weeks at 25 °C to 44 weeks at 15 °C, associated with a reduction of 36% in its oxidation rate, from 0.156 meq/

kg/week at 25 °C to 0.115 meq/kg/week at 15 °C. In storage at 5 °C, none of the different monocultivar VOOs studied reached PV > 20 during the 72-week experimental period (Krichene *et al.*, 2015).

12.2.4 Effects of filtration and moisture content

The results of some studies have shown a gradual loss in stability during the storage of filtered oils mainly due to a significant decrease in the phenolic components (Tsimidou *et al.*, 2005; Gómez-Caravaca *et al.*, 2007; Lozano *et al.*, 2010). At the same time, it has also been reported that filtration and dehydration decrease the hydrolysis rate of the TAG matrix, especially during storage at the higher temperature (40 °C) and in oils with a higher initial free acidity (e.g., free acidity > 0.6%; Fregapane *et al.*, 2006). Moreover, the formation rate of simple phenols due to hydrolysis of their secoiridoid derivatives was also greater in unfiltered olive oils. Thus, from this point of view, filtration and especially dehydration could help prolong the shelf life of some high-quality but less stable VOOs (e.g., the 'Arbequina' and 'Colombaia' cultivars).

12.3 Accelerated oxidative assays and shelf-life prediction

The stability of OO usually ranges up to more than 1 year and a half, and hence accelerated methods are generally employed to estimate the induction period of the oxidation reaction in a relatively short period of time (Frankel, 2005). Indeed, as mentioned in this chapter, the average PV trend observed at 25 °C for VOO (Figure 12.1) is a straight line ascending to the established endpoint (20 meq of O_2/kg, the maximum value established for the extra virgin category by European regulations) in 90–160 weeks (Gómez-Alonso *et al.*, 2007). However, under accelerated oxidative Rancimat conditions, VOO reached this PV value in just 28–56 h (Mancebo-Campos *et al.*, 2007). Furthermore, a reliable and standard Oxidative Stability Index (OSI) should be one of the most important quality markers of edible oils.

Until now, OO stability has commonly been evaluated by accelerated methods employing relatively high temperatures (100–120 °C) and an air flow (10–20 L/h) supply (e.g., Rancimat or the active oxygen method [AOM]) (Baldioli *et al.*, 1996; Aparicio *et al.*, 1999; Koski *et al.*, 2002; Mateos *et al.*, 2003, 2005; Abaza *et al.*, 2005). These rapid tests are very useful for practical purposes and give a good correlation with concentrations of phenolic compounds in OO (Gutfinger, 1981; Salvador *et al.*, 2001; Gómez-Alonso *et al.*, 2002), but OSI is not recognized as a legal parameter due to the unsatisfactory relationship between the results of these accelerated assays and the real shelf life of such food products (Kaya *et al.*, 1993; Velasco & Dobarganes, 2002; Frankel, 2005; Gómez-Alonso *et al.*, 2007). This is probably because the oxidation mechanism is significantly different at temperatures above 60 °C; moreover, at higher temperatures, the oxidation reaction becomes more dependent on oxygen concentration because oxygen solubility decreases. As a result, the oxidation process under these accelerated conditions causes the formation of oxidation products, in particular volatile compounds (i.e., volatile acids), which are not produced in significant amounts under normal storage conditions (Frankel, 2005).

It is well documented that there is a good relationship between the oxidative stability of VOO, determined using accelerated oxidation tests (i.e., AOM or Rancimat), and its initial content of natural antioxidants, especially phenolic compounds (i.e., total polyphenols, o-diphenols, or oleosidic forms of hydroxytyrosol) (Gutfinger, 1981; Salvador *et al.*, 1999, 2001; Gutiérrez *et al.*, 2001; Gómez-Alonso, *et al.*, 2002), as shown in Figure 12.2.

In Figure 12.2, the relationship between oxidative stability, measured by Rancimat, and the content in total phenolic compounds (by Folin-Ciocalteu) and the hydroxytyrosol and its secoiridoids derivatives (o-diphenols by high-performance liquid chromatography [HPLC]) is reported. While a linear relationship is observed in the first case ($R^2 = 0.885$), a polynomial second-degree equation ($R^2 = 0.937$) better fits the nonlinear correlation with hydroxytyrosol and its secoiridoid derivatives, also known as o-diphenols. As discussed above, accelerated assays have a drawback in that the oxidation process takes place under drastic conditions, quite unlike those typically occurring in oil mill storage tanks or even during the marketing of the product. As a consequence, the methods selected to determine the endpoint of the stability assays and the changes observed in the oil have no satisfactory correlation with the autoxidation process that takes place at room temperature (Velasco & Dobarganes, 2002; Frankel, 2005; Mancebo-Campos *et al.*, 2007), or hence with VOO shelf life.

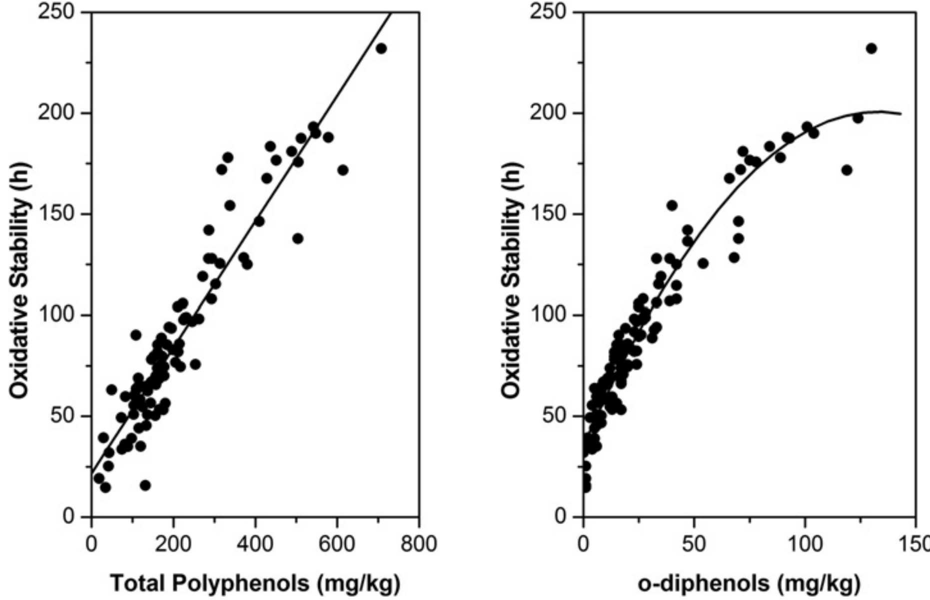

Figure 12.2 Relationship between oxidative stability and the content of phenolic compounds.

The analysis of the evolution of the oxidation indices, for example PV in the VOO and POO samples in the course of oxidation under Rancimat conditions (100 °C and 10 L/h air flow), shows that PV and K_{232} are both suitable indices for monitoring the progress of oxidation and for determining the IP in VOOs under Rancimat conditions, rising linearly until the Rancimat assay endpoint, when a sudden increment was observed. In VOO, the PV increased linearly up to about 80–90% of the IP following a pseudo-zero-order kinetic (R^2 > 0.964; Gutiérrez *et al.*, 2002; Gómez-Alonso *et al.*, 2007; Mancebo-Campos *et al.*, 2007). In contrast, the PV increase for POO was considerably greater from the very first (Gómez-Alonso *et al.*, 2004), fitting a second-grade polynomial equation (R^2 > 0.999). Under these accelerated oxidation conditions, both primary and secondary oxidation indices K_{232}, K_{270}, and p-anisidine value followed a similar trend (Mancebo-Campos *et al.*, 2007).

Moreover, under the Rancimat test conditions, the UV characteristic K_{270}, a marker of secondary oxidation products, was the first oxidation index to reach the legal limit established for the extra virgin olive oil (EVOO) category (European Regulation no. 2568/91 and later amendments, EU, 2013), whereas at 25 °C it was K_{232}, an index of primary oxidation products (Mancebo-Campos *et al.*, 2007). This means that under these accelerated conditions (100 °C and 10 L/h air flow), oxidation was very fast, and hydroperoxides decomposed to secondary oxidation products much more quickly than at 25 °C.

Based on these findings, no correlation was found between the stability at room temperature, measured as the time taken by the first oxidation index to reach the legal upper limit (often K_{232}, as previously mentioned), and Rancimat oxidative stability (R^2 = 0.000005) in several comprehensively studied VOOs (Gómez-Alonso *et al.*, 2007; Mancebo-Campos *et al.*, 2007). A similar plot was obtained using the time taken to reach PV = 20 as the endpoint of the assay (R^2 = 0.15). This means that the Rancimat test cannot be used to predict the real shelf life of OO, probably because of the different oxidation mechanisms that take place under these diverse conditions. A better correlation, although not yet satisfactory (R^2 = 0.55, p = 0.01), was found between stability at 25 °C and under the Rancimat conditions, using the time required to reach PV = 20 as endpoint in both cases. It could be helpful to investigate this matter further to see whether the Rancimat assay could be modified to provide more relevant information regarding the stability and shelf life of OO under normal storage conditions.

It should therefore be very relevant to fully understand the meaning and usefulness of the Rancimat IP and of the good relationship with the content of phenolic compounds. It may be thought that it could be just an index for measuring the content of antioxidants, since under accelerated oxidative conditions, at close to

or higher than 100 °C and using air flow, the phenolic compounds are quickly destroyed, and hence, when they are almost completely used, a very fast increase in slope, characteristic of the IP, is noted. Although such rapid assays are very useful in measuring the relative OSI for olive oils and other edible oils, they also provide a good correlation with the content of phenolic compounds; however, they cannot be used to extrapolate the shelf life of such food products because this correlation has not been confirmed at room temperature (Cinquanta *et al.*, 2001; Gómez-Alonso *et al.*, 2007).

12.3.1 Shelf-life prediction from accelerated stability testing

Although OO can be consumed even years after production without risk to health, its prized sensory and nutritional properties are significantly affected by oxidation, and that is the main cause of quality depreciation. Thus, from commercial and economic standpoints, it should be most useful to be able to predict the stability or shelf life of VOO by means of an accelerated test that can be performed at a mild temperature.

As already discussed, stability or shelf life of OO may be defined and based on the TRUL established by the European regulation for the EVOO category. Research showed that the experimental or extrapolated TRUL values for PV, K_{232}, and K_{270} correlate satisfactorily with temperature (T) by a potential equation, $TRUL = aT^b$ (Mancebo-Campos *et al.*, 2008). On the basis of these experimental results, it can be concluded that it may be feasible to perform an accelerated stability test at a temperature below 60 °C to estimate the shelf life under normal storage temperature conditions. Moreover, it is suggested that K_{232} is the best normalized index for oxidation monitoring in that (a) it is a good marker of primary oxidation products; (b) it is the first normalized index to reach the regulation limits at all the temperatures studied; (c) it presents high linearity in the early stages of oxidation, making it possible to calculate a rate of oxidation from which the shelf life can be estimated; (d) it presents the best correlation with loss of UFA; and finally (e) it is simple to determine, the procedure is highly repeatable and reproducible, and it presents no interference problems with other natural compounds in olive oil (e.g., phenolic compounds, which absorb at 280 nm and can interfere with the parameter K_{270}).

Q_{10} factors (the ratio of the shelf life at two storage temperatures differing by 10 °C) obtained from experimental TRUL values are similar for all OO, as shown by their mean values of 2.10 ± 0.2 for PV, 2.07 ± 0.4 for K_{232}, and 2.51 ± 0.6 for K_{270}. This means that an increase of 10 °C in the experimental temperature reduces the time taken by the test by more than half. For instance, a shelf life of one week at 50 °C is equivalent to 15 weeks of storage at 25 °C (Mancebo-Campos *et al.*, 2008).

12.4 Stability of olive oil components: fatty acids and minor components

12.4.1 Changes in fatty acid profile

The fatty acid profile and the relationship between monounsaturated, polyunsaturated, and saturated fatty acids determine the nutritional quality of a fat substance, apart from its relevant role in oxidative stability, as already explained (Frankel, 2005). Few changes are observed in the UFA composition during a long-term storage period under normal conditions (Gomez-Alonso *et al.*, 2007). This is due to the levels of antioxidant compounds and the mild storage conditions, which reduced the oxidation in the oils, as already discussed. After almost two years of storage, there were no detectable changes in oleic acid, the main fatty acid in OO. The changes observed in the polyunsaturated fatty acids (PUFAs), linoleic and linolenic acids, are higher as expected, ranging between 2.1 and 3.8% for linoleic acid and between 5.8 and 10.0% for linolenic acid (Gomez-Alonso *et al.*, 2007).

12.4.2 Changes in minor compounds

The stability of OO natural antioxidants and functional components is of great relevance regarding the overall quality and the nutritional value of the product during its shelf life. The most important tocopherol in OO is by far α-tocopherol (e.g., Cunha *et al.*, 2006); therefore, the other isomers are not generally investigated. A wide range of α-tocopherol content has been observed in OO (50–350 mg/kg) from different olive varieties

and geographical areas (Boskou, 2006). Only small differences are observed at the beginning of the market period in the α-tocopherol (vitamin E) content in different commercial VOOs and OOs (Fregapane *et al.*, 2013), since it is allowed to add vitamin E to refined oils to recover losses after the refining process and therefore improve oil stability. However, the α-tocopherol content falls significantly after approximately one year of storage, and the loss in vitamin E is about 25–30% of its initial content (Fregapane *et al.*, 2013). In contrast, the decrease in total phenols and o-diphenols throughout the market period is generally statistically insignificant (Fregapane *et al.*, 2013).

12.4.2.1 Behavior of simple phenolics

A significant increase in simple phenolics (hydroxytyrosol and tyrosol) is generally observed during storage due to the degradation of their complex derivatives (Fregapane *et al.*, 2006, 2013; Lavelli *et al.*, 2006; Krichene *et al.*, 2010, 2015). An average increment of 33% in their concentration is observed, with an interquartile range between 20 and 50% (as the 25th and 75th percentiles). Moreover, the complex–simple phenolics ratio decreased in all cases after a one-year market period, with 29% as the median value (between 15 and 33%, as the 25th and 75th percentiles). The behavior of simple phenolics was similar and clearly related to the storage temperature and to the initial concentration of their complex forms that determined the rate of the degradation of their complex derivatives (Krichene *et al.*, 2015).

A plateau is apparently reached, probably because these compounds are also degraded during storage. Indeed, at 50 °C, a decrease in hydroxytyrosol was clearly visible in all VOO varieties studied after reaching a maximum value (Fregapane *et al.*, 2006; Krichene *et al.*, 2010, 2015). This behavior may be explained if we consider the difference in the hydroxytyrosol formation rate, due to the hydrolysis of the secoiridoid derivatives, and the disappearance rate, due to the oxidation and nonoxidative degradation of this compound. This is in fact an interesting question that still needs to be fully addressed, since further research is required to understand the mechanisms of hydrolysis and oxidation of complex and simple phenols.

The increase in the content of simple phenolics, the decrease in their secoiridoid derivatives, and the ratio of simple to secoiridoid phenolics can be used as indices of the oxidative and hydrolytic degradation of VOO phenolics (Fregapane *et al.*, 2006; Krichene *et al.*, 2015). They may also be used as a freshness or aging index of the olive oil during its shelf life. However, the relationship between complex and simple phenolic compounds also depends on the olive variety or blend and the processing conditions. The tyrosol formation rate is generally significantly lower than that of hydroxytyrosol during storage (Lavelli *et al.*, 2006; Krichene *et al.*, 2015).

12.4.2.2 Behavior of complex phenolics

The two most relevant families of complex (secoiridoid) phenolics found in olive oil deserve a more detailed study: hydroxytyrosol secoiridoid derivatives, namely, the dialdehydic and aldehydic forms of elenolic acid linked to hydroxytyrosol (DOA and AOA, also known as 3,4-DHPEA-EDA and 3,4-DHPEA-EA, respectively, or simply o-diphenols); and tyrosol secoiridoid derivatives, which are the dialdehydic and aldehydic forms of elenolic acid linked to tyrosol (DLA and ALA, aka p-HPEA-EDA and p-HPEA-EA, respectively).

Several recent studies have assessed the degradation of VOO phenolic compounds under different storage conditions (Okogeri & Tasioula-Margari, 2002; Rastrelli *et al*., 2002; Lavelli *et al*., 2006; Migliorini *et al*., 2013; Li *et al*., 2014) or different cooking and accelerated-heating conditions (Brenes *et al*., 2002; Gómez-Alonso *et al*., 2003; Carrasco-Pancorbo *et al*., 2007; Daskalaki *et al*., 2009). However, the effect of a lower storage temperature (10–15 °C) until recently has not yet been investigated (Krichene *et al.*, 2015).

The stability of VOO phenolic compounds during long-term storage (18 months) at different temperatures (5, 15, and 25 °C) to verify the advantage of storing VOO at a temperature lower than the usual commercial conditions (20–25 °C) (Krichene *et al.*, 2015) was the purpose of a recent study. Storage at 50 °C was also used to reveal the major changes that phenolic compounds underwent in these conditions. The degradation of secoiridoid phenolics during storage displayed pseudo-first-order kinetics and depended on the initial content of phenolics related to olive oil variety. The initial degradation rate was similar at 5 and 15 °C but increased considerably at 25 °C and was even faster at 50 °C. The shelf life of the studied VOO was considerably extended at a reduced storage temperature (15 vs. 25 °C).

The European Union Commission Regulation 432/2012 (EU, 2012) establishes a list of permitted health claims made on foods. For olive oil phenolics, the claim "Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress" has been approved, and this claim may be used for olive oil that contains at least 5 mg of hydroxytyrosol and its derivatives per 20 g of olive oil (to the consumer, the beneficial effect is obtained with a daily intake of 20 g of olive oil). As a consequence, storage conditions affect OO phenolic content and therefore the expiry date of the health claim for olive oil polyphenols. Taking into account that not many commercial VOOs or OOs contain more than 250 mg/kg (equivalent to 5 mg/20 g) of the sum of hydroxytyrosol and its derivatives, this minimum required amount was maintained for 10 months at 25 °C but up to 18 months in storage at 15 °C. In another monocultivar OO, the initial content of total phenolics (hydroxytyrosol, tyrosol, and their derivatives) was 317 mg/kg 21; this total amount was reduced to 250 mg/kg in just 4.5 months during storage at 25 °C but maintained up to 7 months at 15 °C (Krichene *et al.*, 2015). The effect of storage temperature on the stability of phenolic compounds is therefore of great interest with regard to the nutritional and biological value of OO, since lower storage temperatures considerably increase the length of time for which the health claim is valid. This finding is particularly relevant for high-value products, often defined as premium or superior VOO.

As expected, an increase in storage temperature speeds up the degradation of phenolic compounds, particularly at 50 °C. For example, the residual content of hydroxytyrosol secoiridoid derivatives after three months of storage in OB was much lower at higher temperatures: 77% at 25 °C, but 28% at 50 °C (Krichene *et al.*, 2015). Similarly, the residual content of tyrosol derivatives after six months was 51% at 25 °C but 29% at 50 °C. Moreover, the stability of phenolic compounds is also higher under CB storage conditions compared to OB, similar to what was observed for OO itself, due to oxygen limitation. However, the found difference is not great, and the general behavior of these two phenolic families is quite similar. For example, the residual content of hydroxytyrosol secoiridoid derivatives after 6 months of storage at 25 °C was, respectively, 63% in CB and 52% in OB; for tyrosol derivatives, it was 85 and 67% in CB and OB, respectively. At a lower storage temperature (15 °C), after 12 months the content of hydroxytyrosol derivatives was maintained at 73% in CB and 62% in OB and tyrosol derivative content remained at 73 and 49% in CB and OB, respectively (Krichene *et al.*, 2015). The stability of the tyrosol secoiridoid compounds appeared to be greater than that of hydroxytyrosol derivatives, especially in CB conditions (Lavelli *et al.*, 2006; Daskalaki *et al.*, 2009; Krichene *et al.*, 2015). Furthermore, the stability of the main phenolic secoiridoid compounds (DOA, AOA, DLA, and ALA) fits well an exponential decay curve ($R^2 > 0.96$).

12.4.2.3 Degradation kinetics

The degradation kinetics of secoiridoid phenolics during storage at different temperatures (85–50 °C) is depicted in Figure 12.3; it fits a pseudo-first-order kinetics equation reasonably well ($r > 0.96$ and $p < 0.005$) at all temperatures for the VOO varieties studied (Kaya *et al.*, 1993; Krichene *et al.*, 2015): $\mathrm{Ln}(Ct/Co) = k \cdot t$, where Co is the initial concentration and Ct is the residual concentration at time t. The stability of tyrosol secoiridoid derivatives is greater than hydroxytyrosol derivatives, as demonstrated by the lower slope of the degradation kinetics, especially at higher storage temperatures (i.e., -0.099 ± 0.005 as compared to -0.068 ± 0.002 for hydroxytyrosol and tyrosol derivatives at 25 °C; or -0.452 ± 0.046 vs. -0.184 ± 0.015 for hydroxytyrosol and tyrosol derivatives at 50 °C in another oil variety) (Kaya *et al.*, 1993; Daskalaki *et al.*, 2009; Krichene *et al.*, 2015).

The initial degradation rate was generally similar at storage temperatures of 5 and 15 °C but increases considerably at 25 °C and much faster at 50 °C. For example, the degradation rate of DOA (the dialdehydic form of elenolic acid linked to hydroxytyrosol) increased from 2.52 μmol/kg/month at 15 °C to 3.97 μmol/kg/month at 25 °C and to 17.5 μmol/kg/month at 50 °C. Similarly, ALA in another VOO variety increased from 0.44 μmol/kg/month and 0.46 μmol/kg/month at 5 and 15 °C, to 1.18 μmol/kg/month at 25 °C and 5.63 μmol/kg/month at 50 °C (Krichene *et al.*, 2015).

The observed differences in the initial degradation rate of the secoiridoid compounds in the different OO varieties studied may be explained by the difference in their initial phenolic contents. Indeed, an approximately linear relationship ($r > 0.98$; and at 50 °C, $r = 0.91$; $p < 0.01$) between the initial degradation rate and initial phenolic concentrations is observed at phenolic concentrations lower than 100 μmol/kg at the storage temperatures assayed (5–50 °C; Krichene *et al.*, 2015). Moreover, taking also into account the

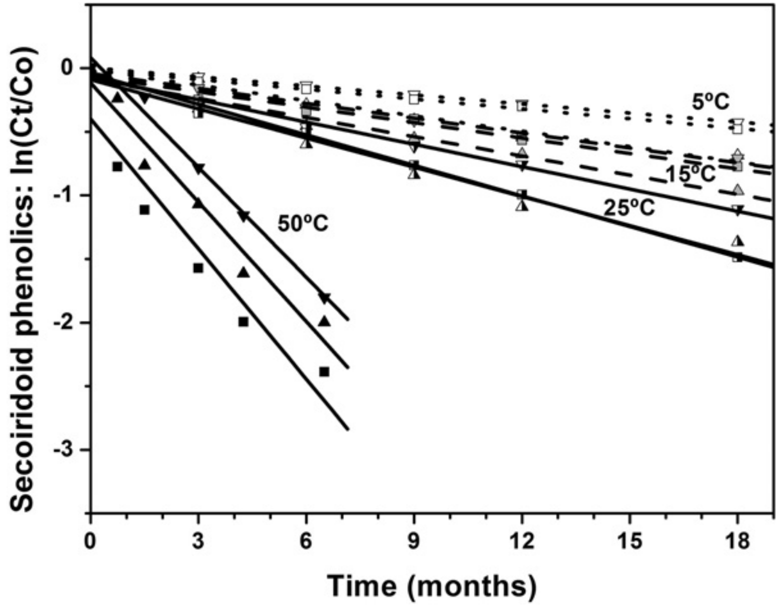

Figure 12.3 Kinetics of degradation of phenolic secoiridoids at different storage temperatures (5–50 °C).

higher phenolic content observed in this study (350 μmol/kg), the initial degradation rate fits an exponential growth curve Ct = Co (1+r)t, where *r* is the growth rate.

Therefore, the effect of the oil variety on the stability of the phenolic compounds may be explained simply by their different initial concentrations. Furthermore, the relationship between initial degradation rate and initial concentration of phenolics was very similar for all the four main complex phenolics compounds (DOA, AOA, DLA, and ALA), and therefore their stability should also be similar.

In contrast to complex phenolics, α-tocopherol degradation apparently follows a simpler zero-order kinetic (Ct = Co + Kt; $r > 0.97$ and $p < 0.01$; Krichene *et al.*, 2015). Moreover, as observed for phenolics, the degradation in CB conditions is lower due to the limited oxygen availability, as is clearly noticeable at 50 °C when a plateau is reached.

12.4.3 Stability of sensory characteristics

It is necessary to protect the oil against lipid oxidation, which can have many deleterious effects on the quality of VOO, including the formation of unpleasant sensory characteristics, the drastic reduction of the bouquet and taste notes, as well as the reduction of naturally occurring antioxidant compounds and therefore shortening of the VOO shelf life (Bendini *et al.*, 2009), as discussed in this chapter. Initially, lipids are oxidized to hydroperoxides, which are odorless and tasteless (Frankel, 1982) and do not account for sensory changes. However, they are susceptible to further oxidation or decomposition into products of secondary reactions, which are responsible for the typical unpleasant sensory characteristics, identified on the whole as a rancid defect.

The concentration and odor threshold of the volatile compounds are indeed crucial to VOO quality. Several researchers (Solinas *et al.*, 1987; Angerosa, 2000, 2002; Kotsiou *et al.*, 2015) have reported that during oxidation, the drastic reduction of the C6 aldehydes, alcohols, and esters from the LOX pathway and the increase of many saturated and unsaturated aldehydes (C5–C11) from chemical oxidation, including hexanal, reduce the perception of the positive attributes and pleasant sensory notes, leading to the kind of off-flavor in VOO recognized as a rancid defect by assessors.

Moreover, as discussed in this chapter, phenolics, particularly secoiridoids, decrease during OO storage, leading to a decrease in bitterness and pungent intensity, positive attributes that are characteristic of a fresh OO and directly related to phenolics content in the oil.

12.5 Antioxidant capacity of olive oil functional components

Antioxidant activity has usually been measured by the effect of antioxidants in delaying the extent of oxidation. In the case of OO, a wide variety of methods are used: the peroxide value, the thiobarbituric acid reactive substance (TBAR) value, the iodine value, free fatty acid content, polymeric compounds, color changes, K_{232}, and K_{270}, among others (Antolovich *et al.*, 2004; Frankel, 2010).

The antioxidant activity of minor bioactive compounds of OO has been reported by several authors using both accelerated oxidative stability tests under strong conditions (e.g., AOM, Rancimat, and OSI) (Vázquez-Roncero *et al.*, 1975; Baldioli *et al.*, 1996; Carrasco-Pancorbo *et al.*, 2005), as well as antiradical capacity assays (e.g., ABTS [2,20-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid], DPPH [2,2-diphenyl-1-picrylhydrazyl], and ORAC [oxygen radical absorbance capacity]) (Espín *et al.*, 2000; Ninfali *et al.*, 2001; Pellegrini *et al.*, 2001; Suárez *et al.*, 2011). A relationship is generally observed between the content in phenolic compounds and the antiradical DPPH assay. Indeed, as expected, the antioxidant activity evaluated by the DPPH method of phenolic-rich VOO was significantly higher ($p < 0.001$) than lower content VOO or OO (Fregapane *et al.*, 2013).

To study the exact role of the OO natural antioxidants, it is necessary to study their effect at normal storage conditions (room temperature) and to add these individual compounds to a POO (Mancebo-Campos *et al.*, 2014). POO may be obtained by stripping pro- and antioxidants to avoid confounding effects, using column chromatography (Yanishlieva & Marinova, 1995; Khan & Shahidi, 1999), to be used as the lipid matrix for making up the spiked oils. POO spiked with different concentrations (typically found in EVOO) of hydroxytyrosol, tyrosol, a mixture of the dialdehydic and aldehydic forms of oleuropein aglycone, and α-tocopherol were prepared by adding aliquots of these antioxidants in methanol (for phenolics) or hexane (for α-tocopherol) (Mancebo-Campos *et al.*, 2014).

The effect of the content in OO natural antioxidants, measured by the increase in shelf life (at 25 °C) or by Rancimat IP (100 °C and 10 L/h), is depicted in Figure 12.4.

Increasing amounts of hydroxytyrosol (H) and oleuropein aglycone (OA) produce an increase in the oxidative stability of the oil, measured by either shelf life or Rancimat IP (Figure 12.4). For example, an increase of 2–2.5 times in OO shelf life is observed at a concentration of 0.5 mmol/kg of hydroxytyrosol or oleuropein aglycone, or between 2.5 and 3 times in the case of the Rancimat IP. These experimental results clearly show that hydroxytyrosol possesses a strong antioxidant effect, similar to its secoiridoids (OA), that increases with its concentration in the POO.

As already established and reported in the literature, tyrosol (T) did not show antioxidant activity at any concentration studied (Figure 12.4), which is consistent with the lack of antioxidant capacity generally attributed to this phenol (Mateos *et al.*, 2003; Fki *et al.*, 2005; Mancebo-Campos *et al.*, 2014). Remarkable behavior of α-tocopherol is observed in this study (Figure 12.4; Mancebo-Campos *et al.*, 2014), since under mild conditions of 25 and 40 °C, this compound acted as a prooxidant, decreasing the shelf life of the oil as its concentration rose (i.e., from 23.8 down to 15.6 weeks when increasing its concentration from 0.10 to 0.50 mmol/kg). In contrast, under accelerated Rancimat conditions (100 °C, 10 L/h), α-tocopherol behaved as an antioxidant, for example increasing the IP from 19.7 up to 29.2 h from 0.10 to 0.50 mmol/kg. This fact makes questionable the use of this kind of accelerated method to assess the antioxidant capacity of different compounds, due to the different reaction mechanisms in such drastic conditions – more so if the results are then extrapolated to real storage conditions.

In OO and edible oils, different natural compounds with antioxidant activity are present at the same time, and, therefore, it is very important to study their combined effect, whether synergistic or antagonistic. From the values of the experimental oxidation rate constants (k) found in a recent study (Mancebo-Campos *et al.*, 2014), it can be concluded that apparently no synergistic or antagonistic effects were observed when more than one compound was present in the POO (Blekas *et al.*, 1995). In fact, the sum of the positive effect (reduction in k) due to the addition of a certain concentration of one antioxidant, hydroxytyrosolorol europein aglycone, and the negative effect (increase in k) produced by the presence of a determined amount of α-tocopherol was close to the oxidation rate observed by spiking POO with a combined concentration of the studied compounds.

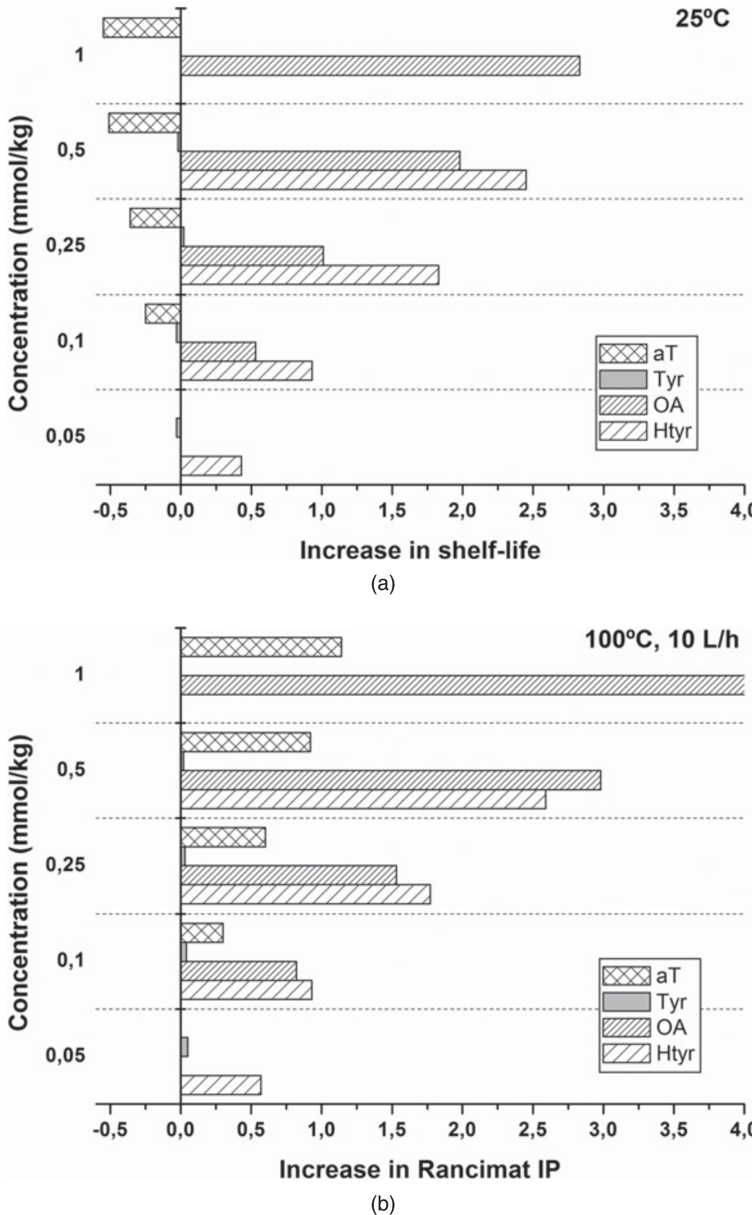

Figure 12.4 Increase in olive oil shelf life (at 25 °C) and in Rancimat IP (100 °C, 10 L/h) at different antioxidants concentrations: αT, α-tocopherol; Tyr, tyrosol; OA, oleuropein aglycone; Htyr, hydroxytyrosol.

12.6 Conclusion

The oxidative stability of olive oil and its minor functional components were described in this chapter, and the primary causes of olive oil deterioration were discussed. Nevertheless, it is very important to note that further investigations are still needed to confirm the role and behavior of these minor compounds with regard to the shelf life and functional quality of olive oil.

References

Abaza, L., Taamalli, W., Temime, S.B., Daoud, D., Gutiérrez, F., & Zarrouk, M. (2005) Natural antioxidant composition as correlated to stability of some Tunisian virgin olive oils. Rivista Italiana delle Sostanze Grasse 82, 12–18.

Angerosa, F. (2000) Sensory quality of olive oils. In: Handbook of olive oil: analysis and properties, ed. J. Harwood & R. Aparicio. Aspen Publications, Gaithersburg, MD.

Angerosa, F. (2002) Influence of volatile compounds on virgin olive oil quality evaluated by analytical approaches and sensor panels. European Journal of Lipid Science and Technology 104, 639–643.

Antolovich, M., Bedgood, D.R., Bishop, A.G., Jardine, D., Prenzler, P.D., & Robards, K. (2004) LC–MS investigation of oxidation products of phenolic antioxidants. Journal of Agriculture and Food Chemistry 52, 962–971.

Aparicio, R., Roda, L., Albi, M.A., & Gutiérrez, F. (1999) Effect of various compounds on virgin olive oil stability measured by Rancimat. Journal of Agriculture and Food Chemistry 47, 4150–4155.

Baldioli, M., Servili, G., Perretti, G., & Montedoro, G.F. (1996) Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. Journal of the American Oils Chemists' Society 73, 1589–1593.

Bendini, A., Cerretani, L., Salvador, M.D., Fregapane, G., & Lercker, G. (2009) Stability of the sensory quality of virgin olive oil during storage: an overview. Italian Journal of Food Science 21, 389–406.

Blekas, G., Psomiadou, E., Tsimidou, M., & Boskou, D. (2002) On the importance of total polar phenols to monitor the stability of Greek virgin olive oil. European Journal of Lipid Science and Technology 104, 340–346.

Blekas, G., Tsimidou, M., & Boskou, D. (1995) Contribution of α-tocopherol to olive oil stability. Food Chemistry 52, 289–294.

Boskou, D. (2006) Olive oil: chemistry and technology, 2nd ed. AOCS, Champaign, IL.

Brenes, M., García, A., Dobarganes, M.C., Velasco, J., & Romero, C. (2002) Influence of thermal treatments simulating cooking processes on the polyphenol content in virgin olive oil. Journal of Agriculture and Food Chemistry 50, 5962–5967.

Calligaris, S., Sovrano, S., Manzocco, L., & Nicoli, M.C. (2006) Influence of crystallization on the oxidative stability of extra virgin olive oil. Journal of Agriculture and Food Chemistry 54, 529–535.

Carrasco-Pancorbo, A., Cerretani, L., Bendini, A., Segura-Carretero, A., Del Carlo, M., Gallina-Toschi, T., Lercker, G., Compagnone, D., & Fernández-Gutiérrez, A. (2005). Evaluation of antioxidant capacity of individual phenolic compounds in virgin olive oil. Journal of Agriculture and Food Chemistry 53, 8918–8925.

Carrasco-Pancorbo, A., Cerretani, L., Bendini, A., Segura-Carretero, A., Lercker, G., & Fernandez-Gutiérrez, A. (2007) Evaluation of the influence of thermal oxidation on the phenolic composition and on the antioxidant activity of extra-virgin olive. Journal of Agriculture and Food Chemistry 55, 4771–4780.

Cert, A., Alba, J., Leon-Camacho, M., Moreda, W., & Perez-Camino, M.C. (1996) Effects of talc addition and operating mode on the quality and oxidative stability of virgin olive oils obtained by centrifugation Journal of Agriculture and Food Chemistry 44, 3930–3934.

Cinquanta, L., Esti, M., & Di Matteo, M. (2001) Oxidative stability of virgin olive oils. Journal of the American Oils Chemists' Society 78, 1197–1202.

Cunha, S.C., Amaral, J.S., Fernandes, J.O., & Oliveira, M.B. (2006) Quantification of tocopherols and tocotrienols in portuguese olive oils using HPLC with three different detection systems. Journal of Agriculture and Food Chemistry 54, 3351–3356.

Daskalaki, D., Kefi, G., Kotsiou, K., & Tasioula-Margari, M. (2009) Evaluation of phenolic compounds degradation in virgin olive oil during storage and heating. Journal of Food and Nutrition Research 48, 31–41.

Di Giovacchino, L., Mucciarella, N., Constantini, N., Ferrante, M.L., & Surricchio, G. (2002) Use of nitrogen to improve stability of virgin olive oil during storage. Journal of the American Oils Chemists' Society 79, 339–344.

Espín, J.C., Soler-Rivas, C., & Wichers, H.J. (2000) Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl-1-picrylhydrazyl radical. Journal of Agriculture and Food Chemistry 48, 648–656.

European Union Commission Regulation (EU) (2012) No. 432/2012 of 16 May 2012 establishing a list of 199 permitted health claims made on foods, other than those referring to the 200 reduction of disease risk and to children's development and health. EU, Brussels.

European Union Commission Regulation EEC/2568/91. (2013) On the characteristics of olive and olive pomace oils and their analytical methods. Off. J. Eur. Communities 1991, L248, 6-36, and later amendments, the latest being Commission Regulation (EU) No. 1348/2013 of 16 December 2013. EU, Brussels.

Fki, I., Allouche, N., & Sayadi, S. (2005) The use of polyphenolic extract, purified hydroxytyrosol and 3,4-dihydroxyphenyl acetic acid from olive mill wastewater for the stabilization of refined oils: a potential alternative to synthetic antioxidants. Food Chemistry 93, 197–204.

Frankel, E.N. (1982) Volatile lipid oxidation products. Progress in Lipid Research 22, 1–7.

Frankel, E.N. (1993) Formation of headspace volatiles by thermal decomposition of oxidized fish oil vs. oxidized vegetable oils. Journal of the American Oils Chemists' Society 70, 767–776.

Frankel, E.N. (2005) Lipid oxidation, 2nd ed. The Oily Press, Dundee, Scotland.

Frankel, E.N. (2010) Chemistry of extra virgin olive oil: adulteration, oxidative stability, and antioxidants. Journal of Agriculture and Food Chemistry 58, 5991–6006.

Frankel, E.N., Huang, S.W., Kanner, J., & German, J.B. (1994) Interfacial phenomena in the evaluation of antioxidants: bulk oils vs. emulsions. Journal of Agriculture and Food Chemistry 42, 1054–1059.

Fregapane, G., Gómez-Rico, A., Inarejos, A.I., & Salvador, M.D. (2013) Relevance of minor components stability in comercial olive oil quality during the market period. European Journal of Lipid Science and Technology 115, 541–548.

Fregapane, G., Lavelli, V., León, S., Kapuralin, J., & Salvador, M.D. (2006) Effect of filtration on virgin olive oil stability during storage. European Journal of Lipid Science and Technology 108, 134–142.

Gómez-Alonso, S., Fregapane, G., Salvador, M.D., & Gordon, M.H. (2003) Changes in phenolic composition and antioxidant activity of virgin olive oil during frying. Journal of Agriculture and Food Chemistry 51, 667–672.

Gómez-Alonso, S., Mancebo-Campos, V., Salvador, M.D., & Fregapane, G. (2004) Oxidation kinetics in olive oil triacylglycerols under accelerated shelf-life testing (25–75 °C). European Journal of Lipid Science and Technology 106, 369–375.

Gómez-Alonso, S., Mancebo-Campos, V., Salvador, M.D., & Fregapane, G. (2007) Evolution of major and minor components and oxidation indices of virgin olive oil during 21 months storage at room temperature. Food Chemistry 100, 36–42.

Gómez-Alonso, S., Salvador, M.D., & Fregapane, G. (2002) Phenolic compounds profile of Cornicabra virgin olive oil. Journal of Agriculture and Food Chemistry 50, 6812–6817.

Gómez-Caravaca, A.M., Cerretani, L., Bendini, A., Segura-Carretero, A., Fernández-Gutiérrez, A., & Lercker, G. (2007) Effect of filtration systems on the phenolic content in virgin olive oil by HPLCDAD-MSD. American Journal of Food Technology 2, 671–677.

Gómez-Rico, A., Fregapane, G., & Salvador, M.D. (2008) Effect of cultivar and ripening on minor components in Spanish olive fruits and their corresponding virgin olive oils. Food Research International 2008, 41, 433–440.

Gutfinger, T. (1981) Polyphenols in olive oil. Journal of the American Oils Chemists' Society 58, 966–968.

Gutiérrez, F., Arnaud, T., & Garrido, A. (2001) Contribution of polyphenols to the oxidative stability of virgin olive oil. Journal of the Science of Food and Agriculture 81, 1463–1470.

Gutiérrez, F., & Fernandez, J.L. (2002) Determinant parameters and components in the storage of virgin olive oil. Prediction of storage time beyond which the oil is no longer of extra quality. Journal of Agriculture and Food Chemistry 50, 571–577.

Gutiérrez, F., Villafranca, M.J., & Castellano, J.M. (2002) Changes in the main components and quality indices of virgin olive oil during oxidation. Journal of the American Oils Chemists' Society 79, 669–676.

Gutiérrez, R., Janer del Valle, C., Janer del Valle, M.L., & Gutiérrez, F. (1977) Relación entre los polifenoles y la calidad y estabilidad del aceite de oliva virgen. Grasas y Aceites 28, 101–105.

Kaya, A., Tekin, A.R., & Öner, D. (1993) Oxidative stability of sunflower and olive oils: comparison between a modified active oxygen method and long term storage. Lebensmittel-Wissenschaft & Technologie 26, 464–468.

Khan, M.A., & Shahidi, F. (1999) Rapid oxidation of commercial extra virgin olive oil stored under fluorescent light. Journal of Food Lipids 6, 331–339.

Koski, A., Psomiadou, E., Tsimidou, M., Hopia, A., Kefalas, P., Wähälä, K., & Heinonen, M. (2002) Oxidative stability and minor constituents of virgin olive oil and cold-pressed rapeseed oil. European Food Research and Technology 214, 294–298.

Kotsiou, K., & Tasioula-Margari, M. (2015) Changes occurring in the volatile composition of Greek virgin olive oils during storage: oil variety influences stability. European Journal of Lipid Science and Technology 117, 514–522.

Krichene, D., Allalout, A., Mancebo-Campos, V., Salvador, M.D., Zarrouk, M., & Fregapane, G. (2010) Stability of virgin olive oil and behaviour of its natural antioxidants under medium temperature accelerated storage conditions. Food Chemistry 121, 171–177.

Krichene, D., Salvador, M.D., & Fregapane, G. (2015) Stability of virgin olive oil phenolic compounds during long-term storage (18 months) at temperatures of 5−50 °C. Journal of Agriculture and Food Chemistry. DOI:10.1021/acs.jafc.5b02187

Lavelli, V., Fregapane, G., & Salvador, M.D. (2006) Effect of storage on secoiridoid and tocopherol contents and antioxidant activity of monovarietal extra virgin olive oils. Journal of Agriculture and Food Chemistry 54, 3002–3007.

Le Tutour, B., & Guedon, D. (1992) Antioxidative activities of Oleaeuropaea leaves and related phenolic compounds. Phytochemistry 31, 1173–1179.

Lee, O.H., Kim, Y.C., Kim, K.J., Kim, Y.C., & Lee, B.Y. (2007) The effects of bioactive compounds and fatty acid compositions on the oxidative stability of extra virgin olive oil varieties. Food Science and Biotechnology 16, 415–420.

Leonardis de, A., & Macciola, V. (1998) Evaluation of the shelf-life of virgin olive oils. Rivista Italiana delle Sostanze Grasse 75, 391–396.

Li, X., Hanjiang, Z., Shoemaker, C.F., & Wang, S.C. (2014) The effect of different cold storage conditions on the compositions of extra virgin olive oil. Journal of the American Oils Chemists' Society 91, 1559–1570.

Litridou, M., Linssen, J., Schols, H., Bergmans, M., Posthumus, M., Tsimidou, M., & Boskou, D. (1997) Phenolic compounds in virgin olive oils: Fractionation by solid phase extraction and antioxidant activity assessment. Journal of the Science of Food and Agriculture 74, 169–174.

Lozano-Sanchez, J., Cerretani, L., Bendini, A., Segura-Carretero, A., & Fernandez-Gutierrez, A. (2010) Filtration process of extra virgin olive oil: effect on minor components, oxidative stability and sensorial and physicochemical characteristics. Trends in Food Science & Technology 21, 201–211.

Mancebo-Campos, V., Fregapane, G., & Salvador, M.D. (2008) Kinetic study for the development of an accelerated oxidative stability test to estimate virgin olive oil potential shelf life. European Journal of Lipid Science and Technology 110, 969–976.

Mancebo-Campos, V., Salvador, M.D., & Fregapane, G. (2007) Comparative study of virgin olive oil behaviour under Rancimat accelerated oxidation conditions and long-term room temperature storage. Journal of Agriculture and Food Chemistry 55, 8231–8236.

Mancebo-Campos, V., Salvador, M.D., & Fregapane, G. (2014) Antioxidant capacity of individual and combined virgin olive oil minor compounds evaluated at mild temperature (25 and 40 °C) as compared to accelerated and antiradical assays. Food Chemistry 150, 374–381.

Mateos, R., Domínguez, M.M., Espartero, J.L., & Cert, A. (2003) Antioxidant effect of phenolic compounds, R-tocopherol and other minor components in virgin olive oil. Journal of Agriculture and Food Chemistry 51, 7170–7175.

Mateos, R., Espartero, J.L., Trujillo, M., Rios, J.J., Leon-Camacho, M.L., Alcudia, F., & Cert, A. (2001) Determination of phenols, flavones, and lignanes in virgin olive oils by solid-phase extraction and high performance liquid chromatography with diode array ultraviolet detection. Journal of Agriculture and Food Chemistry 49, 2185–2192.

Mateos, R., Trujillo, M., Pérez-Camino, M.C., Moreda, W., & Cert, A. (2005) Relationships between oxidative stability, triacylglycerol composition, and antioxidant content in olive oil matrices. Journal of Agriculture and Food Chemistry 53, 5766–5771.

Migliorini, M., Cherubini, C., Cecchi, L., & Zanoni, B. (2013) Degradazione dei composti fenolici durante la conservazione dell'olio extra vergine di olive [degradation of phenolic compounds during extra virgin olive oil shelf-life]. Rivista Italiana delle Sostanze Grasse 90, 71–80.

Montedoro, G.F., Servili, M., Baldioli, M., & Miniati, E. (1992) Simple and hydrolyzable phenolic compounds in olive oil. Note 1. Their extraction, separation, and quantitative and semi-quantitative separation and evaluation by HPLC. Journal of Agriculture and Food Chemistry 40, 1571–1577.

Ninfali, P., Aluigi, G., & Magnani, M. (2001) Antioxidant capacity of extra-virgin olive oils. Journal of the American Oils Chemists' Society 78, 243–247.

Okogeri, O., & Tasioula-Margari, M. (2002) Changes occurring in phenolic compounds and r-tocopherol of virgin olive oil during storage. Journal of Agriculture and Food Chemistry 50, 1077–1080.

Pagliarini, E., Zanoni, B., & Giovanelli, G. (2000) Predictive study on Tuscan extra virgin olive oil stability under several commercial conditions. Journal of Agriculture and Food Chemistry 48, 1345–1351.

Pellegrini, N., Visioli, F., Buratti, S., & Brigherti, F. (2001) Direct analysis of total antioxidant activity of olive oil and studies on the influence of heating. Journal of Agriculture and Food Chemistry 49, 2532–2538.

Porter, W.L., Black, E.D., & Drolet, A.M. (1989) Use of polyamide oxidative fluorescence test on lipid emulsions: contrast on relative effectiveness of antioxidants in bulk versus dispersed systems. Journal of Agriculture and Food Chemistry 37, 615–619.

Pristouri, G., Badeka, A., & Kontominas, M.G. (2010) Effect of packaging material headspace, oxygen and light transmission, temperature and storage time on quality characteristics of extra virgin olive oil. Food Control 21, 412–418.

Rastrelli, L., Passi, S., Ippolito, F., Vacca, G., & De Simone, F. (2002) Rate of degradation of r-tocopherol, squalene, phenolics, and polyunsaturated fatty acids in olive oil during different storage conditions. Journal of Agriculture and Food Chemistry 50, 5566–5570.

Salvador, M.D., Aranda, F., Gómez-Alonso, S., & Fregapane, G. (2001) Cornicabra virgin olive oil: a study of five crop seasons: composition, quality and oxidative stability. Food Chemistry 2001, 74, 267–274.

Salvador, M.D., Aranda, F., & Fregapane, G. (1999) Contribution of chemical components of "Cornicabra" virgin olive oils to oxidative stability: a study of three successive crop seasons. Journal of the American Oils Chemists' Society 76, 427–433.

Servili, M., & Montedoro, G. (2002) Contribution of phenolic compounds to virgin olive oil quality. European Journal of Lipid Science and Technology 104, 602–613.

Silva, S.F., Rodrigues-Anjos, C.A., Cavalcanti, R.N., & Dos Santos Celeghini, R.M. (2015) Evaluation of extra virgin olive oil stability by artificial neural network. Food Chemistry 179, 35–43.

Solinas, M., Angerosa, F., & Cucurachi, A. (1987) Connessionetra I prodotti di neoformazioneossidativadelle-sostanzegrasse e insorgenza del difetto di rancidità all'esameorganolettico, Nota II. Determinazionequantitativa. Rivista Italiana delle Sostanze Grasse 64, 137–142.

Suárez, M., Romero, M.P., Ramos, T., & Motilva, M.J. (2011) Stability of a phenol-enriched olive oil during storage. European Journal of Lipid Science and Technology 113, 894–903.

Tan, C.P., Che Man, Y.B., Selemat, J., & Yusoff, M.S.A. (2001) Application of Arrhenius kinetics to evaluate oxidative stability in vegetable oils by isothermal differential scanning calorimetry. Journal of the American Oils Chemists' Society 78, 1133–1137.

Tsimidou, M.Z., Georgiou, A., Koidis, A., & Boskou, D. (2005) Loss of stability of "veiled" (cloudy) virgin olive oils in storage. Food Chemistry 93, 377–382.

Vázquez-Roncero, A., Janer del Valle, C., & Janer del Valle, M.L. (1973) Determinación de los polifenoles totales del aceite de oliva. Grasas y Aceites 24, 350–354.

Vázquez-Roncero, A., Janer del Valle, C., & Janer del Valle, M.L. (1975) Natural polyphenols and stability of olive oil. Grasas y Aceites 26, 14–18.

Velasco, J., & Dobarganes, M.C. (2002) Oxidative stability of virgin olive oil. European Journal of Lipid Science and Technology 104, 661–676.

Visioli, F., & Galli, C. (1998) Olive oil phenols and their potential effects on human health. Journal of Agriculture and Food Chemistry 46, 4292–4296.

Yanishlieva, N.V., & Marinova, E.M. (1995) Effects of antioxidants on the stability of tryacylglycerols and methyl esters of fatty acids of sunflower oil. Food Chemistry 54, 377–382.

Zanoni, B., Bertuccioli, M., Rovellini, P., Marotta, F., & Mattei, A. (2005) A preliminary approach to predictive modeling of extra virgin olive oil stability. Journal of the Science of Food and Agriculture 85, 1492–1498.

Zhong, Y., & Shahidi, F. (2012) Antioxidant behavior in bulk oil: limitations of polar paradox theory. Journal of Agriculture and Food Chemistry 60, 4–6.

13 Chemical and sensory changes in olive oil during deep frying

George Siragakis and Dafni Karamanavi

13.1 Introduction

Olive oil is the basic ingredient of the daily diet of most people in the Mediterranean region. It is a valuable natural product that provides an excellent and unique flavor and nutritional benefits, thus it is important to preserve these unique characteristics. Needless to say, olive oil is more expensive than other plant oils (Chatzilazarou *et al.*, 2006). Its phenolic composition benefits humans' health by providing antioxidant properties. Deep-fat frying is an attractive and the most common method for fried food preparation, both domestically and industrially (Saguy & Dana, 2003; Akil *et al.*, 2015). The unique sensory properties of deep-fried foods, such as flavor, texture, odor, and appearance, make them highly appreciated by consumers (Santos *et al.*, 2013). Deep-fat frying can be defined as the cooking process in which foods are immersed into an edible oil or fat maintained at a temperature of about 150–200 °C (Yamsaengsung & Moreira, 2002). The layer of the frying oil is about 20–200 mm or deeper, and frying oil is reused several times (Xu, 2000; Pokorny, 2002). The quality of the products cooked by deep-fat frying depends not only on the frying conditions, such as temperature of the heated oil, frying time, food weight, and frying oil volume, but also on the types of oil and the quality of the food used (Sanchez-Gimeno *et al.*, 2008). There are three important factors causing changes in chemical and physical properties of oil during frying; these include exposure to water (coming from food), high temperatures, and oxygen. Under these conditions, oils undergo oxidation, polymerization, hydrolysis (which causes formation of free fatty acids), cyclization, and isomerization (Lalas, 2009). In addition, thermal oxidation of oil could lead to the formation of hydroperoxides, known as primary oxidation products, which degrade into hydrocarbons, aldehydes, and ketones, among other classes of compounds, known as secondary oxidation products. It could also form conjugate di-, tri-, and tetraene groups, and result in loss of unsaturated fatty acids. The polymerization of oils results in an increase in viscosity. All these physical and chemical changes deteriorate the quality of oil (Martínez-Pineda *et al.*, 2011). Secondary oxidation products tend to be volatile and are responsible for the rancid flavor of oxidized oils. The oxidative degradation indices assess these primary and secondary products and are used as surrogate measures for oil quality and oxidative stability (Frankel, 2010). There is a strong relation between the formation of polar compounds and the primary and secondary oxidation that takes place during frying, and this comprises an established quality index for frying oils with a 20–25% limit for rejection or replenishment of the cooking oil (Dobarganes, 2000; Gertz, 2000). Some of these compounds may also be harmful to human health (Pokorny, 2002). The oil needs to maintain a high oxidative stability during the life of the product (Katragadda *et al.*, 2010).

Oxidative stability is a very important factor in oil quality, especially for oils that are used for frying because of the high temperature applied. From a nutritional point of view, it should be taken into consideration that oils with a high amount of saturated fatty acids and fats containing *trans* fatty acids should be rejected (Tabee *et al.*, 2008). Moreover, highly saturated fatty acid composition of some industrial frying oils may represent a problem in case it is necessary to keep the product in the liquid state (De Marco *et al.*,

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2007). Vegetable oils like soybean, sunflower, and corn oils are often considered to be very unstable when exposed to continuous frying due to their reasonably high content of polyunsaturated fatty acids (PUFAs). On the other hand, the presence of natural substances such as tocopherols, sterols, and squalene enhances the oil stability at higher temperatures (Gertz, 2000). With prolonged heating time, the accumulation of deteriorative products leads to organoleptic failures and a decrease of the nutritive value. Deep-fat frying decreases the unsaturated fatty acids and increases polar matters (Marinova *et al.*, 2012).

13.2 Alterations of chemical characteristics in frying olive oil

Numerous studies have been conducted in order to determine changes in olive oil and its constituents during frying. The changes that are examined are in iodine value (IV), peroxide value (PV), viscosity, polar compounds (PCs), free fatty acids (FFAs; acidity), fatty acid methyl esters (FAMEs), tocopherols, total phenols (TPs), total antioxidant activity (TAA), color, and oxidative stability. During deep frying, FFAs, PV, PCs, color, and viscosity are increased, whereas IV, PUFA content, and tocopherol concentration decrease (Tsaknis & Lalas, 2002).

13.2.1 Iodine value

The IV decrease, during frying, is indicative of increased rate of oxidation and could be attributed to oxidation and polymerization reactions involving the double bonds, either through chain reactions adjacent to the double bond to form volatile degradation products or through direct interaction across the bond to form 1,2-diol (Alim & Morton, 1974). Although the decrease in IV is a result of complex physicochemical changes, such a decrease is indicative of the oxidation (Lalas, 2009) and could be a useful quality parameter to control oil quality during frying.

13.2.2 Peroxide value

Peroxides are unstable under frying conditions and break down to secondary oxidation products (Chatzilazarou *et al.*, 2006). An increase in the initial stage of frying is expected to be followed by a decrease with further heating, because the hydroperoxides tend to decompose at 180 °C to form secondary oxidation products (Perkins, 1967). The overall increase in PV is related to the cooling period of the oil. The length of time required for the oils to cool to room temperature (25 °C) after frying is more than 4 h. During the cooling period, the hot oil is exposed to air and more hydroperoxides are formed (Augustin & Berry, 1983). Hydroperoxides are decomposed to alkoxyl and hydroxyl radicals by homolysis of the oxygen–oxygen bond. This would explain why at 160 °C, peroxides increase during the first 20 frying cycles (20 times of frying the olive oil) and then decrease. It also explains why, at a higher temperature (180 °C), PV is lower under severe frying conditions, because the rate of peroxide decomposition becomes higher than the rate of peroxide formation (Marmesat *et al.*, 2009). Although PV is a classical method to evaluate oil oxidation, it does not seem to be an accurate tool for the evaluation of heat-treated oils.

13.2.3 Viscosity

The increase in viscosity of olive oil is significant after 8 h of frying (Chatzilazarou *et al.*, 2006). As the oxidation is accelerated, the viscosity of oil progressively increases (Tyagi & Vasishtha, 1996). The increase in viscosity of frying oils is due to polymerization that results in the formation of higher molecular weight compounds (carbon-to-carbon and/or carbon-to-oxygen-to-carbon bridges) between fatty acids (Al-Harbi & Al-Kabtani, 1993). It is found that an oil rich in linoleic acid is more easily polymerized during deep-fat frying than an oil rich in oleic acid (Takeoka *et al.*, 1997; Valdes & Garcia, 2006).

13.2.4 Polar compounds

The determination of PCs is considered to be one of the most reliable indicators of the oxidative state of the frying oil (Fritch, 1981; Gutierrez *et al.*, 1988). Any heated oil with 27% or more PCs should be discarded. Determination of the total hours of frying before the PCs reach the 27% critical level mark is very critical (Tsaknis & Lalas, 2002).

13.2.5 Free fatty acids

The increase in FFAs during frying could be attributed to oxidation and hydrolysis (Peeled *et al.*, 1975; Abdel-Aal & Karara, 1986). Moreover, FFA is a dynamic value because at the same time that the acids are being produced, they have sufficient vapor pressure at frying temperatures to evaporate from the surface (Peeled *et al.*, 1975).

There are some commercially available kits for the assessment of frying oil quality like Mquant of Merck-Millipore. This inexpensive method requires no special training, lab instruments, or sample preparation. A simple dipping of the test strip into the oil or fat sample is needed, and one can read the results in 30 seconds. The label shows typical threshold values to help make quick decisions.

13.2.6 Fatty acid methyl esters (FAMEs)

Experiments have shown that after 10 h of frying, the saturated fatty acid content of olive oil increases while the PUFA content decreases (Chatzilazarou *et al.*, 2006). However, this decrease was not significant in the case of fried oil. PUFAs in olive oil, such as linoleic acid, are more readily oxidized and polymerized than the less unsaturated fatty acid, the oleic acid. Subsequently, monounsaturated oils such as olive oil are less altered during frying than the polyunsaturated ones such as corn or safflower oil. The triacylglycerol fatty acid content remains relatively unchanged except for a slight decrease in the ratio of PUFA/SFA and MUFA/SFA after 10 times frying (Andrikopoulos *et al.*, 2002a, 2002b) and a slight *trans* fatty acid formation occurs. However, these ratios decrease progressively with an increasing number of frying cycles.

13.2.7 Tocopherols

The α-tocopherol is more stable during frying of oils than γ- and δ-tocopherols. The oxidative stability of olive oil is partly related to the higher amount of α-tocopherol contained in it (Sonntag, 1991). The order of antioxidant activity of tocopherols changes with the quality of the oil used (Lea, 1960).

13.2.8 Conjugated dienes (CDs)

Another factor that indicates chemical alteration in oils is the conjugated CDs, which seem to increase at 160 °C (Chatzilazarou *et al.*, 2006). CDs are formed during oxidation of unsaturated fatty acids containing two or more double bonds to achieve a more stable radical. Oils with high content of PUFAs show the highest increase in CDs. Several studies have shown that the CD formation is faster and more extensive in sunflower oil than in extra virgin olive oil (Smith *et al*., 2007; Sanchez-Gimeno *et al*., 2008; Lalas, 2009; Farhoosh & Tavassoli-Kafrani, 2011).

13.2.9 Color

Alterations also occur in the color of olive oil, and this is related to the degradation processes such as the formation of hydroperoxides, conjugated dienoic acids, ketones, and aldehydes, among others (Fritch, 1981; Gutierrez *et al.*, 1988). Additional cause for a color change in deep-fat frying might be the presence of pigments in the commercial oils together with the solubilization of browning pigments from potatoes during frying (Guillaumin, 1988; White, 1991; Melton *et al.*, 1994).

13.3 Oxidation of olive oil during frying

According to Casal *et al.* (2010), extra virgin olive oil had the highest oxidative stability of 16 h frying, followed by that of 15 h (for refined olive oil) when the Rancimat apparatus was used. The oxidative stability of both extra virgin olive oil and refined olive oil was almost the same after the first 3 h of frying, but a clear reduction was observed after 6 h, while after 18 h of frying their values did not change until the end of the process. Sunflower oil, despite reduced initial levels, was characterized by a smoother reduction, varying between 3.6 and 2.2 h. Virgin olive oil shows a high resistance to oxidative deterioration due to both its triacylglycerol composition (low in PUFA) and the presence of phenolic antioxidants, mainly polyphenols and tocopherols. Polyphenols are of greater importance for virgin olive oil stability when compared with refined oils, because they are eliminated or drastically reduced during the refining process (Velasco & Dobarganes, 2002).

Acrylamide, which is considered as being carcinogenic and genotoxic, is usually formed during the frying process (Totani, 2007; Vrettakou *et al.*, 2014). When different edible oils (corn oil, sunflower oil. and olive oil) were subjected to frying, slices of potatoes fried for 15 min at 180 °C showed lower acrylamide content when fried in olive oil, as determined by liquid chromatography–tandem mass spectrometry (LC-MS-MS). Acrylamide concentration in crisps increased during frying time, but the formation was faster in the oil having the lowest concentration of phenolic compounds (Siragakis *et al.*, 2014).

13.4 Methods for determination of polar compounds and evaluation of the quality of frying olive oil

13.4.1 Determination of polar compounds

For determination of PCs in oils and fats, adsorption chromatography using silica minicolumns is the standard method. For quantification of polymerized triacylglycerols, oxidized triacylglycerols, and diacylglycerols in PCs, high-performance size-exclusion chromatography (HPSEC) is employed. The procedure of adsorption chromatography is sensitive, allowing savings in time, solvents, and reagents, while HPSEC is very rapid, providing detailed information on the main groups of compounds in fats and oils associated with hydrolysis, oxidation, and thermal polymerization. Both of these methods are useful not only for the analysis of used frying oils but also for the analysis of virgin or refined oils.

The quality of the used frying fats and oils is indicated by the level of PCs, which provides information about the total amount of newly formed compounds. These compounds have higher polarity than triacylglycerols and are the basis of the recommendations and regulations, limiting degradation of the used frying fats for human consumption (Firestone, 1996). Complementary determination of polymerized triacylglycerols in fats and oils by exclusion chromatography allows quantification of the main groups of compounds formed during the frying process (Dieffenbacher & Martin, 1987; Dobarganes *et al.*, 1988; Wolf *et al.*, 1991; Márquez-Ruiz, 1996).

13.4.2 Rapid test kit Oleo Test™

This is a colorimetric test for the determination of PCs in frying fats and oils.

During the frying process, which must be controlled adequately, the fat or oil is exposed to atmospheric oxygen and moisture at high temperatures (over 160 °C) for a long time; as a result, many chemical reactions take place in the frying fat, producing a number of harmful compounds that can potentially cause several problems such as lung or stomach cancer.

Several oil samples were collected from restaurants, baking industries, and other food units in order to determine the PCs using the Oleo Test (Teixeira, 2006). The fats used were frying oil (a mixture of different food oils), maize oil, peanut oil, soybean oil, and sunflower oil. A total of 200 samples were analyzed using the ISO 8420:2002 standard and the Oleo Test kit. Tables 13.1 and 13.2 and Figure 13.1 show the representative results. Color 1 represents fat content <5%; Color 2, 5–10%; Color 3, 10–17%; Color 4, 17–25%; and Color 5, >25%. According to ISO 8420:2002, results seem to range between these values.

Table 13.1 Quantification of polar compounds by the ISO method and the Oleo Test™ for several samples of frying oil.

Sample number	Oleo Test		Polar compounds (%)(ISO 8420:2002)
	Color number	Polar compounds (%)	
1	2	5 to 10	7.2
2	2	5 to 10	7.7
3	2	5 to 10	8.1
4	3	10 to 17	9.9
5	3	10 to 17	11.6
6	4	17 to 25	27.3
7	4	17 to 25	22.4

Table 13.2 Relation between the color number and the interval of polar compounds.

Color number	Polar compounds (%)
1	<5
2	5 to 10
3	10 to 17
4	17 to 25
5	>25

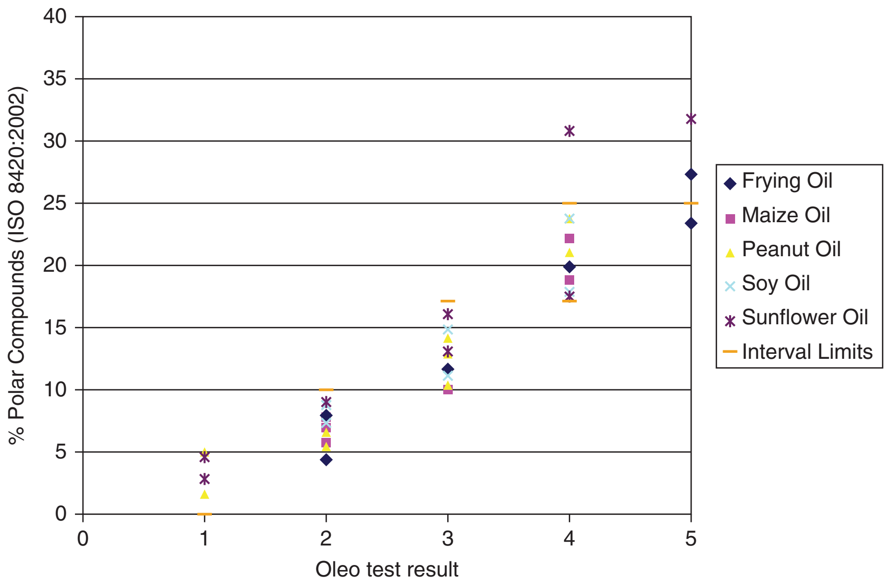

Figure 13.1 Relation between ISO results and the Oleo Test™ results.

The results confirmed that this is a feasible and a simple test for monitoring the quality of frying fats in terms of PCs.

13.5 Evaluation of the quality of frying olive oil

Apart from the standardized method for determination of PCs, which is based on adsorption chromatography, some other rapid and simple methods are also used that are necessary to evaluate the quality of frying oils in fried-food outlets. These rapid tests are based on chemical reactions (Fritest and Oxifritest) and on physical properties (Viscofrit [VF] and food oil sensor).

Rapid tests have been evaluated in a set of 105 used frying oils as alternative methods for the determination of PCs (Marmesat *et al.*, 2007a, 2007b). The results demonstrated that any of the rapid tests assayed are of great utility to determine the point at which frying fats and oils should be discarded. The tests based on physical properties showed better results than those based on chemical reactions, as the number of false results was lower. Furthermore, they can be useful to gain information about the changes in the frying oil quality, as their results showed high correlation coefficients with the determination of PCs.

13.5.1 Viscofrit

The VF test is based on the time necessary to empty a standard funnel-like cone filled with the frying oil. The cone is emptied by gravity through a small calibrated hole at its bottom. The test gives response to the increase of viscosity due to the formation of polymerization compounds. The VF is supplied with two thermometers that are used for monounsaturated and polyunsaturated frying oils (i.e., oils with prevailing amounts of monounsaturated and polyunsaturated fatty acids, respectively). The thermometers are calibrated between 15 and 50 °C and give for each temperature the maximum time in seconds to empty the cone containing the used frying oil. Times longer than those established indicate that the used frying oil has to be discarded.

13.5.2 Food Oil Sensor (FOS)

The FOS is a portable electronic instrument, commercialized by Northern Instruments Corporation (Wauwatosa, WI, USA), measuring the change of the dielectric constant in the fried oil compared to no fried oil. The instrument is calibrated with fresh fat of the same type as the frying fat, and the range of measurement is from 0 to 10. It has been indicated that a FOS reading of 4 is the limit beyond which the frying fat should be discarded (Graziano, 1979).

These methods of rapid testing aim to provide the ability to determine the point at which frying fats and oils should be discarded and to contribute to improving the present situation of the discontinuous frying sector, characterized by a significant number of fats and oils overpassing the limits of degradation established in official regulations. They can also be useful by giving information about the changes in the frying oil quality, as their results show high correlation coefficients with the determination of PCs.

13.6 Prediction of oxidative stability under heating conditions

13.6.1 Rancimat method

The effect of temperature on the Rancimat method for virgin olive oil analysis has been evaluated by measuring the method's reproducibility at each point of temperature (Mateos *et al.*, 2006). Method precision, expressed as the relative standard deviation (RSD), ranged from 1.14 to 5.84%, indicating that the analytical method was repeatable even at 140 °C. The results suggested 110 °C as being a more adequate temperature since the analysis time was shortened by 63% compared to the induction period obtained at 98 °C. In the Rancimat test, an oil sample is heated under atmospheric pressure, and air is allowed to bubble through the oil at a selected temperature (DeMan & DeMan, 1984; Laubli & Bruttel, 1986; DeMan *et al.*, 1987).

13.7 Impact of deep frying on olive oil compared to other oils

A study was carried out in order to investigate the high-temperature performance of some vegetable oils as a function of heating duration at a stimulated frying temperature of 180 °C and to evaluate the oxidative degradation by monitoring PV, induction period (IP), the content of CDs as absorbance at 232 nm, the content of conjugated trienes as absorbance at 270 nm, changes in fatty acid composition (C16:0/C18:2), and total polar components (TPCs). The results revealed that olive oil has better stability against thermal oxidation when compared to polyunsaturated oils, which is due to fatty acid composition. On the other hand, corn and soybean oils (among unsaturated oils) were most resistant to oxidation at frying temperature (Marinova *et al.*, 2012).

The PV is a useful indicator of the initial stage of oxidation, where the primary oxidation products are measured. PVs of analyzed frying oils decreased after 125 min of frying, except corn oil. This decrease can be explained by the formation of secondary oxidation products such as hydrocarbons, alcohols, ketones, and aldehydes from the primary oxidation products, hydroperoxides. PV decreases as oxidation proceeds due to rapid decomposition of hydroperoxides (Bester *et al.*, 2008). The PV of corn oil, however, increased after 125 min of frying, which can be explained as oxidation continuing during 125 min of frying. The PVs of the tested oils are increased in the order of soybean > corn > olive. The use of PVs to show the oxidative process in frying oils is not recommended because peroxides decompose spontaneously above 150 °C (Gordon & Kourimska, 1995; Frankel, 1998). The rates of oxidation for the tested vegetable oils decreased in the order of olive oil ~ sunflower oil > grapeseed oil > soybean oil ~ corn oil. Obviously, the oils rich in γ-tocopherol, like soybean and corn oils (Gliszczynska-Siwglo & Sikorska, 2004), show higher oxidation stabilities while treated at high temperature.

Thermal oxidation of unsaturated fatty acids is accompanied by considerable isomerization of double bonds leading to products containing *trans* double bonds and conjugated double-bond systems (Frankel, 1998). The results showed that the rates of conjugated dienes accumulation (RCDAs) decreased in the order of sunflower > grapeseed > soybean > corn > olive oil. These data indicated that with respect to CD accumulation, olive oil is the most stable of all oils. Conjugated trienes absorbing at 270 nm are produced by linolenate oxidation products or by dehydration of hydroxylinoleate (Frankel, 1998). The results presented a trend of increasing trienes content with the increase in heating time. This process is less pronounced in the case of olive oil. In soybean oil, conjugated trienes decompose more rapidly than in other oils.

Monitoring of fatty acid changes in oils during deep-fat frying is an effective method to assess thermal oxidative changes in the oils (Jawsir *et al.*, 2004). Linoleic acid content is frequently used as an indicator of the degree of oil degradation, since the polyunsaturated linoleoyl chain is highly susceptible to oxidation. It is found that changes in the C18:2/C16:0 ratio was an effective parameter for assessing oxidation of oils. The C18:2/C16:0 ratio declined as time of heating was increased. The results showed that this parameter decreased in the same order as RCDA: sunflower > grapeseed > soybean > corn > olive oil (Augustin *et al.*, 1987).

The total polar compounds (TPCs) used in assessing deep-frying fats is until today an important criterion for monitoring the decrease of fat quality (Firestone, 1996). TPCs are considered to be nonvolatile compounds having higher polarity than triacylglycerols, resulting from thermal, hydrolytic, and oxidative alteration (Firestone *et al*., 1991; Dobarganes *et al*., 1993; Sanchez-Muniz & Bastida, 2003; Mariod *et al*., 2006; Firestone, 2007). The fraction of polar components isolated from oxidized oils is the most toxic to laboratory animals (Pantzaris, 1998). It should be pointed out that the limit of acceptance for the TPC is 25%, and the times required to reach this limit differed for the oils tested and were: olive oil (41.7 h) > corn oil > (37.2 h) > soybean oil (33.5 h) > grapeseed oil (20.0 h) > sunflower oil (10.5 h).

In another study, Karakaya and Simsek (2011) used four oils (hazelnut, corn, soybean, and olive oil) to fry potato chips over two replicates of 125 min. Oils were heated to 90±2 °C, and potato chips were fried in each oil for 8 min. After each frying and cooling process, the experiment was repeated 15 times. The necessary equipment and reagents were used to evaluate oil degradation and oxidation by measuring the TPCs, the PV, and the antioxidant activity.

When the amount of TPCs exceeded the 24% level, oil should be replaced with new oil, as it is considered being thermally degraded (Hara *et al.*, 1994; Xu, 2000). The amount of TPCs in corn oil, soybean oil, and

olive oil increased as the time increased. The amount of TPCs in hazelnut oil didn't increase significantly during frying. After 125 min of frying time, TPCs in all oils analyzed were lower than 5%, which indicated that all the oils analyzed could be used to fry potato chips for longer than 125 min frying time (Gil *et al.*, 2004). It has also been noticed that the amount of polar compounds in extra virgin olive oil and high-oleic sunflower oil increased linearly with the frying cycle, but the increase was faster in high oleic sunflower oil, which is probably related to the absence of phenolic compounds in sunflower oil. After 60 frying cycles, the amount of polar compounds in both oils was about 20–23% (Sanchez-Gimeno *et al.*, 2008).

Frying did not cause any significant change in the total phenol (TP) content of corn oil, soybean oil, and olive oil. Although TP content was expected to decrease with frying time due to the thermal degradation of phenolic compounds, none of the oils' TP content was significantly reduced. A significant decrease in total antioxidant activity (TAA) was observed after 50 min of frying in hazelnut oil (from 67 to 47%) and corn oil (from 87 to 75%). TAA of soybean oil decreased from 86 to 75% after 75 min of frying and for olive oil from 22 to 18% after 25 min of frying. The low TAA of olive oil was probably due to the type of olive oil used, because the Riviera brand contains refined oil and the refining process can cause a decrease or loss in phenols and tocopherols. Virgin olive oil, however, has approximately two times more TAA than olive oil before and after different frying times (Quiles *et al.*, 2002).

The changes in characteristics and composition of the different fried oils showed that olive oil was more stable during frying when compared to polyunsaturated oils. Olive oil has a significant tocopherol content, enough to be considered as a good source of vitamin E (Kiritsakis, 1988; Kalogeropoulos *et al.*, 2007; Siragakis *et al.*, 2012). It should also be mentioned that although olive oil has a lower tocopherol content compared to other vegetable oils, it still seems to be the most stable oil of all vegetable oils during frying (IUPAC, 1987; Cuesta *et al*., 1991; Morales, 1997; Gliszczynska-Swiglo *et al*., 2007).

The smaller decrease of iodine value in the frying olive oils indicates that a lower degree of oxidation of unsaturated fatty acids takes place in this oil. The peroxide value is increased in frying oils, but it does not seem to be an accurate tool for the evaluation of thermally treated oils, because peroxides are easily decomposed. The increase in viscosity of frying oils is a result of polymerization that causes the formation of higher molecular weight compounds.

13.8 Conclusion

Olive oil is an excellent medium of frying. Although chemical and sensory changes occur during deep olive oil frying, its constituents help in retaining its antioxidant activity. The frying life of vegetable oils is accepted until it reaches the 27% level of polar compounds, but it must be noted that olive oil didn't reach that level in any of the reported studies. Conjugated dienes are formed during oxidation of unsaturated fatty acids containing two or more double bonds. Oils with a high content of PUFAs show the highest increase in CDs. Olive oil, rich in monounsaturated fatty acids, however, does not show a high increase in CDs during frying. Olive oil polyphenols provide the main advantage for the oil's antioxidant ability, and this is the reason why the European Union's Regulation 432/2012 permits only foods containing olive oil polyphenols to use in their package the health claim that "Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress." Frying studies showed that olive oil polyphenols are retained during frying, and these are the main functional constituents along with squalene and sterols that make olive oil the best medium of frying among all vegetable oils.

References

Abdel-Aal, M.H., & Karara, H.A. (1986) Changes in corn oil during deep-fat frying of foods. Lebensmittel-Wissenschaft & Technologie 19, 323–327.

Akil, E., Naciuk Castelo-Branco, V., Mesquita Magalhães Costa, A., do Amaral Vendramini, A.L., Calado, V., & Guedes, Torres A. (2015) Oxidative stability and changes in chemical composition of extra virgin olive oils after short-term deep-frying of French fries. Journal of the American Oils Chemists' Society 92, 409–421.

Al-Harbi, M.M., & Al-Kabtani, H.A. (1993) Chemical and biological evaluation of discarded frying palm oil from commercial restaurants. Food Chemistry 48, 395–401.

Alim, H., & Morton, I.D. (1974) Oxidation in foodstuffs fried in edible oils. International Congress of Food Science and Technology 6, 345–556.

Andrikopoulos, N.K., Dedoussis, G.V.Z., Falirea, A., Kalogeropoulos, N., & Hatzinikola, H.S. (2002a) Deterioration of natural antioxidant species of vegetable edible oils during the domestic deep-frying and panfrying of potatoes. International Journal of Food Sciences and Nutrition 53, 351–363.

Andrikopoulos, N.K., Kalogeropoulos, N., Falirea, A., & Barbagianni M.N. (2002b) Performance of virgin olive oil and shortening during domestic deep-frying and pan frying of potatoes. International Journal of Food Science and Technology 37, 177–180.

Augustin, K.A., Asao, T., & Heng, L.K. (1987) Relationships between measurements of fat deterioration during heating and frying in RBD olein. Journal of the American Oils Chemists' Society 64, 1670–1675.

Augustin, M.A., & Berry, S.K. (1983) Efficacy of the antioxidants BHA and BHT in palm olein during heating and frying. Journal of the American Oils Chemists' Society 60, 1520–1522.

Bester, E., Butinar, B., Bucar-Miklavcic, M., & Golob, T. (2008) Chemical changes in extra virgin olive oils from Slovenian Istra after thermal treatment. Food Chemistry 108, 446–454.

Casal, S., Malheiro, R., Sendas, A., Oliveira, B.P., & Pereira J.A. (2010) Olive oil stability under deep-frying conditions. Food and Chemical Toxicology 48, 2972–2979.

Chatzilazarou, A., Gortzi, O., Lalas, S., Zoidis, E., & Tsaknis, J. (2006) Physicochemical changes of olive oil and selected vegetable oils during frying. Journal of Food Lipids 13, 27–35.

Cuesta, C., Sanchez-Muniz, F.J., & Hernandez, I. (1991) Evaluation of non-polar methyl esters by column and gas chromatography for the assessment of used frying olive oils. Journal of the American Oils Chemists' Society 68, 443–445.

DeMan, J.M., & DeMan, L. (1984) Automated AOM test for fat stability. Journal of the American Oils Chemists' Society 61, 534–536.

DeMan, J.M., Tie, F., & DeMan L. (1987) Formation of short chain volatile organic acids in the automated AOM method. Journal of the American Oils Chemists' Society 64, 993–996.

De Marco, E., Savarese, M., Parisini, C., Battimo, I., Falko, S., & Sacchi, R. (2007) Frying performance of a sunflower/palm oil blend in comparison with pure palm oil. European Journal of Lipid Science and Technology 109, 237–246.

Dieffenbacher, A., & Martin E. (1987) Determination of emulsifiers in foods: separation of polar and non-polar lipids by chromatography in silica-gel microcolumns. Revue Franchais des Corps Gras 34, 323–328.

Dobarganes, M.C., Márquez-Ruiz, G., & Perez-Camino, M.C. (1993) Thermal stability and frying performance of genetically modified sunflower seed (*Helianthus annuus* L.) oils. Journal of Agricultural and Food Chemistry 41, 678–681.

Dobarganes, M.C., & Perez Camino, M.C. (1988) Systematic evaluation of heated fats based on quantitative analytical methods. Journal of the American Oils Chemists' Society 65, 101–105.

Dobarganes, M.C., Velasco, J., & Dieffenbacher, A. (2000) Determination of polar compounds, polymerized and oxidized triacylglycerols, and diacylglycerols in oils and fats: results of collaborative studies and the standardized method (technical report). Pure Applied Chemistry 72, 1563–1575.

Farhoosh, R., & Tavassoli-Kafrani, M.H. (2011) Simultaneous monitoring of the conventional qualitative indicators during frying of sunflower oil. Food Chemistry 125, 209–213.

Firestone, D. (1996) Regulation of frying fats and oils in deep frying: chemistry, nutrition and practical applications. AOCS Press, 323–334.

Firestone, D. (2007) Regulation of frying fats and oils in deep frying: chemistry, nutrition and practical applications, 2nd ed. AOCS Press, 373–385.

Firestone, D., Stier, R.F., & Blumenthal, M. (1991) Regulation of frying fats and oils. Food Technology, 45, 90–94.

Frankel, E.N. (1998) Antioxidant activity by headspace gas chromatography of volatile oxidation products of ω-3 and ω-6 polyunsaturated lipids. Methods in Enzymology 299, 190–201.

Frankel, E.N. (2010) Chemistry of extra virgin olive oil adulteration, oxidative stability, and antioxidants. Journal of Agricultural and Food Chemistry 58, 5991–6006.

Fritch, C.W. (1981) Measurements of frying fat deterioration: a brief review. Journal of the American Oils Chemists' Society 58, 746–750.

Gertz, C. (2000) Chemical and physical parameters as quality indicators of used frying fats. European Journal of Food Science and Technology 102, 566–572.

Gil, B., Cho, Y.J., & Yoon, S.H. (2004) Rapid determination of polar compounds in frying fats and oils using image analysis. Lebensmittel-Wissenschaft & Technologie 37, 657–661.

Gliszczynska-Swiglo, A., & Sikorska, E. (2004) Simple reversed-phase liquid chromatography method for determination of tocopherols in edible plant oils. Journal of Chromatography A 1048, 195–198.

Gliszczynska-Swiglo, A., Sikorska, E., Khmelinskii, I., & Sikorski, M. (2007) Tocopherol content in edible plant oils. Polish Journal of Food and Nutrition Sciences 57, 157–161.

Gordon, M.H., & Kourimska, L. (1995) Effect of antioxidants on losses of tocopherols during deep-fat frying. Food Chemistry 52, 175–177.

Graziano, V.J. (1979) Portable instrument rapidly measures quality of frying fat in food service operations. Food Technology 33, 50–56.

Guillaumin, R. (1988) Kinetics of fat penetration in food. In: Frying of food: principles, changes, new approaches, ed. G. Varela, A.E. Bender, & I.D. Morton. Ellis Horwood Ltd, Chichester, 82–90.

Gutierrez, R., Gonzalez, O., & Dobarganes, M.C. (1988) Analytical procedures for the evaluation of used frying fats. In Frying of food: principles, changes, new approaches, ed. G. Varela, A.E. Bender, & I.D. Morton. Ellis Horwood Ltd, Chichester, 141–154.

Hara, K., Hasegana, K., Endo, Y., & Fugimoto K. (1994) Polymer and polar material content determination as basis for assessing thermally oxidative deterioration of soybean oil for potato heat cooking. Journal of Japan Oil Chemists' Society 43, 57–60.

International Union of Pure and Applied Chemistry (IUPAC). (1987) Standard Method 2.507: Determination of polar compounds in frying fats. In: Standard methods for the analysis of oils, fats and derivatives, 7th ed., ed. IUPAC. Blackwell, Oxford.

Kalogeropoulos, N., Mylona, A., Chiou, A., Ioannou, M.S., & Andrikopoulos, N.K. (2007) Retention and distribution of natural antioxidants (a-tocopherol, polyphenols and terpenic acids) after shallow frying of vegetables in virgin olive oil. Lebensmittel-Wissenschaft & Technologie 40, 1008–1017.

Karakaya, S., & Simsek, S. (2011) Changes in total polar compounds, peroxide value, total phenols and antioxidant activity of various oils used in deep fat frying. Journal of the American Oils Chemists' Society 88, 1361–1366.

Katragadda, H.R., Fullana, A., Sidhu, S., & Carbonell-Barrachina, A.A. (2010) Emissions of volatile aldehydes from heated cooking oils. Food Chemistry 120, 59–65.

Kiritsakis, A. (1988) Olive oil: from the tree to the table, 2nd ed. Trumbull, CT: Food and Nutrition Press.

Lalas, S. (2009) Quality of frying oil. In Advances in deep-fat frying of foods, ed. S. Sahin & S.G. Sumnu. CRC Press, Boca Raton, FL, 57–80.

Laubli, M., & Bruttel, P. (1986) Determination of the oxidative stability of fats and oils: Comparison between the active oxygen method (AOCS Cd 12-57) and the Rancimat method. Journal of the American Oils Chemists' Society 63, 792–795.

Lea, C.H. (1960) On the antioxidant activities of the tocopherols. II-influence of substrate, temperature and level of oxidation. Journal of Food Science and Agriculture 11, 212–215.

Marinova, E.M., Seizova, K.A., Totseva, I.R., Panayotova, S.S., Marekov, I.N., & Momchilova, S.M. (2012) Oxidative changes in some vegetable oils during heating at frying temperature. Bulgarian Chemical Communications 44, 57–63.

Mariod, A., Matthaus, B., Eichner, K., & Hussein, I.H. (2006) Antioxidant activity of extracts from *Sclerocarya birrea* kernel oil cake. Grasas y Aceites 57, 361–366.

Marmesat, S., Morales, A., Velasco, J., Ruiz-Méndez, M.V., & Dobarganes, M.C. (2009) Relationship between changes in peroxide value and conjugated dienes during oxidation of sunflower oils with different degree of unsaturation. Grasas y Aceites 60, 155–160.

Marmesat, S., Rodrigues, E., Velasco, J., & Dobarganes, M.C. (2007a) Quality of used frying fats and oils: comparison of rapid tests based on chemical and physical oil properties. International Journal of Food Science and Technology 42, 601–608.

Marmesat, S., Velasco, J., Márquez-Ruiz, G., & Dobarganes, M.C. (2007b) A rapid method for determination of polar compounds in used frying fats and oils. Grasas y Aceites 58, 179–183.

Márquez-Ruiz, G., Jorge, N., Martín-Polvillo, M., & Dobarganes, M.C. (1996) Rapid, quantitative determination of polar compounds in fats and oils by solid-phase extraction and size-exclusion chromatography using monostearin as internal standard. Journal of Chromatography A 749, 55–60.

Martínez-Pineda, M., Ferrer-Mairal, A., Vercet, A., & Yagüe C., (2011) Physicochemical characterization of changes in different vegetable oils (olive and sunflower) under several frying conditions. CyTA – Journal of Food 9, 301–306.

Mateos, R., Ucedam, M., Aguilera, M.P., Escuderos, M.E., & Maza, G.B. (2006) Relationship of Rancimat method values at varying temperatures for virgin olive oils. European Food Research and Technology 223, 246–252.

Melton, S.L., Jafar, S., Sykes, D., & Trigiano, M.K. (1994) Review of stability measurements for frying oils and fried food flavor. Journal of the American Oils Chemists' Society 71, 1301–1308.

Pantzaris, T.P. (1998) Comparison of monounsaturated and polyunsaturated oils in continuous frying. Grasas y Aceites 49, 319–325.

Peeled, N., Gutfinger, T., & Letan, A. (1975) Effect of water and BHT on stability of cottonseed oil during frying. Journal of Food Science Agriculture 26, 1655–1668.

Perkins, E.G. (1967) Non-volatile decomposition products in heated fats and oils. Food Technology 21, 611–616.

Pokorny, J. (2002) Frying. In: The nutrition handbook for food processors, ed. C.J.K. Henry & C. Chapman. CRC Press, New York, 162–178.
Quiles, J.L., Ramirez-Tortosa, M.C., Gomez, Y.A., Huertas, J.R., & Mataix J. (2002) Role of vitamin E and phenolic compounds in the antioxidant capacity, measured by ESR of virgin olive, olive and sunflower oils after frying. Food Chemistry 76, 461–468.
Saguy, I.S., & Dana, D. (2003) Integrated approach to deep fat frying: engineering, nutrition, health and consumer aspects. Journal of Food Engineering 56, 143–152.
Sanchez-Gimeno, A.C., Negueruela, A.I., Benito, M., Vercet, A., & Oria, R. (2008) Some physical changes in Bajo Aragon extra virgin olive oil during the frying process. Food Chemistry 110, 654–665.
Sanchez-Muniz, F.J., & Bastida, S. (2003) Frying oil discarding: polar content vs. Oligomer content determinations. Forum of Nutrition 56, 345–347.
Santos, C.S.P., Cruz, R., Cunha, S.C., & Casal, S. (2013) Effect of cooking on olive oil quality attributes. Food Research International 54, 2016–2024.
Siragakis, G., Maistrou, E., & Christofakis, M. (2012) Profile of Tocopherols in Greek Olives and Olive oils. Ann of Nutrition & Metabolism 60, 131–145.
Siragakis, G., Miliadis, G., & Vrettakou, A. (2014) Reduction of acrylamide in potato chips using olive oil as frying mean. Immunoassay vs LC-MS/MS techniques for acrylamide determination, Food Allergens Laboratory Olivebioteq 2014, Book of Abstracts, 147.
Smith, S.A., King, R.E., & Min, D.B. (2007) Oxidative and thermal stabilities of genetically modified high oleic sunflower oil. Food Chemistry 102, 1208–1213.
Sonntag, N.O.V. (1991) Erucic, behenic: Feedstocks of the 21st century. Inform 2, 449–463.
Tabee, E., Azadmard-Dimirchi, S., Jagerstad, M., & Dutta, P.C. (2008) Effects of α-tocopherol on oxidative stability and phytosterol oxidation during heating in some regular and high-oleic vegetable oils. Journal of the American Oils Chemists' Society 85, 857–867.
Takeoka, G.R., Full, G.H., & Dao, L.T. (1997) Effect of heating on the characteristics and chemical composition of selected frying oils and fats. Journal of Agricultural and Food Chemistry 45, 3244–3249.
Teixeira, J.A., (2006) Report Oleo Test^{TM}. University of Minho, Department of Biological Engineering, Braga, Portugal.
Totani, N., Yawata, M., Takada, M., & Moriya, M. (2007) Acrylamide content of commercial frying oil. Journal of Oleo Science 56, 103–106.
Tsaknis, J., & Lalas S. (2002) Stability during frying of Moringa oleifera seed oil variety "Periyakulam 1." Journal of Food Composition and Analysis 15, 79–101.
Tyagi, V.K., & Vasishtha, A.K. (1996) Changes in characteristics and composition of oils during deep fat frying. Journal of the American Oils Chemists' Society 73, 499–506.
Valdes, A.F., & Garcia, A.B. (2006) A study of the evolution of the physicochemical and structural characteristics of olive and sunflower oils after heating at frying temperatures. Food Chemistry 98, 214–219.
Velasco, J., & Dobarganes, C. (2002) Oxidative stability of virgin olive oil. European Journal of Lipid Science and Technology 104, 661–676.
Vrettakou, A., Miliadis, G., & Siragakis, G. (2014) Determination of acrylamide in light and high fat content food by different variations of the quenchers method. In: 8th European Congress on Pesticides and Related Organic Micropollutants: book of abstracts, 53–54.
White, P. (1991) Methods for measuring changes in deep-fat frying oils. Food Technology 45, 75–80.
Wolf, I.P., Mordret, F.X., & Dieffenbacher A. (1991) Determination of polymerized triglycerides in oils and fats by high performance liquid chromatography. Pure Applied Chemistry 63, 1163–1171.
Xu, X.-Q. (2000) A new spectrophotometric method for rapid assessment of deep frying oil quality. Journal of the American Oils Chemists' Society 77, 1083–1086.
Yamsaengsung, R., & Moreira, R.G. (2002) Modelling the transport phenomena and structural changes during deep fat frying. Part II: model solution and validation. Journal of Food Engineering 53, 11–25.

14 Olive oil packaging: recent developments

Michael G. Kontominas

14.1 Introduction

Virgin olive oil (VOO) and extra virgin olive oil (EVOO) are natural fruit juices obtained from olive drupes, using exclusively mechanical procedures, without chemical treatments. They are produced in numerous countries and regions around the world, including southern Europe, north Africa, the Middle East, the United States, and Australia, among others. The Mediterranean basin, including Spain, Italy, Greece, Tunisia, Turkey, Morocco, Algeria, Syria, and Portugal, account approximately for 95% of the world production of olive oil, which recently (2003–2012) averaged 2946 kilotons globally per year (Abbadi *et al.*, 2014). Of this, 85% is consumed in the Mediterranean countries.

Given olive oil's health-promoting properties, it is expected that its demand, especially that of EVOO, will increase in the immediate future (Accorsi *et al.*, 2015). In order, however, for it to retain its long-term beneficial properties, it must be properly processed, packaged, and stored under specified conditions because of its sensitivity to various factors such as oxygen, light, and elevated temperatures. Such conditions ensure that the product will not be subjected to deterioration or changes regarding its nutritional content as well as its sensory attributes (Kiritsakis, 1998; Kiritsakis *et al.*, 2002).

Quality of olive oil is defined as the combination of those attributes that determine the degree of its acceptability by the consumer. It may also be defined by both nutritional and sensory perspectives. Based on the above quality parameters, olive oil is considered superior to other vegetable oils.

The quality of olive oil decreases during both processing and storage due to autoxidation and photooxidation that lead to rancidity and due to hydrolytic degradation leading to the production of free fatty acids (FFAs). Preserving the positive attributes of olive oil is of paramount importance for the olive oil industry during the time elapsing from production to purchasing and consumption. Proper packaging and bottling of olive oil are considered very important for preventing its quality deterioration before the time of consumption.

14.2 Migration aspects during packaging

Migration is an important safety aspect to be considered when selecting food-packaging materials (Goulas *et al.*, 2007). Considerable work has been carried out on the migration of plastics additives, processing aids, and residual monomers or oligomers from food-grade polyvinylchloride (PVC) and polyvinylidenechloride (PVDC) packaging materials into aqueous and fatty foods, including olive oil (Kondyli *et al.*, 1990; Badeka & Kontominas, 1996; Goulas *et al.*, 1998; Zygoura *et al.*, 2011). Such low-molecular-weight compounds possess high mobility as compared to the macromolecular polymer backbone and tend to migrate into the food-contacting medium. Migration can thus adversely affect the flavor, acceptability, and even safety of the food. The chemical nature and polarity of the packaging material have notable effects on migration. According to Kondyli *et al.* (1990, 1992), Badeka *et al.* (1999), Goulas *et al.* (2000, 2007, 2008), and Zygoura *et al.*

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(2011), edible oils and fatty foodstuffs should not be stored in PVC or PVDC plastic materials because additives such as plasticizers and stabilizers can migrate into the food, resulting in food product contamination.

In particular, Kondyli *et al.* (1990) studied the migration of dioctylphthalate (DOP) and dioctyl-adipate (DOA) plasticizers from food-grade PVC films into commercial olive oil. Olive oil was chosen as a fat simulant. Experiments were carried out at 6, 22, and 30 °C with and without agitation as a function of time (0–100 h). Two different PVC films were used containing 31.5% DOA and 29.5% DOP, respectively. Results showed a significantly higher amount of plasticizer migration into oil with agitation than without (30 vs. 22.2% for DOP after 100 h). The equilibrium amount of DOA migrated was higher than the respective amount of DOP, whereas diffusion coefficients determined were slightly higher for DOP than DOA. The findings were related to the solubility, compatibility, and molecular weight of the plasticizer.

Castle *et al.* (1991) investigated the preferential migration of oligomers from PVC into olive oil according to size. The smaller oligomers migrated 90-fold more readily than the bulk of the plasticizer. PET, on the other hand, is one of the most inert among plastics, and in recent years packaging of oil into PET bottles has increased. Nevertheless, PET monomers, oligomers, and colorants as well as degradation products are all prone to migration. The migration of acetaldehyde from PET bottles is a major problem, as its presence may affect the sensory properties of oil (Tsimis & Karakasides, 2002). Migrating PET oligomers have been measured, and the cyclic trimer was the most dominant. Relatively limited data concerning the influence of migration processes from PET on the quality of olive oil are available; in general, PET bottles are usually considered suitable to contain not only seed oil but also olive oil (Kaya *et al.*, 1993; Cecchi *et al.*, 2010).

14.3 Flavor scalping

In recent years, plastics have been increasingly employed to package vegetable oils, due to their low weight, ease of handling, and competitive cost (Kaya *et al.*, 1993; Gambacorta *et al.*, 2004). Plastic packaging materials, depending on their polarity, have the tendency to absorb compounds of similar polarity from the food, a phenomenon known as "scalping" (sorption) (Brody, 2002; Revi *et al.*, 2013). In particular, "flavor scalping" is a term used to describe the loss of quality of a packaged food due to the absorption of a part of its volatile flavor compounds by the packaging material. Besides volatiles, nonvolatile compounds may also be absorbed by the packaging material, but in this case, these may affect the packaging material characteristics such as permeability and mechanical properties. Kanavouras *et al.* (2004) studied the role of plastic materials as potential flavor sorbents for olive oil. Flavors dissolved in the oil were readily absorbed by low-density polyethylene (LDPE), with the flavor concentration and storage temperature affecting the absorption of aroma compounds.

Sorption of oil by nonpolar packaging materials causes swelling of the polymer, which in turn increases migration. The sorption of fatty acids increases with increasing chain length due to increased van der Waals bonds between the polymer and fatty acid. The sorption of olive oil flavor compounds by polymeric plastic materials during storage can result in a considerable decrease in oil quality due to partial loss in oil aroma.

Several investigations have shown that considerable amounts of aroma compounds can be adsorbed by plastic packaging materials, resulting in loss of aroma intensity or unbalanced flavor of the food (van Willige *et al.*, 2000a, 2000b; Revi *et al.*, 2013). Sorption may also indirectly affect food quality by causing delamination of multilayer packaging materials (Olafsson & Hildingsson, 1995) or by altering the barrier and mechanical properties of plastic packaging materials (Tawfek & Huyghebaert, 1999). Oxygen permeability through the package may increase as a result of such interactions, but unfortunately, very little information is available in the literature on this subject. Van Willige *et al.* (2002) studied the effects of flavor and off-flavor adsorption on the oxygen permeability of LDPE, PP, polycarbonate (PC), and PET. Absorption of some volatile substances (limonene, decanal, 2-nona-none, and hexylacetate) increased oxygen permeability of PP and LDPE. The oxygen permeability of PET was not affected by the presence of flavor compounds.

14.4 Effect of packaging materials on olive oil quality

According to Regulation 1019/2002/CE (European Union, 2002) olive oil shall not be sold in bulk but only as a standardized, packaged product. Until retail packaging, olive oil must be stored at constant temperature,

about 14–15 °C, protected from light and oxygen. The preferred packaging material for such applications are large vessels (capacity 10–20 tons) made of stainless steel (bulk packaging), with the capability of flushing their headspace with nitrogen.

There has been considerable research during the past decade regarding the suitability of various retail packaging materials for maintaining the quality of olive oil. The major commercial packaging materials used for this purpose include glass, metals (tinplate and stainless steel), plastics (mainly PET), and composites (Tetra Brik/Tetra Prisma, bag-in-box, and single-use multilayered sachets). These will be further discussed in detail in this section.

14.4.1 Glass

The first glass containers were produced as early as 3000 BC. Glass is formed principally by silica obtained from sand, flint, or quartz. Silica is fused at very high temperatures (about 1720 °C) to form silicate glass. In most cases, silica is mixed at variable proportions with several raw materials or additives: that is, sodium and potassium carbonates (acting as stabilizers and for the protection of glass from water solubilization), lead (conferring transparency and lightness), and aluminum (increasing its hardness). Glass is neither a solid nor a liquid but rather a glassy material. The basic structural unit of glass is that of the silica–oxygen tetrahedron, in which a silicon atom is surrounded by four oxygen atoms to form a tetrahedron. Large groups in glass structure tend to increase disorder (amorphous structure), giving the glass fragility with the tendency to rupture if submitted to an excessive tension (Robertson, 2009). Glass bottles (Figure 14.1) can be produced in different forms and sizes, and their color can vary largely from colorless to green and more dark tints to protect the contents from the effect of light. The most popular bottle volume is 0.75 L, followed by 0.5 L. Research carried out in 2005 on 48 producers of organic olive oil in Italy indicated that the type of bottle largely used is that of 0.75 L (about 85% of the total), followed by 0.50 L (about 58%) and 0.1 L sizes (about 17%) (Paffarini, 2007).

According to Ricci (2007), 64% of Italian olive-bottling plants use green glass, while only 10% of the total choose colorless glass. It is advisable to use very dark-tinted glass for better protection from light. On the other hand, the consumer appreciates transparent glass that reveals the oil color. In such cases, it is advisable to supply the bottle with a paperboard case (usually a cylindrical drum) that protects the product from light. Nitrogen is most often used to protect oil from contact with air and to remove oxygen from the container headspace.

During commercial bottling, special apparatus are used that saturate the bottle headspace with nitrogen in order to avoid the presence of oxygen above the oil. Alternatively, during the bottle-filling operation, bottles are hooked up by a special machine accessory, which inverts them and blows liquid nitrogen inside, which evaporates when it comes in contact with the environment and removes the oxygen present inside the bottle. Then, the bottle is straightened up and proceeds toward the filling operation, during which nitrogen

Figure 14.1 Olive oil packaging in glass bottles or glass–paperboard combinations.

stays inside because it is heavier than air. As the bottle is filled up and the oil level is raised, nitrogen exits the bottle, avoiding oxygen penetration. The speed of successive capping assures the presence of nitrogen between the cap and the oil (Soressi, 2009).

Bottle capping can be carried out by means of cork or more frequently by metal caps. A good-quality cork should be nonporous and prevent fungal contamination. It may, however, cause early deterioration, due to its high oxygen permeability. Metal screw-type caps including a plastic soft liner provide a perfect airtight seal (Ricci, 2007).

Studies have demonstrated, in fact, that EVOO collected from the previous harvesting season and stored under nitrogen atmosphere could be packaged in glass bottles without appreciable quality changes, as compared to seasonal EVOO packaged in similar bottles (Guil-Guerrero & Urda-Romacho, 2009).

These researchers ran a shelf-life study carried out on olive oils produced from the 'Picual', 'Hojiblanca', and 'Arbequina' cultivars packaged in dark and transparent glass bottles. The oils showed a decrease in some quality parameters during storage (i.e., the variation of peroxide value [PV] was significant in EVOOs stored in transparent glass). Several studies (as discussed further in this subsection) conducted on olive oil shelf life reported glass to be the best material for storage (Kanavouras *et al.*, 2006; Pristouri *et al.*, 2010; Rababah *et al.*, 2011). Finally, in a comparative study using, along with glass, high-density polyethylene (HDPE) and polyethyleneterephthalate (PET) containers, the results clearly indicated that glass was the best in the order of glass > HDPE > PET (Ben Tekaya *et al.*, 2007).

Vacca *et al.* (2006) studied changes in quality parameters, antioxidant compounds, oxidative stability, and antioxidant activity of EVOO from the 'Bosana' cultivar, exposed to light and dark during storage for a period of 18 months. Analysis of data showed that all the parameters underwent significant changes during storage: free acidity, PV, and ultraviolet (UV) spectrophotometric indices remained below the limits reported in EEC Regulations 2568/91 and 1989/03. Phenol and α-tocopherol contents decreased during storage (42.0 and 29.6%, respectively), while chlorophylls and carotenoids underwent a decrease until eight months of storage (49 and 30%, respectively); after that, the values of these parameters remained constant. Oxidative stability and antioxidant activity did not change substantially during the entire storage period. Phenols were significantly correlated with antioxidant activity of the oil, while oxidative stability measured by Rancimat did not show any correlation with carotenoids, chlorophylls, phenols, and α-tocopherol. Regarding exposure conditions, storage in the dark was more effective in retaining the quality of the oil.

14.4.2 Metals

14.4.2.1 Tinplate

Steel is an iron–carbon alloy. Tinplate is a sheet of soft steel that is more workable because of its lower carbon content. It is coated on both sides by layers of tin oxides and on the food-contact side by organic synthetic (usually epoxy-related) lacquers. Tinplate containers used in olive oil packaging are shown in Figure 14.2. Their side seam is welded and protected with a food-approved special varnish. The welding method assures a secure side seam, avoiding the dissolution of lead into the product (Tsimis & Karakasides, 2002).

For the storage of large volumes of olive oil, stainless steel is the suggested packaging material. This is an alloy containing 11% chrome, which reacts with oxygen to form an auto-passivation condition due to the formation of chrome oxides (Pergiovanni & Limbo, 2010). Compared to glass, it has the same light, oxygen, and water vapor barrier properties. Tinplate is used for the retail packaging of olive oil, both in larger volume sizes (3–5 L) and in smaller containers (0.5–1 L).

Rababah *et al.* (2011) evaluated changes in physicochemical (PV, acidity, total phenolics, antioxidant activity and % sedimentation) and sensory attributes of olive oil stored in different packaging materials at 25 °C for a period up to 60 days. The olive oil samples from a local company (Ajlon-Jordan) were stored in 2.5 L containers made from clear glass, tinplate, and polypropylene (PP). Results showed that as time increased from 0 to 60 days, the acidity and PVs of the oil increased while antioxidant activities, total phenolics, and sensory attributes decreased. The samples in the glass container exhibited the lowest acidity values (1.25–1.53%) and PVs (6.13–7.17 meq/kg), followed by those stored in the PP and tinplate containers. The lowest antioxidant activities and total phenolics values were recorded in oil from the tinplate container, while no significant difference ($p \leq 0.05$) was found for samples in the glass and PP containers. The oil in the tinplate container had the highest values of sedimentation (0.17%). The glass container provided the

Figure 14.2 Olive oil packaging in cylindrical and parallelepipedal tinplate containers.

best protection to oil samples as indicated by the best sensory properties, followed by the plastic and tinplate containers.

In a study conducted by the Fraunhofer Institute for Process Engineering and Packaging in Germany (Fraunhofer Institute, n.d.; www.oliveoilone.com), the following packaging materials were tested to package EVOO: 1 L PET plastic bottles, 500 mL clear glass bottles, and 1 L tinplate containers. EVOO was provided in large 5 L tinplate containers. The different types of bottles and containers were filled with olive oil and sealed. Half-filled bottles were also tested. These were not tightly sealed, but just as one would seal them at home. Bottles and containers were then stored at 20 °C temperature, 50% humidity, and under ample light for three months. Periodically, the following analytical parameters were monitored: (a) the induction period, which corresponds to the oxidative stability of the remaining olive oil (using a Rancimat test at 120 °C); (b) the content of tocopherols in the olive oil, which shows the rate at which the vitamins in the oil decompose (using high-performance liquid chromatography, after separation of tocopherols from methanol); (c) the detection of hexanal and pentane, the two malodorous volatile compounds that are formed during the oxidative process and are responsible for the oil's distinct rancid taste (using gas chromatography); and (d) color evaluation that visually shows the quality deterioration of the olive oil (using a Minolta or similar chromatometer). The study showed that in the sealed tinplate containers, a minimal decrease in tocopherols occurred, which stabilized with time. With the sealed PET and glass bottles, the decrease in tocopherols did not stabilize but, on the contrary, intensified over time, especially with the PET bottles, which besides being transparent are also somewhat permeable by oxygen. The tinplate containers that were not tightly sealed showed a greater decrease in the amount of tocopherols. This most likely occurred because of the self-oxidizing process taking place inside the container due to the excess amount of atmospheric oxygen present in the container headspace. In the PET and glass bottles, the tocopherols in olive oil decreased sharply at the same rate and approached zero, since the diffusion of atmospheric oxygen inside the bottles no longer played a significant role.

Regarding the induction period, the study showed that in the sealed containers, the oxidative stability in the olive oil decreased along with a decrease in tocopherol concentration. Based on these results, it is obvious that the tinplate containers ensure the best possible protection for olive oil, followed by the glass bottles, and then the PET bottles (where things became worst due to the penetration of oxygen through this material).

The situation with the unsealed containers was similar, with the tinplate containers providing the best possible protection of the olive oil. In samples that were not tightly sealed, the adequate atmospheric oxygen contained therein allowed for very minor differences between the glass and PET bottles.

Pentane is one of the end products of the oxidative process and represents the deterioration of the olive oil that becomes noticeable regarding taste. Due to the high concentration of pentane, it should only be

measured in sealed containers. This is because in the containers that were not tightly sealed, part of the pentane that formed had already evaporated from the bottles and containers before the predetermined sampling time even began. The study showed that in the sealed tinplate containers, no pentane was detected. On the contrary, significant amounts of pentane were detected in the glass and PET bottles, making the progress of the oxidative process detectable in the taste of the olive oil. The fact that the concentration of pentane appeared in smaller amounts in the PET bottles is misleading and may be explained by the ability of this volatile substance to partially escape through permeation from the plastic containers. Regarding color, the study showed no significant differences in the color of the olive oil among the samples, with the exception of the non-sealed plastic bottles, which showed a deeper green color after only one week.

The study concluded that the best packaging material for the retail distribution of olive oil is the tinplate container. This is due to the complete blockage of light and oxygen, rendering autoxidation and photooxidation impossible. The latter reaction is catalyzed by chlorophyll being responsible for the deterioration of the quality of the olive oil. Because tinplate containers are impermeable to light, the complete removal of oxygen during packaging of the product is not necessary. The saturated fatty acids that are found in olive oil and especially the monounsaturated oleic acid are not particularly prone to autoxidation. On the contrary, since PET and glass bottles are penetrable to light, they must be sealed airtight to keep oxygen away from the oil. At the same time, tinplate containers combine the desirable characteristics of the other two packaging materials, specifically the light weight and durability of plastic and the complete airtightness of glass.

14.4.2.2 Stainless steel

Stainless steel provides excellent protection from oxygen, light, humidity, and microorganisms. It is the best bulk storage option for olive oil producers and processors. However, its high cost and heavy weight make it less suitable for commercial retail packaging (Wang *et al.*, 2014).

Dabbou *et al.* (2011) investigated the effect of packaging materials (stainless steel, clear and dark glass bottles, clear PET, and clay jars) on quality attributes (acidity, PV, K_{232}, K_{270}, fatty acid analysis, pigments, and total phenols) of EVOO from Tunisia as a function of storage time (0 to 12 months) under diffused light and room temperature. The results showed significant effects of storage time and the type of container on the acidity of oils.

In clear glass, PVs increased up to a maximum (reached after nine months of storage) and then decreased. This behavior can be explained by the initial formation of hydroperoxides (odorless, flavorless compounds produced during the primary step of oxidation) and their subsequent decomposition into aldehydes and ketones. These latter compounds are responsible for off-flavors (secondary oxidation products).

The least stable oils are those stored in the clay jars with a progressive decrease in quality attributes. A clear reduction in antioxidant content (carotenes, chlorophylls, and total phenols) was observed in the oils stored in clay jars and PET. Overall, the results showed that the best packaging material for olive oil was stainless steel, followed by glass. PET and clay jars proved to be unsuitable for such an application. Exposure of olive oil samples to light and room temperature caused substantial deterioration in product quality parameters.

14.4.2.3 Aluminum

Aluminum packaging for olive oil has not been studied extensively. Aluminum provides excellent protection from light and oxygen, but its high cost is a disadvantage. It is suggested to coat the interior of the aluminum container with a food-grade enamel to prevent the migration of toxic aluminum ions from the package to the oil; such a migration causes the deterioration of olive oil quality. Aluminum is nowadays used as an internal layer in bag-in-box type containers and single-use multilayer sachets for the adequate protection of olive oil against oxygen and light (Wang *et al.*, 2014).

14.4.3 Plastics

Plastics are either synthetic or natural organic substances. They are composed of a macromolecular backbone made of repeating monomer units to which a number of additives and processing aids are added to improve

Figure 14.3 Olive oil packaging in PET, tinplate, single-use, and multi-use multilayer plastic pouches.

mechanical, physical, and chemical properties. Plastics have gained a large number of applications due to their versatility, low production cost, light weight, good performance, and recyclability.

Plastic containers are formed by various processes, including thermoforming, extrusion blow-molding, and injection blow-molding in the case of rigid materials, and extrusion, co-extrusion, and lamination in the case of flexible materials. Disadvantages of plastics include permeability to gases, vapors, and light (in case they are transparent) (Robertson, 2009); migration (Kondyli *et al*., 1990; Badeka *et al*., 1999; Tsimis & Karakasides, 2002; Goulas *et al*., 1995, 2007; Zygoura *et al*., 2011) and flavor scalping (van Willige *et al*., 2000a, 2000b; Revi *et al*., 2013). In the past, HDPE, PP, and PVC have been used to package olive oil (Robertson, 2009). After 1985, all of the above plastics have been replaced by PET in the olive oil market. PET bottles are produced by extrusion stretch-blow molding. Their advantages include low water vapor and oxygen permeability, light weight, clarity, exceptional mechanical properties, and resistance to fats and oils (Figure 14.3).

Pristouri *et al.* (2010) demonstrated that between PET and PP, PET provided better protection to olive oil than PP due to its significantly lower oxygen transmission rate. Moreover, Kiritsakis and Dugan (1984) reported that peroxide values were higher for olive oil packaged in polyethylene bottles as compared to those packaged in glass bottles stored in the dark.

Abbadi *et al.* (2014) evaluated different packaging materials in terms of their ability to protect the quality of Palestinian EVOO stored at two different temperatures in a six-month stability study. Olive fruits of the cultivar ‘Nabali Baladi’ were collected from the district of Salfeet in Palestine. Olives were washed and processed using a stone mill and hydraulic press. The extracted oil had PV < 20, acidity < 0.8%, K_{232} < 2.5, and K_{270} < 0.22, meeting the criteria of EVOO.

The EVOO was bottled (300 mL each) in different packaging materials (amber glass bottles, PET, HDPE, hermetically sealed tinplate cans, and pottery jars with covers), maintaining 2% headspace in each bottle. Bottled oil was stored under different storage temperatures (18±1 and 37±1 °C) inside incubators with 100 Lux normal white light for around 10 hours daily (simulating conditions on supermarket shelves). The samples were rearranged weekly to ensure uniform light exposure of the bottles. The effect of each of these factors (packaging materials and temperature storage conditions) on the stability of the EVOO was studied in a non-orthogonal design by monitoring oil quality indicators that included: acidity (percentage as oleic acid), PV, ultraviolet extinction coefficients (K_{232} and K_{270}), total phenolic content (expressed as mg of gallic acid/kg^{-1} oil), and sensory attributes. Sampling was carried out at 0, 30, 60, 90, and 180 days of storage. Results showed that the acidity of EVOO increased with storage time in all packaging materials. At 18 °C, acidity remained below 0.6% in all cases. At 37 °C, acidity reached the limit value of 0.8% in both tin cans and glass, while in HDPE, PET, and pottery it remained below 0.6%.

PVs progressively decreased because of the degradation of primary products into secondary products. The oil samples packaged in pottery and stored at both room and elevated temperatures had higher PVs

compared to those stored in other kinds of containers. After 90 days of storage, the PV of oil packaged in pottery exceeded the limit value of 20 meq oxygen/kg of oil. K_{232} values increased with storage time and temperature. At 18 °C, K_{232} values remained below the limit value of 2.5 throughout storage. At both temperatures, the lowest K_{232} values were recorded for the glass and tin can containers. At 37 °C, K_{232} exceeded this value in oils packaged in the HDPE and pottery containers. Likewise, at 18 °C, K_{270} remained below the limit value of 0.22 throughout storage for all containers. At 37 °C, K_{270} values exceeded this limit value after 180 days for HDPE, PET, and pottery containers. K_{270} values were affected by temperature more than K_{232}.

Phenolic content of EVOO decreased during storage in all packaging materials due to their degradation. At the end of the storage period, phenolic compounds of samples stored at 37 °C showed a significantly higher reduction than those stored at 18 °C in all types of packaging materials except those stored in PET bottles. The smallest reduction in phenolic compounds was recorded in glass and tin can containers (8–13%). Phenolic compounds act as natural antioxidants in oil and inhibit oxidation of lipids by trapping intermediate peroxyl radicals. Their reduction during storage is the result of oil oxidation.

Regarding sensory evaluation, at 18 °C the best type of container was either glass or HDPE, followed by cans and PET, while the worst container was pottery. At 37 °C, glass containers scored the highest and pottery scored the lowest; other types of containers scored in between.

It was concluded that, at 18 °C, K_{270} along with sensory evaluation were the dominant quality determinants for olive oil; while at 37 °C, the respective dominant parameter was K_{270} followed by sensory evaluation. Grading of stored olive oil under investigation using sensory evaluation without chemical analysis proved to be insufficient. It was shown that EVOO stored in glass bottles at room temperature maintained the extra virgin quality, whereas EVOO stored at elevated temperatures (37 °C) resulted in a sharp decrease in sensory score after less than two months of storage, losing its positive attributes (e.g., fruity apple, green) while developing negative attributes (e.g., winy, muddy, rancid).

Savarese *et al.* (2013) investigated the changes occurring in bottled EVOO in different PET containers. The changes of quality indices, sensory parameters, phenolic antioxidants, and pigments of EVOO were evaluated in relation to prolonged storage (12 months). Two EVOO samples were selected on the basis of their different acidity levels and phenolic contents and were bottled in transparent or red PET bottles. Bottles were placed in dark or light conditions at 25 °C, the latter simulating the normal conditions found in the supermarket. Lighting conditions were monitored using a digital chromatometer. During the night, bottles of EVOOs were exposed to a constant light intensity of 300 LUX provided by eight artificial neon lights, while daytime light intensity reached 500 LUX due to the effect of daylight.

Results showed a significant decrease in the contents of α-tocopherol and chlorophylls during the first month of storage. Light exposure greatly influenced these parameters. Tocopherols seem to have a crucial role in controlling and slowing down oil oxidation. α-Tocopherol showed a greater effect as compared to phenolics in reducing photooxidation processes. In relation to the container characteristics, it was observed that colored red bottles did not show a sufficient protection against light as compared to transparent PET bottles, while good protection of overall quality was obtained only through storage in the dark. Results suggest the importance of finding containers with adequate barrier properties against light and oxygen in order to extend the shelf life of EVOO packaged in PET containers.

Pristouri *et al.* (2010) compared the effects of packaging parameters (transmission to light and oxygen, headspace volume) and storage temperature on quality characteristics of EVOO as a function of storage time (0–12 months). Packaging materials tested included clear glass, clear PET, clear PET + UV blocker, clear PET covered with aluminum foil, and clear PP bottles. Quality parameters monitored over the 12-month storage period included acidity, PV, spectrophotometric indices (K_{232}, K_{270}), and color. Results showed that the best packaging material for olive oil packaging was glass followed by PET. PP proved to be unsuitable for such an application. Exposure of olive oil samples to light, high storage temperatures (35 °C), and large headspace volumes caused substantial deterioration in product quality parameters. The most pronounced effects were those of temperature and light, while the smallest effect was that of headspace volume and packaging material permeability to oxygen. Olive oil color was not substantially affected by storage conditions, with the exception of storage of olive oil at 35 °C exposed to light for 12 months. The shelf life of EVOO was six months when packaged in clear glass in the dark at temperatures up to 22 °C, three months in clear PET in the dark at 22 °C, and less than three months in clear PP in the dark at 22 °C. When exposed to light, the shelf life of olive oil was nine months when packaged in PET + aluminum foil, three months

in PET + UV blocker, and less than three months in clear PET at 22 °C. Product shelf life was less than three months at 35 °C. Finally, oxygen in the headspace of olive oil resulted in deterioration of product quality. The relative contribution of parameters studied to the retention of olive oil quality was in the order of temperature ≈ light > container headspace > packaging material oxygen permeability.

Vekiari *et al.* (2007) studied the effect of packaging material on the quality of Greek olive oil ('Koroneiki' cultivar) collected from the island of Crete. Olive oil was packaged in 1 L plastic containers made of PVC and transparent glass bottles, and stored at the temperature of 28 °C under different light conditions for ten months. Three different light intensities were studied; these were (a) intense artificial light, (b) diffused daylight, and (c) darkness. During storage, the acidity, PV, extinction coefficients K_{232} and K_{270}, and phenol content were determined once a month. The samples packaged in PVC presented higher PVs compared to those packaged in glass containers and exposed to all the light intensities. This finding was expected due to the permeability of PVC to oxygen.

Generally, during the first months of storage, an increase in PV in both packaging materials was observed as a consequence of the actions of both oxygen permeation and headspace oxygen in the containers, and of light, which induce a rapid deterioration. Then, the PV was progressively reduced because of the degradation into secondary products. In samples exposed to artificial light and diffused daylight, the maximum PV values were recorded during the second or third month of storage. On the contrary, samples stored in the dark and packaged in both glass and plastic containers attained their maximum PV during the sixth month of storage. This delay could be attributed to the absence of light.

Regarding the absorbance to UV, it was shown that K_{232} values were maintained under the limit of 2.5 units which has been established by IOOC while K_{270} values in some cases exceeded the limit of 0.22 units during the ten months of storage. Higher values of K_{232} were found in samples stored in PVC bottles due to the joint action of light and oxygen permeation that catalyzes the oxidation reaction. In samples stored in the dark in both packaging materials, the absorption at 232 and 270 nm was much lower.

Based on the data, but also on migration and environmental/recycling issues, PVC bottles do not comprise a suitable packaging material for olive oil. The study concluded that glass bottles, stored in the dark, preserve the quality of olive oil by providing superior protection from both autoxidation and photooxidation compared to PVC containers.

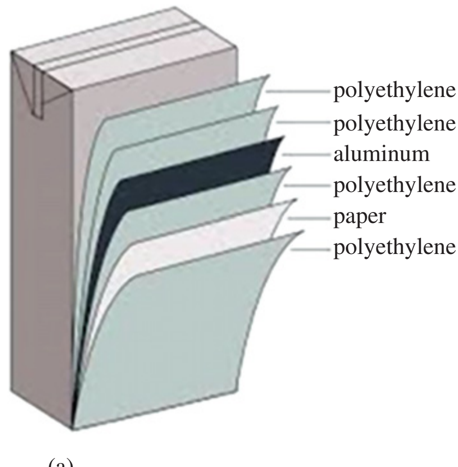
14.4.4 Composites

14.4.4.1 Tetra Brik and Tetra Prisma type containers

These are proprietary parallelepipedal-shaped multilayered packaging materials used for the packaging of fruit juices, milk, tomato juice, wine, and olive oil (Figure 14.4). From the outside to the inside, they are composed of LDPE, then paperboard, then LDPE acting as the adhesive between paperboard and aluminum, then aluminum, and then one or two layers of LDPE. In this structure, aluminum provides the high barrier to water vapor and oxygen. Tetra Brik and Tetra Prisma are both contemporary packaging materials that guarantee superior protection to olive oil's chemical, physical, and sensory properties for up to two years. Besides preserving the quality, Tetra Brik type containers provide the producer with logistic efficiency, lower probabilities of rupture during distribution, and 100% recyclability. It is used in olive oil packaging in countries such as Spain and Italy (Soressi, 2007).

In a study conducted by Mendez and Falque (2007), EVOO was packaged in clear PET bottles, PET bottles covered with aluminum foil, glass bottles, tin cans, and Tetra Brik containers. Olive oil was analyzed in order to evaluate the effects of packaging material and storage time on quality. The parameters used after three and six months of storage were acidity, peroxide index, absorption coefficients K_{270} and K_{232}, humidity (%), impurity content (%), phenol content, iodine value, saponification value, color, and fatty acid content. Olive oil was stored at room temperature with the same surface area of exposition to air and light. Results showed a gradual loss of quality during storage, especially in plastic or glass bottles. The best containers for commercial packaging of EVOO were tin cans and Tetra Brik. Performance of these two materials was based on their high barrier to light and oxygen as compared to transparent glass and PET bottles.

Four commercial samples of EVOO were analyzed in order to evaluate the influence of storage time on quality. The parameters used after three and six months of storage were again acidity, peroxide index, absorption coefficients K_{270} and K_{230}, humidity (%), impurity content (%), phenols content, iodine value,



(a)

(b)

Figure 14.4 Olive oil packaging in (a) Tetra Pak and (b) Tetra Prisma type containers.

saponification value, color, and fatty acid content. At the same time, the effects of container type on the deterioration in quality were studied. Each olive oil was stored in five different containers at room temperature with the same surface area of exposure to air and light; the containers were a clear PET bottle, a PET bottle covered with aluminum foil, a glass bottle, tin, and Tetra Brik. The results showed a gradual loss of quality during storage, especially in plastic or glass bottles. The best containers for commercial packing of EVOO were tin and Tetra Brik.

14.4.4.2 Bag-in-box type containers

Bag-in-box packaging consists of a multilayer plastic bag including a layer of aluminum (usually metallized polyester) placed inside a corrugated-fiber box. Aluminum provides a high barrier to oxygen, light, and water vapor. The type of plastics used for the bag can influence the shelf life of the oil. Bag-in-box packaging is similar to coated paperboard, additionally providing excellent protection from oxygen. It is inexpensive, lightweight, but not reusable. Bag-in-box packaging has been widely used in the wine industry (Revi *et al.*, 2013), but it is recently becoming more common in the commercial packaging of olive oil. It has the potential to become one of the best commercial packaging options (Wang *et al.*, 2014).

Mailer *et al.* (2012) designed a study in Australia to determine the effects of short- to long-term storage in collapsible containers (pouches) (Figure 14.5) on olive oil quality. A variety of collapsible and rigid materials composed of different polymers were selected for testing. The testing took place over a 12-month period. Packaging materials tested included four types of collapsible containers (clear LDPE, coextruded nylon laminate, medium-density polyethylene [MDPE] metallized polyester, and SF aluminum foil) and one rigid plastic container (white HDPE with a UV filter) of 20 L capacity. Each of the polymers had different porosity and oxygen transmission rates. A HDPE tap was fitted to the container to access the contents. The oil was dispensed into each of the four collapsible sample containers and the rigid plastic container. Each container was loaded to capacity to remove any oxygen from the container. The collapsible sample containers were stored in sealed black boxes in the laboratory under controlled temperature conditions. The rigid white plastic container was stored in the laboratory under the same temperature conditions. Throughout the study, samples were collected from all of the containers, including the original drum, to determine changes in oil quality. A sample from the original drum was analyzed at the time $t = 0$ to set a baseline for the quality of the original product. All of the containers were sampled and tested after 2, 4, 8, 16, 32, and 52 weeks. After sampling, each container was sparged with nitrogen to retard oxidation. The temperature was maintained at a constant 21 °C.

Figure 14.5 Olive oil packaging in bag-in-box type containers (outer paperboard carton is shown on the right).

Parameters monitored included PV, FFAs, total phenolic content, induction time, tocopherol content, UV absorption coefficients, pheophytin-α, pyropheophytin-α, diacylglycerols, and fatty acid profile.

Results showed that PV only in the LDPE container was significantly degraded during storage. Within only 12 weeks, the PV had started to increase, and by 48 weeks it had exceeded the limit value of 20 meq of oxygen/kg of oil. Although there was some reduction in quality for the aluminum foil collapsible container and the HDPE bottle, nylon and metallized polyester maintained almost the same initial PV throughout the storage period. The changes that occurred in LDPE, aluminum foil, and HDPE were the result of oxygen permeation through the polymer. For FFAs, their formation relies mostly on enzymatic reactions during processing. Since lipases were not present in processed olive oil, as expected, there was virtually no change in acidity in any of the samples.

The ability of the container to restrict the diffusion of air through its walls can be measured by the limited change in reduction of the content of antioxidant phenolics. This in turn is also observed by the limited change in PV. The changes in phenolic content and PV showed an inverse trend, highlighting the very strong effect of phenolic compounds on oil oxidation. No other component of the oil has such a close relationship to oil oxidation, and this suggests that PV is one of the best and simplest methods to determine oil deterioration.

The rapid increase in PV and the decrease in phenolic compounds of the LDPE container show the unsuitability of this container to store olive oil. In contrast, there was almost no change in either PV or phenolic content in the nylon or MDPE metallized polyester containers, showing their suitability for the packaging of olive oil when stored under appropriate environmental conditions over 12 months.

Induction time is a measure of the production of by-products as a result of accelerated oxidation. The induction time test produced data that are almost identical to those representing the change in phenolic compounds. The comparison of the increase in PV and the changes in induction time should be considered when estimating the shelf life of olive oil.

α-Tocopherol (vitamin E) is an effective antioxidant. The results obtained for α-tocopherol were quite different from those shown for phenolic antioxidants. In this case, α-tocopherol decreased in all containers despite the apparent absence of oxygen even in the containers providing the best protection. Within eight weeks, for oils in the four containers, its concentration decreased from around 340 to only 280 mg/kg of oil. From eight weeks onward, the reduction proceeded more slowly, with the LDPE container showing the most significant change. However, the change in α-tocopherol from 8 weeks to 52 weeks was relatively small, and even in the worst case, it was only reduced to 210 mg/kg oil. The rapid change in α-tocopherol in the initial stages may suggest that this compound is more sensitive to oxidation than other phenolics. It may have actually protected the phenolics, which did not show any substantial change for the first eight weeks. The PV also showed little change in the early period, but increased dramatically in the LDPE container after 8–12 weeks. This result would indicate that α-tocopherol has a very important role in protecting the oil between processing and bottling when it is exposed to oxidation sources. Once the oil is sealed from oxygen, phenolic compounds can maintain oil quality and relatively long-term stability.

Regarding the specific extinction coefficient K_{232}, there was a relatively rapid increase in absorption after eight weeks, particularly for the less suitable containers of LDPE and SF aluminum foil in line with the increased PV, due to the formation of primary oxidation products, mainly the peroxides. The subtler changes in K_{268} nm would suggest that there was little conversion of these primary into secondary products during this period. The lack of change in the K_{268} may be attributed to the low temperature at which the containers were stored based on the fact that all containers performed similarly. It would be expected that the K_{268} values would increase over time as secondary products develop. The slow increase in K_{268} values in all containers over the 12-month storage period provides valuable information when evaluating the quality of supermarket oils. Oil obtained from supermarkets is often found to have high UV 268 absorbance levels. Based on the present findings, it may be suggested that oils with high K_{268} values have been stored under poor conditions for considerable time, thus contributing to the production of the secondary products associated with off-flavor development.

Chlorophyll is the green pigment in fresh olive oil. The green color changes as the oil ages. Chlorophyll is converted to yellow and brown pigments, known as pheophytins and pyropheophytins (PPPs), respectively. The German Fat Society (DGF) has indicated that the proportion of PPPs to the total pheophytins is a good indicator of aged oil. In this study, the level of PPPs increased from around 3 to approximately 10%. Remarkably, the LDPE container, which had shown the least ability to maintain oil quality, produced the lowest level of PPPs. The HDPE container, which was generally a more suitable packaging material, showed the largest increase in PPPs. Other containers, including the light-impermeable MDPE metallized polyester container, all increased at similar rates. These data present new information on how PPPs may be used to evaluate olive oil quality. A high level of PPPs does not relate to an increase in peroxide or a decrease in antioxidant. PPPs can increase in the light or dark at the same rate, regardless of temperature. The relationship between PPPs and sensory quality was significantly lower than the relationship with diacylglycerols (DAGs) or K_{268}. It may be that there would be a much greater change in PPPs at higher temperatures, particularly those at which oil is generally refined.

DAGs are formed as fatty acids are lost from oil molecules (triacylglycerols). Although the fatty acids are lost to form 1,2-diacylglycerol, in time the fatty acids move to the 1,3- diacylglycerol position. The proportion of 1,3-diacylglycerol may serve as an indicator of the age of the oil. As the formation is accelerated at higher temperatures, it may also indicate some refining. As for PPPs, oil in all containers exhibited the same results with a continuous reduction in 1,2-diacylglycerol over the 52-week period.

As for PPPs, no relationship with the container was found, which indicates that the presence of oxygen (which is higher in the LDPE container) did not contribute to a decrease in 1,2-diacylglycerol. The most important outcome regarding the DAG experiment, however, is the continuous decline in DAG over time. Within 12 months, the level of 1,2-diacylglycerol decreased from 90 to 60%. Although all oils showed deterioration with no clear relationship to the plastic container, this method is an excellent indicator of oil that has been stored for a long time period. This aging can be accelerated if the oil is stored at higher temperatures. Regardless of the temperature, oil quality is reflected by low 1,2-diacylglycerol. This was also shown by Frankel *et al.* (2010, 2011). Fatty acid profiles determine the proportion of individual fatty acids within the total oil complex. Despite the changes in triacylglycerols and oxidative processes, there was no significant variation in the fatty acid profile.

Most containers tested in the study were adequate in maintaining the quality of EVOO over a 12-month period. The oil in the LDPE and silver aluminum foil containers did show some signs of oxidation occurring during the 12-month study. By the end of the study, the PV for the LDPE container was outside the legal limit set by the International Olive Oil Council (IOOC), which led to the K_{232} also being outside the legal limit.

Finally, it is noteworthy to mention a recent trend involving the marketing of 10 mL single-dose sachets, containing either 100% EVOO or olive oil mixed with vinegar, used as salad dressings. These containers have a similar structure to that of the Tetra Brik container with the exception of using paper rather than paperboard (Paffarini, 2007). Multilayer barrier pouches are also used for olive oil packaging (Figure 14.3).

14.4.4.3 Active packaging

In order to reduce the diffusion of O_2 into bottled olive oil, various solutions have been suggested. The most popular one involves the use of oxygen scavengers (OSs), which remove dissolved oxygen in the oil and

that present in the bottle headspace. In the case of plastic packaging, an OS also adsorbs oxygen permeating through the wall of the bottle into the oil during storage. OSs can be easily incorporated into the packaging material without altering its other properties. Sacchi *et al.* (2008) studied the autoxidation of EVOO and sunflower oil (SO) stored in PET bottles with two different OS concentrations (1 and 5%). The shelf-life study was carried out for six months at 25 °C under a constant illumination of 400 LUX. During the first three months of storage, the effect of scavengers was already evident as oils bottled in PET loaded with 5% of OS showed a dissolved oxygen (DO) content lower than that of oils bottled in PET with 1% OS and in standard PET. At the time between three and six months, the level of DO remained almost constant in all packages, indicating that the O_2 consumed during storage was practically limited to the initial content of the oil.

Gambacorta *et al.* (2004) evaluated the shelf life of EVOO packaged in bottles having different oxygen barrier properties and stored in the dark at room temperature and at 37 °C for 12 months. Five different materials were tested: PET, PET including 1% oxygen scavenger (PETA), PET including 3% oxygen scavenger (PETB), PET coated with high barrier resin (PETC), and PET with high barrier resin including oxygen scavenger (PETD); glass was used as the control. The quality indices monitored during the storage were acidity, PV, K_{232}, K_{270}, sensory assessment, and (E)-2-hexenal/hexanal ratio. A significant effect of packaging material on quality decay kinetics of oil was found. Acidity did not change during storage, except for oil packaged in glass stored at 37 °C, where approximately a 50% increase was observed. K_{232} and peroxide values of samples packaged in glass, PETC, and PETD were lower than the ones of oil packaged in PET, PETA, and PETB. K_{270} increased in all samples, whereas the sensory score decreased from 7.69 (initial value) to about 6.5 for oils stored at room temperature and to about 6.0 for the ones stored at 37 °C. The ratio of (E)-2-hexenal/hexanal remained above 1, indicating the absence of off-flavors.

Del Nobile *et al.* (2003) presented a two-dimensional mathematical model able to predict the time course of hydroperoxides and oxygen concentration profile inside bottled virgin olive oil during storage. By simulating the behavior of the bottled virgin olive oil, it was possible to assess the effect of the bottle's shape and size on the quality decay kinetics of virgin olive oil bottled in glass and plastic containers. In particular, five geometrically different containers were used to predict the storage behavior of bottled virgin olive oil. The obtained results showed that the quality decay kinetics of bottled virgin olive oil greatly depended on container geometry. Furthermore, the extent to which the container's geometrical factors affect the quality decay kinetics depends on the material used to make the bottle, and on the initial value of the oxygen partial pressure in the bottle headspace.

Savarese *et al.* (2012) worked on the optimization of PET packaging for EVOO. Several solutions based on the addition of OSs and UV-visible (UV-Vis) absorbers were evaluated, in order to improve the functional properties of the bottles. The effectiveness of the different active packaging solutions in preserving the quality of EVOO during storage and their influence on prolonging shelf life were assessed by the analysis of the main olive oil quality parameters.

Sensory evaluation showed an earlier generation of off-flavors (rancid) in oils stored in bottles without the addition of OS and UV-Vis absorber. The active OSs based on aromatic co-polyamide (MXD-6) did not alter the volatile profile of oils but contributed very little to improvement of the shelf life of EVOO. Best results were obtained with OSs that were able to absorb higher amounts of oxygen during the first weeks of storage. The packaging with the UV-Vis light radiation barrier effect showed the best performance in limiting the oil quality deterioration and oxidative degradation.

14.5 Conclusions

A number of contemporary materials are suitable for packaging of olive oil. Among these, dark-tinted glass preferably placed inside a paperboard or corrugated fiberboard container is the best means for olive oil packaging. Glass provides chemical inertness and excellent protection from oxygen. When combined with an outer fiberboard container, it also very effectively protects the oil from light. Alternatively, olive oil can be packaged in tinplate containers that, even though they are less inert than glass, adequately protect olive oil from both oxygen and light. Finally, plastics (PET) and composites (bag-in-box and Tetra Brik/Tetra Prisma type containers) are acceptable packaging materials for olive oil, even though the latter requires further in-depth investigation for this purpose.

References

Abbadi, J., Afaneh, I., Ayyad, Z., Al-Rimawi, F., Sultan, W., & Kanaan, K. (2014) Evaluation of the effect of packaging materials and storage temperatures on quality degradation of extra virgin olive oil from olives grown in Palestine. American Journal of Food Science and Technology 2(5), 162–174.

Accorsi, R., Versar, L., & Manzini, R. (2015) Glass vs. plastic: life cycle assessment of extra-virgin olive oil bottles across global supply chains. Sustainability 7, 2818–2840.

Badeka, A., & Kontominas, M.G. (1996) Effect of microwave heating on the migration of dioctyladipate and acetyltributyl- citrate plasticizers from food-grade PVC and PVDC/PVC films into olive oil and water. Zeitschrift für Lebensmittel-Untersuchung 202, 313–317.

Badeka, A., Pappa, K., & Kontominas, M.G. (1999) Effect of microwave versus conventional heating on the migration of DOA and ATBC plasticizers from food grade PVC and P(VdC-VC) films into fatty foodstuffs. Zeitschrift für Lebensmittel-Untersuchung 208, 429–433.

Ben Tekaya, I., Ben Tekaya Ben Amor, I., Belgaied, S., El Atrache, A., & Hassouna, M. (2007) Étude du conditionnement de l'huile d'olive dans les emballages en plastique. Sciences des Aliments 27(3), 214–233.

Brody, A.L. (2002) Flavor scalping: quality loss due to packaging. Food Technology 56(6), 124–128.

Castle, L., Mercer, A.J., & Gilbert, J. (1991) Migration from plasticized films into foods. 5. Identification of individual species in a polymeric plasticizer and their migration into foods. Food Additives & Contaminants 8, 565–576.

Cecchi, T., Passamonti, P., & Cecchi, P. (2010) Study of the quality of extra virgin olive oil stored in PET bottles with or without an oxygen scavenger. Food Chemistry 120, 730–735.

Dabbou, S., Gharbi, I., Dabbou, S., Brahmi, F., Nakbi, A., & Hammami, M. (2011) Impact of packaging material and storage time on olive oil quality. African Journal of Biotechnology 74, 16937–16947.

Del Nobile, M.A., Bove, S., La Notte, E., & Sacchi, R. (2003) Influence of packaging geometry and material properties on the oxidation kinetics of bottled virgin olive oil. Journal of Food Engineering 57, 189–197.

European Union (2002) Commission regulation (EC) No 1019/2002 of 13 June 2002 on marketing standards for olive oil. Official Journal European Union. EU, Brussels.

Frankel, E.N., Mailer, R.J., Wang, S.C., Shoemaker, C.F., Guinard, J-X., Flynn, J.D., & Sturzenberger, N.D. (2010) Tests indicate that imported "extra virgin" olive oil often fails international and USDA standards. UC Davis Report, July. Available from olivecenter.ucdavis.edu.

Frankel, E.N., Mailer, R.J., Wan, S.C., Shoemaker, C.F., Guinard, J-X., Flynn, J.D., & Sturzenberger, ND. (2011) Evaluation of extra-virgin olive oil sold in California. UC Davis Report, April. Available from olivecenter.ucdavis.edu.

Fraunhofer Institute for Food Process Engineering and Packaging. (N.d.) Report on olive oil packaging. Available from www.oliveoilone.com

Gambacorta, G., Del Nobile, M.A., Tamagnone, P., Leopardi, M., Faccia, M., & La Notte, E. (2004) Shelf life of extra virgin olive oil stored in packages with different oxygen barrier properties. Italian Journal of Food Science 16, 417–428.

Goulas, A.E., Anifantaki, K.I., Kolioulis, D.G., & Kontominas, M.G. (2000) Migration of Dioctyladipate plasticizer from food-grade PVC film into hard and soft cheeses. Journal of Dairy Science 83, 1712–1718.

Goulas, A., Kokkinos, A., & Kontominas, M.G. (1995) Effect of γ-radiation on migration behavior of dioctyladipate and acetyltributyl-citrate plasticizers from food-grade PVC and PVDC/PVC films into olive oil. Zeitschrift für Lebensmittel-Untersuchung 201, 74–78.

Goulas, A.E., Riganakos, K.A., Ehlermann, D.A.E., Demertzis, P.G., & Kontominas, M.G. (1998) Effect of high dose electron beam irradiation on the migration of DOA and ATBC plasticizers from food-grade PVC and PVDC/PVC films respectively into olive oil. Journal of Food Protection 61, 720–724.

Goulas, A.E., Salpea, E., & Kontominas, M.G. (2008) "Di(2-ethylhexyl) adipate migration from PVC-cling film into packaged sea bream (*Sparus aurata*) and rainbow trout (*Oncorhynchus mykiss*) fillets: kinetic study and control of compliance with EU specifications. European Food Research and Technology 226, 915–923.

Goulas, A.E., Zygoura, P., Karatapanis, A., Georgantelis, D., & Kontominas, M.G. (2007) Migration of di(2-ethylhexyl) adipate and acetyltributyl citrate plasticizers from food-grade PVC film into sweetened sesame paste (halawa tehineh): kinetic and penetration study. Food and Chemical Toxicology 45, 585–591.

Guil-Guerrero, J.L., & Urda-Romacho, J. (2009) Quality of extra virgin olive oil affected by several packaging variables. Grasas y Aceites 60, 125–133.

Kanavouras, A., Hernandez-Münoz, P., & Coutelieres, F. (2004) Shelf life predictions of packaged olive oil using flavor compounds as markers. European Food Research and Technology 219, 190–198.

Kanavouras, A., Hernandez-Münoz, P., & Coutelieres, F.A. (2006) Packaging of olive oil: Quality issues and shelf life predictions. Food Reviews International 22, 381–404.

Kaya, A., Tekin, A.R., & Öner, M.D. (1993) Oxidative stability of sunflower and olive oils: Comparison between a modified active oxygen method and long term storage. Lebensmittel-Wissenschaft & Technologie 26, 464–468.

Kiritsakis, A.K. (1998) Olive oil: from the tree to the table, 2nd ed. Food & Nutrition Press, Trumbull, CT.

Kiritsakis, A.K., & Dugan, L.R. (1984). Effect of selected storage conditions and packaging materials on olive oil quality. Journal of the American Oils Chemists' Society 61, 1868–1870.

Kiritsakis, A., Kanavouras, A., & Kiritsakis, K. (2002) Chemical analysis, quality control and packaging issues of olive oil. European Journal of Lipid Science and Technology 104, 626–638.

Kondyli, E., Demertzis, P.G., & Kontominas, M.G. (1990) Migration of dioctylphthalate and dioctyladipate from polyvinylchloride films into olive oil. Food Chemistry 36, 1–10.

Kondyli, E., Demertzis, P.G., & Kontominas, M.G. (1992) Migration of dioctylplthalate and dioctyladipate plasticizers from food-grade PVC films into ground-meat products. Food Chemistry 45, 163–168.

Mailer, R.J., Graham, K., & Ayton, J. (2012) The effect of storage in collapsible containers on olive oil quality. RIRDC Publication No. 12/008, August.

Mendez, A.I., & Falque, E. (2007) Effect of storage time and container type on the quality of extra-virgin olive oil. Food Control 18, 521–529.

Olafsson, G., & Hildingsson, I. (1995) Sorption of fatty acids into LDPE and its effect on adhesion with aluminum foil in laminated packaging material. Journal of Agriculture and Food Chemistry 43, 306–312.

Paffarini, C. (2007) Così il packaging aiuta a catturare il consumatore. Olivo e Olio 10(3), 20–22.

Pergiovanni, L., & Limbo, S. (2010) Packaging and shelf life of vegetable oils. In: Food packaging and shelf life: a practical guide, ed. G. Robertson G. CRC Press, Boca Raton, FL, 317–338.

Pristouri, G., Badeka, A., & Kontominas, M.G. (2010) Effect of packaging material headspace, oxygen and light transmission, temperature and storage time on quality characteristics of extra virgin olive oil. Food Control 21, 412–418.

Rababah, T.M., Feng, H., Yang, W., Eriefej, K., & Al-Omoush, M. (2011) Effects of type of packaging material on physicochemical and sensory properties of olive oil. International Journal of Agricultural and Biological Engineering 4, 66–72.

Revi, M., Badeka, A., & Kontominas, M.G. (2013) Effect of packaging material on quality parameters and aroma profile of dry white wine. Food Chemistry 152, 331–339.

Ricci, A. (2007) Serbevolezza, i segreti di un olio che dura di più. Olivo e Olio 10, 48–57.

Robertson, G.L. (2009) Food packaging: principles and practice. Taylor & Francis Group, Boca Raton, FL, 229–242.

Sacchi, R., Savarese, M., Del Regno, A., Paduano, A., Terminiello, R., & Ambrosino, M.L. (2008) Shelf life of vegetable oils bottled in different scavenging polyethylene-terephthalate (PET) containers. Packaging Technology & Science 21, 269–277.

Savarese, M., De Marco, E., Caporaso, N., & Sacchi, R. (2013) Extra virgin olive oil overall quality assessment during prolonged storage in PET containers. Global Virtual Conference 8–12 April. http://www.gv-conference.com.

Savarese, M., Porpora, R., D'Antuoni, I., De Marco, E., Paduano, A., Ambrosone, A., Oliva, V., Galdi, M.R., & Sacchi, R. (2012) Monitoring of volatile cmpounds and overall quality of extra virgin olive oil packed in functioalized polyethyleneterephthalate. 5th Intl. Symposium on Food Packaging: Scientific Developments supporting Safety and Innovation. 14–16 November 2012, Berlin, Germany.

Soressi, M. (2007) Brik, sullo scaffale arriva un outsider. Olivo e Olio 2, 8–9.

Soressi, M. (2009) Quando l'azoto previene l'ossidazione dell'olio. Olivo e Olio 4, 1–8.

Tawfek, M.S., & Huyghebaert, A. (1999) Interaction of packaging materials and vegetable oils: oil stability. Food Chemistry 64, 451–459.

Tsimis, D.A., & Karakasides, N.G. (2002) How the choice of container affects olive oil quality: a review of the research. Packaging Technology & Science 15, 147–154.

Vacca, V., Del Caro, A., Poiana, & M., Piga, A. (2006) Effect of storage period and exposure conditions on the quality of Bosana extra-virgin olive oil. Journal of Food Quality 29, 139–150.

Van Willige, R.W., Linssen, J.P.H., Meinders, M.B.J., Van der Stege, J., & Voragen, A.G.J. (2002) Influence of flavour absorption on oxygen permeation through LDPE, PP, PC and PET plastics food packaging. Food Additives & Contaminants 19, 303–313.

Van Willige, R.W.G., Linssen, J.P.H., & Voragen, A.G.J. (2000a) Influence of food matrix on absorption of flavour compounds by linear low density polyethylene: proteins and carbohydrates. Journal of the Science of Food and Agriculture 80, 1779–1789.

Van Willige, R.W.G., Linssen, J.P.H., & Voragen, A.G.J. (2000b) Infuence of food matrix on absorption of flavour compounds by linear low density polyethylene: oil and real food products. Journal of the Science of Food and Agriculture 80, 1790–1797.

Vekiari, S.A., Papadopoulou, P., & Kiritsakis, A. (2007) Effects of processing methods and commercial storage conditions on the extra virgin olive oil quality indexes. Grasas y Aceites 58, 237–242.

Wang, S., Li, X., Rodrigues, R., & Flynn, D. (2014) Packaging influences on olive oil quality: a review of the literature. UC Davis Report, UC Davis Olive Center.

Zygoura, P.D., Riganakos, K.A., & Kontominas, M.G. (2011) Study of the migration behavior of acetyl tributyl citrate from PVDC/PVC film into fish fillets as affected by intermediate doses of electron beam radiation. European Food Research and Technology 232, 1017–1025.

15 Table olives: processing, nutritional, and health implications

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15.1 Introduction

Table olives are prepared from the bitter drupe fruit of the woody species *Olea europaea* L. Olive fruit differs from other drupe fruits in that it has very low sugar levels and high oil content. Over the past 25 years (from 1990–1991 to 2014–2015), global table olive consumption has risen nearly 170% to around 2.5 million tons (t)/year. This increase can be in part attributed to greater consumption around the world as well as among those living in traditional olive-growing countries.

Table olives are a high source of energy, phytochemicals, and substances with therapeutic benefits. Phytochemicals include: phenolic and related compounds (polyphenols), phytosterols, tocopherols, squalene, and terpenoids as well as pigments such as carotenoids, chlorophylls, and anthocyanins. The bitterness of raw olives is predominantly due to the polyphenol oleuropein, which in most table olive products is much reduced. At the usual harvest time for table olive and oil production, the pulp contains about 60% water, 30% oil, up to 4% sugars, 3% protein, and the rest are fiber and ash (minerals). Proteins are well balanced in all essential amino acids. Olive pericarp is rich in potassium but low in sodium; contains β-carotene (vitamin A precursor), vitamin E (tocopherols, tocotrienols), together with vitamin B group (thiamine) as well as hemicellulose, the more complex carbohydrate component. In general, there is a greater amount of fiber in raw olive fruits than in processed olives, due to degradation of some of its components during processing. Apart from potassium, the mineral content of raw olive pericarp is modest in calcium > phosphorus > sodium > magnesium and sulfur, with lesser amounts of iron, zinc, copper, and manganese. Nutrients, especially those that are water soluble, are lost through soaking and washing processes. The endocarp (stone) contains 10% water, 30% cellulose, 40% other carbohydrates, and about 2% oil. The seed has 30% water, 27% oil, 27% carbohydrates, and 10% protein, with a small amount of tocopherols. Although seeds are a non-consumable part of olive fruit, during processing by spontaneous fermentation (especially with turning-color olives of the 'Kalamon' cv.), the seeds can impart a nutty flavor to the flesh (S.G. Kailis, personal observation). Most table olive products are prepared from olives with discrete maturation characteristics. With ripening, at the naturally black ripe stage, anthocyanins predominate in skin and flesh as other pigments have degraded.

The health benefits of table olives are mostly extrapolated from research on virgin olive oil and the Mediterranean diet, derived mainly from the favorable fat profile and minor components that include a number of antioxidants.

This chapter describes the different types of table olives existing internationally, their composition, and their functionality.

15.2 Olive maturation stages for table olive processing

For table olives, raw olive fruits are mostly processed at the green ripe, turning-color. or naturally black ripe stages. During ripening, flesh texture changes rapidly over a period of one to two weeks. At the

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green-colored stage (i.e., pre–green ripe), the fruit is difficult to squeeze, indicating it is not suitable for table olive processing. However, from the green ripe stage to turning-color stage (cf. veraison, the point where the skin changes color), the fruit becomes easier to squeeze and olive juice is released, a signal that olives are ready for processing. At this stage, antioxidant levels, polyphenols, and tocopherols are at their highest level. As the fruit ripens further, the amount of dry matter increases, but slowly. In the case of nine Italian olive cultivars, the overall trend in oil composition of olive fruit was a decrease in palmitic and linoleic acids in all cultivars and an increase of oleic acid in most cultivars.

During olive fruit maturation, as oil content increases the sugar content decreases (Kiritsakis, 1998; Kiritsakis *et al.*, 1998; Salas *et al.*, 2002). Squalene, a hydrocarbon intermediate metabolite of sterol synthesis, accumulates in olive flesh in the early stage of fruit development, peaking before 21 weeks from flowering. From 21 to 26 weeks after flowering, its levels decrease greatly, and when completely mature the amount of squalene is around 1.25 g/kg in the oil fraction. When olives overripen, past the black ripe stage, polyphenol levels radically fall to around half the original level, so the naturally black ripe olive fruits are less bitter and pungent. Furthermore, the level of linoleic acid in the flesh of naturally black olives increases at the expense of oleic acid, hence the ratio of oleic to linoleic acids falls.

Maturation of raw olive fruit is a slow process lasting several months or longer according to cultivar, growing conditions, temperature, water availability, and farming practices. Weight of fresh olive fruit increases during maturity, reaching a near maximum size at the green ripe stage. As the overall composition of raw olive flesh changes during maturation, the starting material for table olive processing will therefore be different. Also, heavy crop loads can significantly increase the maturation period and reduce fruit size, factors important to the table olive industry. From a practical perspective, ripeness descriptions that are commonly used are described in subsequent sections of this chapter.

15.2.1 Green ripe olive stage

Green ripe olives are used to produce green table olives by spontaneous fermentation in brine, black ripe olives (olives treated with lye and artificially darkened by oxidation), and Greek-style *tsakistes* (cracked olives). These olives must be firm, sound, resistant to slight pressure between the fingers, and with no marks other than natural pigmentation. Depending on the cultivar, solar radiation level, and nutritional state of the tree, skin color varies from pale green to yellow green, whereas flesh color varies from pale green to cream.

15.2.2 Turning-color olive stage

Turning-color olives have partially pigmented skin with anthocyanins, patchy or overall, signaling veraison and cream or green flesh. Olives at this stage can be processed by spontaneous fermentation, then aromatized as specialty olives. Olives picked at this stage can be used to prepare California-style black ripe olives and Greek-style *tsakistes*.

15.2.3 Naturally black ripe olive stage

Naturally black ripe olives are picked when the skin and flesh are fully pigmented, purple-brown to black, with anthocyanins. They are used for producing Greek-style naturally black-ripe table olives by spontaneous fermentation in brine. In some centers, raw olives are harvested when pigmentation of flesh is nearly complete, a practice that delivers firmer olives. In cooler climates, some cultivars will not complete this stage of ripening. For salt-dried or heat-dried olives, overripe black olives are used.

15.2.4 Acylglycerols in raw olive fruit during growth, maturation, and ripening

As olive fruit develops, matures, and ripens, its oil content increases significantly, reaching a maximum level at veraison, then falls (Salvador *et al.*, 2001). Generally, as olive fruits mature, the composition of fatty acids changes in the oil fraction with an increase in the relative proportion of polyunsaturated fatty acids (PUFAs), with small reductions in monounsaturated (MUFAs) and saturated fatty acids (SFAs). In addition, the proportion of monounsaturated fats to saturated fats in the oil fraction of olive fruit is greater when they are grown at lower versus higher temperatures. Fruit of olive cultivars from Greece, Italy, and Spain

generally produce oil with lower linoleic and palmitic and higher oleic acid levels, whereas with Tunisian cultivars the oleic acid levels are lower and linoleic and palmitic acid levels are higher.

15.2.5 Secondary metabolites in raw olive fruit

Olive fruit accumulates a number of diverse groups of secondary metabolites, especially polyphenols. They make up around 2–3% of the drupe, of which 90% are present in the skin and flesh with the remainder in the seed. At veraison, when polyphenol levels peak, oleuropein may reach 0.5–1%, hydroxytyrosol 70–350 mg/kg, tyrosol 0.2–0.5 mg/kg, and verbascoside 0.1–1.3 mg/kg, depending on the cultivar (Garrido Fernández *et al.*, 1997).

15.2.5.1 Polyphenols

The polyphenol fraction of raw olives and table olives differs quantitatively and qualitatively from that of virgin olive oils. Levels are greater in raw olive fruit and table olives than virgin olive oils, as only 2% of total phenolic compounds partition into the oil phase during processing. Furthermore, table olive processing methods markedly influence polyphenol levels in the final products.

Olive polyphenols are categorized as hydrophilic or lipophilic. The hydrophilic fraction includes secoiridoids, phenolic acids, phenolic alcohols, flavonoids, and lignans (Table 15.1). Water solubility of complex polyphenols is due to the presence of sugars in their structure (glycosides) and multiple hydroxyl groups. Cresols are the principal lipophilic polyphenols in olive fruit. Phenolic acids, phenolic alcohols (hydroxytyrosol and tyrosol), flavonoids, and lignans are present in all parts of the olive fruit, especially in the flesh. The fruit of some olive cultivars contains high levels of verbascoside, a hydroxycinnamic acid derivative, with other specific phenolic alcohols and acids present at maturity. Specific polyphenols occurring in the olive seed are nüzhenide and salidroside (Ryan *et al.*, 2002), whereas flavonoids such as quercetin, rutin, and luteolin-7-glucoside are in the peel (Servili *et al.*, 1999). Free hydroxytyrosol is a significant polyphenol in green and black olives, whereas tyrosol is present at lower levels (Malik & Bradford, 2006). Verbascoside present in olive flesh increases during ripening, overlapping degradation of oleuropein, then declines. Anthocyanin polyphenols that accumulate after veraison include cyanidin-3-O-rutinoside and cyanidin-3-O-glucoside.

Polyphenol composition and levels change during growth, maturation, and ripening of olive fruit. They gradually accumulate in olive fruit, reaching maximum levels at veraison, the point when the skin changes color (Chimi & Atouati, 1994; Monteleone *et al.*, 1995), then they decline. Indicative values for turning-color olives are oleuropein 0.5–1 g/kg, hydroxytyrosol 70–350 mg/kg, tyrosol 0.2–0.5 mg/kg, verbascocide 0.1–1.3 mg/kg, and luteolin 7-glucoside 0.4–0.6 mg/kg.

Olive fruit grown at higher altitudes has higher total polyphenol compounds than fruit from lower altitudes. Similar observations have been made for olives grown in cool climates versus hot climates. Furthermore, frost-damaged olives have lower levels of secoiridoids (Goulas *et al.*, 2012). Total polyphenol levels in olive fruit are inversely related to water availability. However, vanillin and vanillic acid increase in olive fruit of

Table 15.1 Polyphenols present in raw olive fruit.

Category	Phenolic compounds present in raw olives
Secoiridoids	Oleuropein, oleuropein aglycone, demethyloleuropein, ligstroside, nüzhenide (only found in olive seeds), 11-methyl oleoside, and elenolic acid (oleuropein metabolite)
Phenolic acids	Caffeic, chlorogenic, ferulic, vanillic, homovanillic, p-coumaric, o-coumaric, syringic, and verbascoside (hydroxycinnamic acid derivative)
Phenolic alcohols	Hydroxytyrosol (hydrolysis product of oleuropein) – (3,4-dihydroxyphenyl) ethanol (3,4-DHPEA); tyrosol (hydrolysis product of ligstroside) – (p-hydroxyphenyl) ethanol (p-HPEA)
Flavonoids	Luteolin-7-*O*-glucoside, cyanidin-3-*O*-glucoside, cyanidin-*O*-3-rutinoside, rutin, apigenin-7-*O*-glucoside, quercetin-3-rhamnoside, quercetin-3-rutinoside, and luteolin-5-glucoside and luteolin
Lignans	Pinoresinol, 1-acetoxypinoresinol

irrigated olive trees compared to those under water stress. Infestation with olive fly also lowers the total polyphenol content of olive fruit (Goulas *et al.*, 2012).

15.2.5.2 Phytosterols

Phytosterols are an important non-acylglycerol component of the lipid fraction of olive flesh. Sterols such as β-sitosterol, cyclartenol, and 24-methylenecyclartenol accumulate increasingly when olive fruit reaches its final size and when veraison begins. Investigation of sterol levels in raw olive fruit of 10 cultivars grown in Australia revealed that 'Arbequina' and 'Barnea' levels were greater than those of 'Corregiolla', 'Frantoio', 'Leccino', and 'Picual', and 'Manzanillo', which in turn were greater than those of 'Coratina', 'Koroneiki', and 'Pendolino'. In another study with three cultivars, 'Barnea', 'Frantoio', and 'Picual', total sterol levels in the oil fraction were within the range of 1.7–2.0 g/kg of oil. For raw olives, the approximate sterol levels were around 1400 mg/kg of oil for 'Hojiblanca' cv. and 1100 mg/kg of oil for 'Manzanilla' cv. olives. In another study of green ripe olives as a proportion of total sterols, β-sitosterol in the fruit of 'Manzanilla' and 'Hojiblanca' cvs. was around 84 and 87%, respectively; Δ-5-avenasterol was 5.6 and 4.6%, respectively; and campesterol was around 2.8% for both cultivars.

15.2.5.3 Tocopherols and tocotrienols

In the raw state, naturally black ripe olives contain significantly higher quantities of α-tocopherol equivalents compared to green ripe olives, mainly due to cultivar characteristics and possibly the maturation state. In a study of the following cultivars, green ripe 'Konservolia' and 'Chalkidikis' as well as naturally black ripe 'Konservolia' and 'Kalamon', α-tocopherol predominated. Both cultivar and maturity state affect tocopherol and tocotrienol composition and levels, with β-tocopherol and α-tocotrienol in trace amounts in green ripe olives, δ-tocopherol in trace amounts in all olives, and trace amounts of γ-tocopherol in green ripe 'Konservolia' cv. olives. Calculated α-tocopherol equivalents (tocopherol + tocotrienol adjusted for activity of each) in the pulp were 21.6, 29.7, 36.8, and 39.4 μg/g pericarp or 138, 132, 189, and 196 μg/g lipid for green ripe 'Konservolia', green ripe 'Chalkidikis', naturally black ripe 'Konservolia', and naturally black ripe 'Kalamon' (Hassapidou & Manoukas, 1993). Furthermore, metabolite analysis pointed to the first and intermediate stages of olive fruit development of 'Koroneiki' cv., 6–22 weeks after flowering and onward, which had greater tocopherol levels than during the final on-tree stages (24 weeks after flowering onward). The approximate α-tocopherol concentration, which ranged from around 16 to 32 mg/100 g flesh, was up to 100 times greater than other tocopherols. With respect to tocotrienols, only γ-tocotrienol was detected (Georgiadou *et al.*, 2015). α-Tocopherol predominated in all types of olives, followed by γ-tocopherol. Green raw olives of 'Konservolia' cv. contained only α-tocopherol, whereas green olives of 'Chalkidikis' cv. contained α- and δ-tocopherols.

15.2.5.4 Terpenoids

Two triterpenic acid compounds, oleanolic and maslinic acids, products of squalene metabolism, comprised 26 and 38% of the total cuticular wax of green ripe and naturally black ripe olives of 'Coratina' cv., respectively. Significant quantities of oleanolic and maslinic acids (31–44 and 55–68%, respectively) were present in the total wax fraction of cvs. 'Cipressino', 'Drittam', and 'Leccino'. These were present in developing olive fruit and reached maximum levels after veraison, then decreased with maslinic acid as the predominant member. The proportion of maslinic to oleanolic acid was cultivar dependent with 'Picual' > 'Hojiblanca' > 'Arbequina'. Erythrodiol and uvaol triterpenic diols were present in young fruit but only appeared in trace amounts in the mature fruit.

15.3 Olive cultivars suitable for table olive processing

With some 2500 or more recognized cultivars, olive fruit comes in all shapes and sizes, from spherical to oval to cylindrical, with or without points or nipples. However, only a relatively small number of these are

of commercial value. Table 15.2 lists numerous cultivars that are used for table olive production. Terms used in the table are indicative, relative but not absolute, and should only be used as a guide. Medium to large olives with a high flesh-to-stone ratio (5:1–8:1), thin delicate skin, optimal flesh characteristics (firm and nonfibrous), and ease of flesh–stone separation (freestone vs. clingstone) are best for table olives, whereas for olive oil production, oil content and ease of oil removal are most important. Some cultivars have dual purpose. Cultivars with freestone fruit are suitable for destoning (e.g., traditional green Sicilian olives) and facilitate flesh separation in the mouth. Cultivars with clingstone fruit are suitable for Greek style *tsakistes*, where the flesh does not separate from the stone during cracking or bruising operations. Olives suitable for fermentation need a sugar content of 2–4%. Large olives with a high flesh–stone ratio tend to produce soft final products, are susceptible to gas pockets, and are more sensitive to surface shriveling if brine strength is too high, whereas those with a low ratio are harder to destone. Only sound olives (i.e., no disease, frost, or harvest/postharvest storage damage) are suitable, and these are best processed within one to two days of harvesting. Furthermore, olive fruits with low levels of polyphenols, because of cultivar or maturation state, generally require less processing time than those with high levels.

Most olive cultivars can be processed into table olive products. Those economically important for table olive production include 'Manzanilla', 'Sevillana', 'Hojiblanca', 'Ascolana Tenera', 'Konservolia', 'Kalamata', 'Chalkidikis', 'Gemlik', 'Ayvalik', and 'Picholine'. Specialty table olives are prepared from 'Arbequina', 'Koroneiki', 'Taggiasca', 'Cerignola', 'Frantoio', and 'Leccino'. Although larger fruit with a high flesh–stone ratio may be preferred by consumers, smaller fruit such as 'Taggiasca' and 'Frantoio' (Ligurian style), 'Koroneiki' (Greek style), and 'Arbequina' (turning color in brine) are also very popular (see also Table 15.2). Commonly, fruits from olive cultivars with low oil content are used for table olive production, whereas those with high oil content are crushed for olive oil. Consumers generally have a preference for large table olives. Olive size depends on the cultivar; growing conditions, especially water availability; and crop load. Each olive tree has a range of olive sizes, with larger ones on parts of the tree that receive the greatest exposure to sunlight. For commercial table olive production, uniform olives are size graded. Olive fruits from large varieties are called "queen olives," such as 'Ascolana', 'Chalkidikis', 'Gordal Sevillana' ('Spanish Queen'), and UC13A6 ('Californian Queen'), or "jumbo olives" ('Cerignola', 'Azapa', and 'Uovo di Piccione').

The texture of olives depends on many factors, including cultivar, maturity state, oil content, and growing conditions. Fruit from some olive cultivars have flesh with a fibrous texture (e.g., 'Domat' and 'Jumbo Kalamata' cvs.), whereas others have a coarse granular texture (e.g., 'Barnea' cv.). Fruit from 'Gordal Sevillana' and 'Konservolia' cvs. have thin skin, and with the latter cultivar, fruit can resist shriveling in high salt brines (12 to 19%). Some cultivars such as 'Ascolana' need to be handled carefully as the fruit bruises easily during handling.

15.4 Factors affecting raw olive fruit for table olive processing

Regular irrigation increases olive weight, an economically important factor in table olive production. It has also been noted that olive fruit from cooler regions has higher levels of unsaturated fatty acids than the fruit from dry and warm areas. When damaged through disease, infestation, or mechanical operations, the enzyme β-glucosidase (Table 15.3) promotes hydrolysis of oleuropein in the flesh to its aglycone. Heavy crop loads generally result in smaller size olives, thus reducing the value of the crop for table olive production. In contrast, crop thinning results in larger sized olives. Restricted watering during the growing season increases phenol levels in olive fruit, especially tyrosol. Limiting water availability also reduces photosynthetic activity, hence reducing yield and oil synthesis as well as marginally retarding olive fruit maturity.

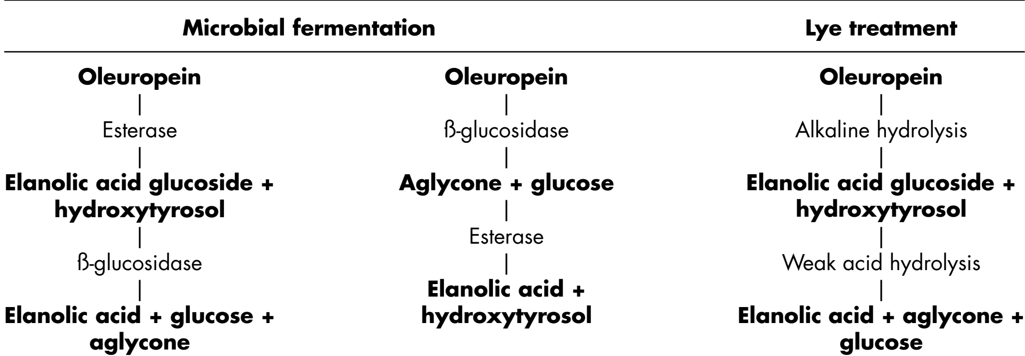
Ideally and preferably, all raw olives for table olive processing, in order to prevent skin and flesh damage, should be picked by hand. To prevent damage, the olives are harvested into stackable slatted plastic crates, then delivered to the processing facility. The olives are then sorted and color graded. Depending on the style and processing method, raw olives are harvested and processed at three stages: green ripe, turning-color, and naturally black ripe. Fruit from some cultivars such as 'Arbequina', 'Frantoio', and 'Taggiasca' are often processed from a harvested crop with all three maturation stages. Harvesting time is significant because if

Table 15.2 Cultivars for table olive processing.

Cultivar	Fruit weight	Stone weight	F:S ratio	Oil content	Polyphenol levels	Country
'Aglandau'	Medium	Medium clingstone	Medium	Medium/high	Medium	France
'Arauco'	Very high	High freestone	High	Medium	Medium	Argentina
'Arbequina'	Low	Low clingstone	Low	Medium/high	Low	Spain
'Ascolana Tenera'	Very high	High	Very high	Low/medium	Medium	Italy
'Ayvalik'	High	High clingstone	Medium	High	Low/medium	Turkey
'Azapa'	Very high	High clingstone	High	Low	High	Chile
'Barnea'	Medium	Medium freestone	High	Medium to high	Medium	Israel
'Carolea'	High	High/clingstone	Medium	Medium to high	Low/medium	Italy
'Chalkidikis'	Very high	High freestone	High	Low/medium	High	Greece
'Coratina'	Medium/high	High clingstone	Medium/high	Very high	Very high	Italy
'Domat'	High	High clingstone	Medium to high	Medium	Low/medium	Turkey
'Frantoio'	Medium	Medium clingstone	Medium	Medium/high	Medium/high	Italy
'Galega Vulgar'	Medium	Medium freestone	Low	Medium	Low	Portugal
'Gemlik'	High	High freestone	*Low/medium*	High	Low/medium	Turkey
'Gordal Sevillana'	Very high	High clingstone	Medium	Low	Low	Spain
'Hojiblanca'	High	High clingstone	Medium	High	Medium	Spain
'Itrana'	High	High freestone	*Medium*	Medium	Medium	Italy
'Kalamon'	High	High freestone	High	High	Low	Greece
'Konservolia' ('Conservolea')	High	High freestone	High	Medium/high	Medium	Greece
'Koroneiki'	Low	Low freestone	Low/medium	High	Very high	Greece
'Ladoelia'	Medium	Medium clingstone	Low	Low/medium	Medium	Cyprus
'Leccino'	Medium	Medium freestone	Medium	Medium/high	Medium	Italy
'Manzanilla de Sevilla'	Medium	High freestone	Medium/high	Medium	High	Spain
'Memecik'	High	High clingstone	High	Medium/high	Medium	Turkey
'Meski'	Medium/high	High freestone	High	Low	Low	Tunisia
'Mission' (Californian)	Medium	Medium freestone	Medium	Medium	Medium/high	USA
'Nocellara de Belice'	High	High freestone	High	Medium	Low	Italy
'Oblitza' ('Oblica')	Variable: low to high	High freestone	Medium/high	Medium/high	Medium	Former Yugoslavia
'Oliva di Cerignola'	Very large	High clingstone	Low/medium	Low	Low	Italy
'Picholine Languedoc'	Medium	Medium freestone	Medium/high	Low/medium	High	France
'Picholine Marocaine'	Medium	Medium freestone	Medium	Low/medium	High	Algeria
'Picual'	Medium	High clingstone	Medium/high	High	Very high	Spain
'Sigoise'	Medium	High freestone	Medium/high	Low	High	Algeria
'Tanche'	Medium	High freestone	Low/medium	High	Low	France
'Toffahi'	High	High clingstone	High	Low	Low	Egypt
UC13A6	Very high	High clingstone	High	Low	Low	USA

Note: Fruit weight range: < 2 g to > 6 g. Stone weight range: < 0.3 g to > 0.45 g. Fruit:stone (F:S) ratio range. Oil content (fresh weight) range: < 18% to > 22%.

Table 15.3 Oleuropein degradation under different conditions.



Microbial fermentation		Lye treatment
Oleuropein	**Oleuropein**	**Oleuropein**
|	|	|
Esterase	ß-glucosidase	Alkaline hydrolysis
|	|	|
Elanolic acid glucoside + hydroxytyrosol	**Aglycone + glucose**	**Elanolic acid glucoside + hydroxytyrosol**
|	|	|
ß-glucosidase	Esterase	Weak acid hydrolysis
|	|	|
Elanolic acid + glucose + aglycone	**Elanolic acid + hydroxytyrosol**	**Elanolic acid + aglycone + glucose**

picked too early, the olives are too hard, causing problems in fermentation, whereas late-harvested olives when processed may end up too soft.

Recommendations for storage of green ripe olives under controlled atmosphere are as follows. Optimum controlled atmospheres, of 2–3% $O_2$with 0–1% CO_2, delay senescence and softening for up to 12 weeks at 5 °C (41 °F) or nine weeks at 7.5 °C (45.5 °F). Note that O_2 levels below 2% can cause off-flavors, whereas CO_2 levels greater than 5% may increase the severity of chilling injury if olives are kept below 7.5 °C. Several effects have been observed when raw green ripe olives have not been stored appropriately – chilling injury, nail-head, and carbon dioxide injury. Chilling injury is characterized by deterioration of flesh if olives are stored for longer than two weeks at 0 °C, five weeks at 2 °C, or six weeks at 3 °C. Damage includes internal browning of flesh, starting around the stone and then radiating toward the skin over time. Cultivar susceptibility is 'Sevillano' > 'Ascolano' > 'Manzanillo' > 'Mission' (Californian). "Nail-head" is characterized by surface pitting and spotting. When epidermis dies, air pockets are created underneath the skin, whereby a browning reaction occurs. Here damage is seen if olives are stored at 10 °C for six weeks or longer or at 7.5 °C for 12 weeks or longer. With carbon dioxide injury, internal browning and decay occur when olives are exposed to >5% CO_2 for more than four weeks (Crisosto *et al.*, 2015).

A study supporting green olive storage in cold rooms with controlled atmosphere was undertaken by Nanos *et al.* (2002). Here, raw green ripe 'Konservolia' cv. olives were stored for up to 37 days at 5 °C in air and up to 22 days at 7.5 °C and 2 kPaO_2 plus 5 kPaCO_2, whereas green 'Chondrolia' cv. olives were very sensitive to cold storage, developing symptoms of chilling injury with excessive internal browning, skin discoloration, and pitting. In another study, when olives were stored for seven days at 15 °C, polyphenol levels fell in three days, probably due to oxidoreductase enzymes in olive flesh and produced by microorganisms associated with the skin (Zullo *et al.*, 2014).

15.5 Table olive processing

The aim of table olive processing is to produce a safe, palatable, and nutritious foodstuff (Kailis & Harris, 2007). Processing involves one or more physical, chemical, or biological treatments resulting in some changes in the physicochemical properties of their pericarp. Some flesh components are modified, influencing aroma, flavor, and texture, whereas water-soluble nutrients such as sugars, organic acids, minerals, amino acids, and vitamins may be lost, hence affecting the nutritional value of the original raw olive positively or negatively. Debittering of some cultivars can occur naturally if the fruit is allowed to overripen and dehydrate on the olive tree.

In most cases where fermentation is involved, processes are largely spontaneous, whereas methods for drying olives by salt and/or heat are largely empirical. At the industrial level, a number of table olive processing technologies, together with controls for sensory characteristics and safety, are well recognized; whereas artisan or home-processing methods such as debittering raw olives by soaking in water or weak brine, or by dehydration, are empirical.

15.5.1 Commercial table olive processing methods

There are four main commercial methods for processing raw olives into table olives; these are spontaneous fermentation, as in Greek-style black olives; lye (sodium hydroxide in potable water) treatment followed by fermentation, as with Spanish-style green olives; and lye-treated oxidized olives, as with California-style black ripe olives and the Greek-style Kalamon method. Other methods involve the use of lye solutions or dehydration of raw olives, generally black ripe, with heat or salt. In recent years, there has been strong preference by consumers for natural foods, including low-salt olives and those not processed with harsh caustic chemicals such as lye. Table 15.4 outlines the sequence involved for a number of processing methods.

15.5.2 Olives processed by spontaneous fermentation

Spontaneous fermentation of olive fruit takes advantage of the microbial population living on their skin that includes yeast, fungi, and both Gram-positive and Gram-negative bacteria; but throughout the fermentation process, Enterobacteriaceae, lactic acid bacteria (LAB), and yeasts are the most relevant microorganisms (Garrido Fernández *et al.*, 1997). However, other organisms may also be present. Green ripe, turning-color, or naturally black ripe olives can all be processed and debittered by spontaneous fermentation in brine (4–10%), usually under anaerobic conditions, at 25 °C.

When placed in brine, untreated olives release polyphenols into the fermentation brine that initially slow the growth of LAB. Oxidative yeasts and molds can accumulate on brine surfaces if fermentation tanks are left uncovered, bringing on spoilage of the olives. To reduce this problem, tanks must be closed, and the air layer in the headspace reduced to a minimum. Where yeasts are involved in table olive fermentation, they generate compounds important for table olive preservation (organic acids) as well as taste and aroma (alcohols including ethanol, and esters such as ethyl acetate and aldehydes) (Alves *et al.*, 2012). Overproduction of these latter compounds can result in unpalatable products categorized as "winey." Yeasts can also produce spoilage, such as olive softening, malodors, off flavors, and clouding of fermentation brine (Arroyo-López *et al.*, 2008). A number of yeast species may increase LAB production by generating compounds essential for their growth. This includes vitamins, amino acids, purines, as well as metabolites of complex carbohydrates.

Microflora that support olive fermentation of raw olives can vary between cultivars and their maturation state. Yeasts are always present throughout processing, as are LAB, especially at lower salt concentrations. *Lactobacillus plantarum* and *Lactobacillus pentosus* are the major bacterial species. Other species have been identified, such as *Lactobacillus casei*, the dominant species in naturally fermented Sicilian green olives, together with heterofermentative strains of *Lactobacillus brevis* and *Lactobacillus plantarum* (Caggia *et al.*, 2004). Addition of specific cultures of *Lactobacillus plantarum* and/or *Lactobacillus pentosus* that have oleuropeinylitic activity (Table 15.3) and good acidifying qualities, even in the presence of high salt and/or polyphenol levels, has been found to degrade oleuropein at faster rates than native flora, hence reducing debittering time. The use of specific mixed bacterial–yeast starter cultures has been considered, but these are not commonly used at the industrial level.

Fermentation of turning-color or naturally black ripe olives can also be undertaken under aerobic conditions. Here air is injected through a central column in the fermentation tank, whereby the proportion of fermentative and oxidative yeasts changes.

15.5.2.1 Changes occurring during fermentation

During fermentation in brine, water-soluble substances including fermentable substrates, polyphenols, and other nutrients diffuse from olives to brine while salt passes into the flesh. Fermentable substrates such as glucose, fructose, sucrose, mannitol, and malic acid, the main energy sources for fermentative bacteria and yeasts, are converted mainly to lactic and acetic acids, essential for stability and preservation during processing and subsequent storage. When lye-treated green ripe fruit from four cultivars – 'Picholine Marocaine', 'Picholine Languedoc', 'Ascolana', and 'Sevillana' – underwent lactic fermentation a final pH between 4.4 and 4.6 was achieved. Significant amounts (60–80%) of total polyphenols, flavonoids, and sugars were lost from the flesh by the end of fermentation. Hydroxytyrosol, tyrosol, (+) catechin, and quercetin were identified at the end of fermentation. The highest total polyphenol content (≈2600 mg tyrosol equivalents/L)

Table 15.4 Common table olive processing methods.

Naturally processed table olives				Lye-treated table olives		
Fermentation	**Kalamata-style**	**Salt-dried**	**Heat-dried**	**Spanish-style green**	**Picholine-style**	**Californian-style black**
Green ripe turning color naturally black ripe olives	Naturally black ripe olives	Naturally black ripe olives	Naturally black ripe olives	Green ripe olives	Green ripe olives	Green ripe olives to start of turning color
Wash olives with potable water	Wash olives with potable water	Wash olives with potable water	Wash olives with potable water	Wash olives with potable water	Wash olives with potable water	Wash olives with potable water
Size grade	Size grade	Size grade (optional)	Size grade (optional)	Size grade	Size grade	Size grade
Slit, crush, or bruise as required	Slit olives	Add dry salt	Blanch olives with hot water	Lye treatment	Lye treatment	Lye treatments (several)
Place in brine to ferment	Place in brine to ferment or multiple daily rinsing with water	Complete or partial dehydration of olives	Add dry salt (optional)	Wash with potable water several times to remove excess lye	Wash with potable water	Air oxidation in alkaline medium to darken olives
Complete or partial fermentation	Water soak or complete or partial fermentation	Rinse in vinegar (optional)	Oven dry at 50 °C	Place in brine in 8–10% brine to ferment	No fermentation	Wash olives with potable water
Aerate black olives to darken			Complete or partial dehydration of olives	Complete or partial fermentation	Place in acid brine	Ferrous salt treatment for color fixation Place in brine, bulk-pasteurize
Remove defective olives	Remove defective olives			Remove defective olives	Remove defective olives	Remove defective olives
Size grade	Size grade			Size grade	Size grade	Size grade
Pack in acid brine	Pack in vinegar brine + olive oil	Pack in containers without added brine	Pack in containers without added brine	Pack in acid brine	Pack in acid brine	Pack into cans with weak brine at neutral pH (or acid brine)
Hot pack or pasteurize	Hot pack or pasteurize	Pasteurize (regular or tunnel)	Pasteurize (regular or tunnel)	Pasteurize	Pasteurize	Sterilize or pasteurize if packed in acid brine
Ready for consumption						

and antioxidant activity was in 'Picholine Marocaine' cv., brined for ten weeks after fermentation (Kiai & Hafedi, 2014).

Major constituents of raw olives, oil water, sugars, protein, pigments, and polyphenols are influenced during processing. With water treatment and fermentation in brine, sugar content decreases significantly while sodium increases. Relative fat content can increase marginally, whereas nitrogen (cf. protein amino acids) and carbohydrate decrease during lactic fermentation (Bravo-Abad & Inigo, 1988). Tocopherol levels also decrease (Hassapidou & Manoukas, 1993).

15.5.2.2 Flavor and aroma of olives formed by spontaneous fermentation

The taste, flavor, and aroma of table olives, apart from salt, acid, and fruit, are closely related to polyphenols, volatile compounds produced by bacteria and yeasts during fermentation together with acid, salt, and other additives or fillings. Compounds identified by Sabatini and Marsilio (2008) in olives prepared by spontaneous fermentation included acids, mainly acetic acid and propionic acid as well as aldehydes such as hexanal and nonanal; ketones such as 3-hydroxy-2-butanone and butanone; esters, namely ethyl acetate, ethyl propanoate, propyl propanoate, and propyl acetate; and alcohols such as 1-butanol, 1-hexanol, 1-pentanol, 1-propanol, 2-butanol, 2-pentanol, isobutanol, ethanol, 3-pentanol, 4-penten-1-ol, cis-3-hexen-1-ol, and isopentanol.

15.5.3 Greek-style black olives

Naturally black ripe olives are placed in brine and then undergo fermentation supported by natural bacteria and yeasts. Debittering is slow and incomplete. Once processed, the olives are exposed to air to darken and hence improve skin color. High salt levels are used (8–14%) that promote yeast fermentation and control the growth of malodorous fermentations by microorganisms such as *Clostridium* and *Propionibacterium*.

When presented for sale to consumers, Greek-style (naturally) black ripe olives are packed into suitable containers with acid brine. The packed products are then either pasteurized and/or sodium sorbate is added to give a final sorbic acid level at equilibrium of 0.05%. Pasteurized products can have much lower salt levels. The end product is slightly bitter. In Spain, a popular non-fermentation processing method for naturally black ripe olives is by placing them in hot brine and allowing them to stand until edible.

15.5.3.1 Suggested cultivars for Greek-style (naturally) black ripe olives

Suggested cultivars for Greek-style (naturally) black ripe olives are 'Arauco', 'Ayvalik', 'Azapa', 'Azeradj', 'Barnea', 'Bosana', 'Buga', 'Carolea', 'Cassanese', 'Chalkidikis', 'Çelebi', 'Çekiste', 'Chétoui', 'Cornicabra', 'Cucco', 'Empeltre', 'Erkence', 'Galega Vulgar', 'Gemlik', 'Gerboui', 'Giarraffa', 'Grossane', 'Hojiblanca', 'Itrana', 'Istrska Belica', 'Kalinjot', 'Konservolia', 'Ladoelia', 'Lechín de Granada', 'Lechín de Sevilla', 'Memeli', 'Manzanilla', 'Manzanilla Cacereña', 'Mara', 'Mastoides', 'Memecik', 'Meski', 'Mission', 'Menara', 'Oblica', 'Picholine', 'Picholine Marocaine', 'Picual', 'Rasi'I', 'Sigoise', 'Soury', 'Tanche' ('Olive de Nyons'), 'Uslu', 'Verdale', 'Villalonga', and 'Zizula'.

15.5.3.2 Some features of Greek-style naturally black ripe olives

As brining/fermentation progresses, salt passes into the flesh, and brine salt levels fall. Polyphenols diffuse out of olive flesh into the surrounding brine, with higher levels occurring at lower salt concentration, as do the sugar levels. High brine salt levels lead to low acidity and relatively high pH levels (4.2–4.5). Brine salt levels of 11–12%, are reflected in flesh salt levels. As fermentation is often incomplete, residual sugar levels could range between 0.3 and 0.6% of olive flesh. With some cultivars, such as 'Ednecik' and 'Gemlik', where initial sugar levels in the flesh are high, residual sugar levels ranged around 0.7–1.0% (Borcaki *et al.*, 1993). At least a one-year storage period is required to ensure complete fermentation. As polyphenol levels in naturally black ripe olives are lower than in green ripe and turning-color olives, inhibition of LAB is not as great. Olive flesh also contains polyphenols with sugar residues that are released slowly by microbial and

weak acid hydrolysis that can also be used during fermentation. Anthocyanins also diffuse from the flesh into the fermentation brine, giving it a deep red color.

Major volatile compounds (at μg/kg levels in olive fruit) associated with olive style are acetic acid, 2-butanol, propionic acid, ethanol, isopentanol, 2-butanone, and minor compounds hexanal, nonanal, 3-hydroxy-2-butanone, ethyl acetate, ethyl propanoate, propyl propanoate, propyl acetate, 1-butanol, 1-hexanol, 1-pentanol, 1-propanol, 2-pentanol, isobutanol, 3-pentanol, 4-penten-1-ol, and cis-3-hexen-1-ol (Sabatini & Marsilio, 2008). The main effect during fermentation of black ripe olives is acid hydrolysis of glucosides in olive juice and aglycones in olive oil phase (Romero *et al.*, 2004). Principal anthocyanins found in black ripe raw olives, cyanidin 3-rutinoside and cyanidin 3-glucoside, were not present in brine or olives after one month of storage due to their polymerization. Naturally black ripe olives fermented under aerobic or anaerobic conditions develop different colors, possibly through alternative polymerization patterns. At the beginning, hydroxytyrosol-4-beta-glucoside, oleuropein, hydroxytyrosol, tyrosol, salidroside, and verbascoside can be identified in olive juice; and after one year, the main polyphenol, hydroxytyrosol. For the oil phase, the dialdehydic form of elenolic acid linked to hydroxytyrosol and tyrosol, oleuropein aglycone, and ligstroside aglycone were the main polyphenols at the beginning of fermentation, but were absent after three months.

15.5.4 Kalamata-style olives

Kalamata-style olives are prepared traditionally from naturally black ripe olives of the cv. ‘Kalamon’, although in some centers when this cultivar is unavailable similar size olives of other cultivars such as cvs. ‘Memecik’, ‘Mission’, ‘Leccino’, or ‘Barouni’ are used. Two processing methods are available. With the first method, olives are size-graded and slit, and bitterness is removed by repeated washing with potable water or 2–3% brine. The debittered olives are rinsed with red wine vinegar, then preserved in 8–10% brine with added virgin olive oil, oregano, and lemon slices. A second method is to prepare as for Greek-style black olives, then add the red wine vinegar, virgin olive oil, oregano, and lemon slices after fermentation.

In a study involving slit pink/purple/black olive fruit of the ‘Memecik’ cv., they were debittered by multiple water changes, then packed in 8% brine where they underwent fermentation. The moisture content of raw olives increased from around an initial 52% to around 55% after they had been placed in brine, and reached about 60% over 24 months of storage. Their initial ash content was around 1.4%, which increased twofold after debittering and rose steadily, stabilizing at around 4.5%. Initial acidity of the raw olives was around 0.25% and after debittering fell by half, then stabilized to around 0.45% from four months onward. Initial crude protein value was around 1.4%, and after 24 months fell to around 1.3%.

15.5.4.1 Suggested cultivars for Kalamata-style olives

Suggested cultivars for Kalamata-style olives are ‘Kalamon’, ‘Barouni’, ‘Leccino’, ‘Memecik’, and ‘Mission’.

15.5.5 Spanish-style green olives

With Spanish-style green and California-style black ripe olives, bitterness is removed from green ripe olives by alkaline hydrolysis of oleuropein, rinsed with water to remove excess lye, then fermented in brine (Spanish-style) or oxidized under alkaline conditions to a black color (California-style). Table 15.4 provides an outline of the processing methods.

For Spanish-style green olives, green ripe olives are treated with 2–5% lye to hydrolyze oleuropein and as a result partially remove their bitterness and increase skin permeability. They are left in lye for around 6–10 h until the lye has penetrated most of the flesh. After lye treatment, the pH of olive flesh ranges from 11 to 13. Olives are then washed up to three times to remove excess lye, reducing pH to 8–9. If strong lye solutions are used, longer washing periods are required. Washed olives are then placed in 8–10% salt brine at around 25 °C, where they undergo fermentation mainly by LAB. The brine pH falls rapidly to around 6 within a few days. Brine pH is monitored, and citric acid is added to maintain the pH at 6. Other food-grade acids or carbon dioxide can be used. The initial treatment is cultivar dependent with respect to flesh texture and olive size. Water-soluble compounds such as sugars, minerals, hydrophilic polyphenols, and

other nutrients diffuse from olive to brine, and salt from the brine accumulates in the flesh. Acids formed during fermentation lower brine pH to around 4.5. Under alkaline conditions, polyphenols undergo alkaline hydrolysis, and in the microbial stage, degradation of oleuropein occurs and less bitter end products such as hydroxytyrosol and elenolic acid glucoside are formed (Table 15.3). If the lye concentration is too strong, the olive fruit flesh softens, whereas if too weak, removing bitterness can take longer, which can negatively affect subsequent fermentation. Lye solutions can be reused several times as long as lye concentration is maintained. Reusing lye solutions has commercial and environmental implications. Fruit of the 'Gordal Sevillana', 'Manzanilla', and 'Hojiblanca' cvs. are commonly processed by this method. Characteristic to 'Gordal Sevillana' cv. fruit are white spots on the skin. Because of a favorable flesh-to-stone ratio, fruit from this cultivar is suitable for destoning and stuffing. During processing, the fruit of 'Manzanilla' cv. is prone to sloughing when placed immediately into lye solution, which is avoided by storing the fruit for 1–2 days before treatment. Spanish-style green olives are ready to eat in around one month.

Volatile compounds formed during fermentation include ethanol, acetaldehyde, and ethyl acetate. Major volatile compounds (μg/kg olive fruit) associated with olive style were acetic acid (3347), propionic acid (1994), 2-butanol (670), 1-propanol (534), ethanol (200), isopentanol (96), and minor compounds 2-butanone, hexanal, nonanal, 3-hydroxy-2-butanone, ethyl acetate, ethyl propanoate, propyl propanoate, propyl acetate, 1-butanol, 1-hexanol, 1-pentanol, 1-propanol, 2-pentanol, isobutanol, 3-pentanol, 4-penten-1-ol, and cis-3-hexen-1-ol (Sabatini & Marsilio, 2008).

15.5.5.1 Suggested cultivars for Spanish-style green olives

For Spanish-style green olive cultivars, use the following cultivars: 'Arauco', 'Aggeizi Shami', 'Aloreña', 'Arauco', 'Ascolano/a', 'Azapa', 'Cacereña', 'Cararrasqueña', 'Chalkidikis', 'Domat', 'Gordal Sevillana', 'Hojiblanca', 'Konservolia', 'Manzanilla/o', 'Memecik', 'Merhavia', 'Meski', 'Mission', 'Morona', 'Nocellara Etnea', 'Oblica' ('Oblitza'), 'Oliva di Spagna' ('Cerignola'), 'Picholine Marocaine', 'Sant'Agostino', 'Sigoise', 'Virdial', and 'Yamalak'.

15.5.5.2 Changes during processing of Spanish-style green olives

Lye treatment of green ripe olives increases skin permeability, degrades oleuropein, and hydrolyzes protein in the flesh releasing amino acids. Reducing sugars that pass from flesh to brine are utilized during fermentation, producing mainly lactic acid together with acetic and succinic acids and ethanol. Polyphenols are lost through diffusion, washing, brining, and fermentation. Generally, olive polyphenols are hydrolyzed during lye treatment and a proportion is lost. After brining and fermentation, a considerable drop in fruit polyphenol levels occurs with a corresponding decrease of polyphenols in the brine. After fermentation, hydroxytyrosol and tyrosol are present in the flesh but not oleuropein. Skin color changes from green to yellow due to degradation of chlorophylls and the presence of carotenoids. Other effects can be a loss of texture in addition to that occurring through well-controlled processing caused by excessive lye treatment, overheating, or microbial spoilage (Kiai & Hafidi, 2014).

In a study with fruit of five olive cultivars, 'Zard', 'Fishomi', 'Ascolana', 'Amigdalolia', and 'Konservolia', processed as Spanish-style green olives, there were changes in oil quality and fatty acid levels as well as their proportions. The highest changes in saturated and unsaturated fatty acids were in the fermentation step. Major changes occurred during lye treatment, when polyphenols fell to one-tenth of their original levels. There were no changes in sterol levels (Nikzad *et al.*, 2013).

15.5.6 Olives darkened by oxidation (California-style black ripe olives)

California-style black ripe olives, artificially blackened by oxidation under alkaline conditions, are called black ripe olives, a contradictory description. Green to turning-color olives are processed immediately after picking, or after storage in brine or specially formulated acid solution when processing capacity at the enterprise is overloaded. Raw olives are repeatedly treated, four to five times, with low-strength lye solutions to improve skin penetration and remove bitterness. In between these treatments, the olives are placed in potable

water through which air is injected to transform the green-colored olives to black. Blackened olives are then stabilized with ferrous gluconate (0.1%) or ferrous lactate over 1–12 h depending on the cultivar and specific procedures. After washing with potable water, the now black olives are placed in 3% brine for 1–3 days to stabilize the salt level in the olive flesh, then bulk pasteurized (60 °C for 45 min) to avoid bacterial spoilage, then packed in glass jars or cans with 2–4% salt brine (neutral pH) and sterilized. If the processed olives are packed in acid brine, pasteurization is sufficient for their preservation. Black-ripened olives are radically different from Greek-style black olives in aroma, taste, and texture. They have a characteristic salty, ocean-like, and earthy flavor. Off flavors and abnormalities include metallic, cheesy with astringent notes, gassy, soapy/medicinal, rancid, winey oak barrel, and buttery (Lee *et al.*, 2012).

15.5.6.1 Suggested cultivars for olives darkened by oxidation

Use the following cultivars: 'Arauco', 'Azapa', 'Cacereña', Californian 'Mission', 'Hojiblanca', 'Manzanilla', 'Manzanilla Chilena', 'Picholine Marocaine', 'Sevillano', and 'Verdial de Huevar'.

15.5.6.2 Effect of processing on olives darkened by oxidation

This processing method requires large amounts of potable water (35 L/kg olives). After treating the raw olives with lye, hydroxytyrosol and caffeic acid are prominent in the flesh. Caffeic acid is a hydrolysis product of verbascoside. Other polyphenols present are vanillic acid and *p*-coumaric esters. The brown-black color of olives by this process is due to oxidative polymerization of hydroxytyrosol and caffeic acid. If these olives are packed in low-pH solutions, an acerbic flavor predominates. Apart from oil, protein, and fiber, black ripe olives have vitamins, essential amino acids, and minerals. Sodium is by far the greatest because of brining and packaging. As this type of olive is processed with ferrous salts, its iron content (7 mg/100 g flesh) is over 20 times greater than that of Greek-style black olives, and it can sometimes have a metallic taste. During the alkaline phase and washing procedures, pigments degrade and sugars are lost. The lye treatment causes degradation of cell walls because of the generalized loss of pectic and hemicellulosic polysaccharides and cellulose, caused by the breakage of ester and hydrogen bonds. On the other hand, the lye treatment introduces shifts in the solubilization of polysaccharides, rendering them more difficult to extract by alkali solutions and enabling their retention in the cellulosic residue, which should contribute positively to cell wall firmness. Overall sterol content is broadly similar throughout the relatively complex processing procedure except that the total sterol content of sterilized olives of 'Hojiblanca' cv. had increased to 1.5 times that of the original raw olives (to around 2500 mg/kg) due to increases in ß-sitosterol, whereas for sterilized olives of 'Manzanilla' cv., the sterol content actually fell by about 20% to around 1100 mg/kg due to losses of most of the individual sterols. Erythrodiol was detected in sterilized California-style black ripe olives.

During California-style black ripe olive processing under certain conditions, acrylamide is generated (Charoenprasert & Mitchell, 2014). Approximate levels of acrylamide in California-style black ripe olives were 400–500 µg/kg. For comparison, California-style green olives had 40–100 µg/kg; Greek style olives had <1.4 µg/kg; and for Spanish olives, none was detected. Acrylamide is most likely produced during the high-temperature sterilization step for both Californian green and black ripe olives, but to a greater degree in the latter. Taking into account the following factors can minimize the problem. Air oxidation during lye treatment before sterilization significantly increases acrylamide levels in finished products, as does storing in brine for <30 days. Longer preprocessing storage periods (>30 days) result in lower acrylamide levels in the finished product. Also, the presence of calcium in storage solutions results in higher levels in finished products. Potential health problems resulting from acrylamide consumption, based mainly on animal studies, relate to nervous system, reproduction, cancer, and developmental effects.

15.5.7 Table olive processing methods that have limited commercial application

15.5.7.1 Green ripe and turning-color olives

Green ripe or turning-color olives are traditionally prepared by spontaneous fermentation in a similar way to Greek-style black olives or by soaking methods. Processed green ripe olives, sometimes called

"Sicilian-style olives," have a distinctive bitter flavor but generally turn gray/green in color if exposed to air while packing. They retain an olive-green color if packed with an antioxidant such as ascorbic acid (0.2 g/kg of total olives + brine). Processed turning-color olives are sometimes a pale pink color, possibly related to small amounts of anthocyanins in the raw material.

Variations to this method include slitting, bruising, cracking, or scratching the olives, prior to fermentation; placing them in water; then changing the water every second day until the desired taste is achieved; then placing them in brine, where they undergo a weak fermentation. Green ripe or turning-color olives can also be processed by daily changes of potable water/weak brines for 7–10 days, then packed in vinegar/brine. To avoid salt-induced shrivel with fruit of some cultivars, such as 'Ascolana' and 'Sevillana', low-strength brine solutions (4–5%) are used. 'Manzanilla' and 'Hojiblanca' cvs. olives do not suffer from this problem. Cracked natural green olives in Greece, Cyprus, and elsewhere are called *tsakistes*.

In Italy, Itrana-style green ripe and black ripe olives are produced from 'Itrana' cv. olives using either green/turning-color olives (*oliva bianca di itri*) or naturally black ripe ones (*oliva nera di gaeta*). Olives are placed in containers with potable water for a month or so, then salt is added (7 kg/100 kg of olives). Bianca olives are ready to eat 6–8 months later. Gaeta olives are ready to eat after 4–6 months.

15.5.7.2 Changes in green ripe olives during fermentation

During the brining/fermentation phase, there is a radical fall in flesh oleuropein with a commensurate increase in hydroxytyrosol in brine. Turning-color olives are prone to gassy, floater, and fish-eye spoilage, most likely due to anomalous bacteria. Olives float due to accumulation of gasses released during fermentation that collects under the skin as blisters. If blisters burst, the olives appear shriveled or develop fissures. In a study of natural fermentation of green ripe olives of the 'Tonda di Cagliari' cv. brined at 4% and 7% NaCl, puncture (skin) and texture (flesh) tests revealed that skin hardness of the olives was less at lower salt levels than at the high levels, whereas texture was unaffected.

Raw olives of 'Ednecik' and 'Gemlik' cvs. prior to fermentation had reducing-sugar and moisture contents of around 6 and 60% for 'Ednecik' and 4.5 and 43% for 'Gemlik'. After fermentation, the reducing-sugar levels in the olives had decreased to less than 1% for both cultivars. The ash content was higher in the fermented olives as they had taken up substantial amounts of salt during processing, whereas changes in protein levels were marginal.

When investigations were made on the effect of natural fermentation on the oxidative and hydrolytic degradation of the lipid fraction of three Italian table olive cultivars 'Bella di Cerignola', 'Termite di Bitetto', and 'Peranzana', the total polyphenols in the lipid fraction of around 400 mg/kg oil fell nearly fourfold. At the end of processing, the level of primary oxidation was comparatively low. A low-level secondary oxidation took place, accounting for a small increase in triacylglycerol oligopolymers. However, a significant increase occurred in fatty acid content as a result of hydrolytic degradation (Pasqualone *et al.*, 2014). Similar changes occur with early turning-color olives.

15.5.7.3 Suggested cultivars for fermented green ripe olives

Use the following cultivars: 'Aggezi Shami', 'Alfafara', 'Amygdalolia', 'Arauco', 'Ayvalik' (split), 'Azapa', 'Azeradj', 'Baladi', 'Barnea', 'Barouni', 'Blanquette', 'Buga', 'Carolea', 'Carrasquenha', 'Çekiste', 'Çelebi', 'Cordovil de Serpa', 'Cornicabra', 'Cucco', 'Domat', 'Erkence', 'Gemlik', 'Gerboui', 'Giarraffa', 'Gordal Sevillana', 'Grimski-172', 'Hamed', 'Haouzia', 'Hojiblanca', 'Istrska Belica', 'Itrana', 'Karolia Lesvou', 'Kadesh', 'Kaissy', 'Karydolia', 'Konservolia', 'Ladoelia', 'Lucques', 'Manzanilha Algarvia', 'Manzanilla', 'Manzanilla Cacereña', 'Mara', 'Memecik', 'Memeli', 'Menara', 'Meski', 'Mission', 'Morisca', 'Nabali', 'Nikitski II', 'Nocellara del Belice', 'Nocellara Etnea', 'Oblica', 'Oliva di Cerignola', 'Picholine', 'Picual', 'Pizz Carroga', 'Rasi'i, 'Redondal', 'Saloneque' (split), 'Santa Caterina', 'Sant'Agostino', 'Sigoise', 'Souryi', 'Toffahi', 'Vasilikada, 'Verdale', 'Villalonga', and 'Zizula'.

15.5.7.4 Suggested cultivars for turning-color olives

Use the following cultivars: 'Barnea', 'Edremit', 'Gaidourelia', 'Gordal Sevillana', 'Hojiblanca', 'Itrana', 'Kalamon', 'Kolybada', 'Manzanilla', 'Manzanilha Algarvia', 'Memecik', 'Picual', and 'Verdale'.

15.5.8 Dehydrated table olives

Traditionally, in and around the Mediterranean Basin, olives have been debittered by dehydration using heat from the sun or an oven; by packing olives in coarse salt; or even by allowing them to dehydrate on the olive tree. Dried olives are usually dark brown to black in color and shriveled. Colloquially, salt-dried olives have been called *throumbes*, *alatsolies*, *staphythoelies*, shriveled olives, raisin olives, date olives, and Kalahari olives. Drying reduces the water content and decreases the water activity of the olives, hence aiding their preservation.

15.5.8.1 Heat-dried olives

A well-recognized heat-drying method in Italy involves 'Majatica di Ferrandina' cv., where the olives are blanched by dipping them in very hot water (95 °C) for a few minutes in order to disrupt their skin, followed by storage in brine (7–8%) for a few days, then oven drying at 50 °C. Variations of the method include placing the olives in 10% salt brine for a few days after blanching, then oven drying them; or dipping the olives (e.g., 'Ascolana Tenera' cv.) in hot 10% salt brine for 10 min, followed by dehydration in a hot-air oven (50 °C) until they lose their bitterness in around 2–3 days. Heat-dried olives are slightly bittersweet, suggesting that substantial amounts of oleuropein have degraded and sugars have concentrated after the heating step.

A variant to this method is the Sardinian 'Scabecciu' olive. Here, the skin of naturally black ripe olives of either 'Tonda di Cagliari' or 'Pizz'e Carroga' cvs. are scratched in three places, then placed in brine. After three days, the olives are washed with potable water, blanched with vinegar water, then dried in the sun. They are ready to eat after a month or so. When ready, the olives are fried with garlic and parsley, then packed in extra virgin olive oil (EVOO).

In Azerbaijan, naturally black ripe olives ('Gara zytun' and 'Shigin zytun' cvs.) are chilled, then dried at 30–40 °C until moisture levels reach 25–30%.

15.5.8.2 Cultivars for heat-dried olives (oven or sun)

The following cultivars can be used for heat-dried olives: 'Arauco', 'Ascolano/a', 'Dolce Agogia', 'Majatica di Ferrandina', 'Pizz'e Carroga', 'Memecik', 'Sevillana' ('Olives of Criolla'), and 'Tonda di Cagliari'.

15.5.8.3 Some changes in heat-dried olives during processing

Heat-dried 'Cassanese' cv. olives processed by the *Ferrandina* method when oven dried had little effect on the wax layer covering the skin and limited shriveling. Changes occurred in the pectin components: their content decreased during initial heating and paradoxically increased during oven dehydration.

15.5.9 Salt-dried olives

To prepare traditional salt-dried olives, naturally black ripe olives, preferably overripe, are packed with alternating layers of food-grade coarse salt at a rate of 10–20% (w/w). Salt draws out moisture from the olives by osmosis, which is allowed to drain away. As a result of salting, the flesh tissue ruptures, bringing polyphenols such as oleuropein into proximity with polyphenoloxidase. Here, phenols are oxidized to highly reactive quinones that polymerize. There is limited leaching of oleuropein into the vegetable water from the olives. The olives shrivel, have a bitter salty taste, and can be eaten after one to two months. In Lebanon, green ripe olives are used for this style. The traditional product has a high salt content, and the salt is the preservative. Olives dehydrated at low salt levels will need further treatment such as pasteurization to facilitate their preservation.

15.5.9.1 Suggested olive cultivars for salt-dried olives

The following cultivars are used for salt-dried olives: 'Alats', 'Edremit', 'Kalamon', 'Thasitiki' ('Thrumba'), 'Thrubolea Lesbos', 'Thrubolea Chios', 'Thrubolea Samos', 'Thrubolea of Cyclades Islands', and 'Thrubolea of Ampadias Rethimnon'.

15.5.10 Naturally dehydrated olives

As oleuropein levels in olive fruit fall significantly when fully ripe, the olives from some cultivars can be eaten directly off the tree. Where these olives are retained on the tree, they dehydrate naturally and can be eaten as is or can be salted, rinsed with vinegar, then packed with olive oil. Other naturally dehydrated overripe olives are *thruba style* (Greek Islands) or *passuluna style* cv. 'Cerasuola' (Sicily). Thruba-style olives are produced from 'Thrubolea' cv., which grows in the Aegean Islands, Crete, and some parts of Attica. It has been speculated that fruit of this variety loses bitterness through the action of an enzyme produced by *Phoma oleae*, a fungus. Following harvesting, the olives are washed with potable water and then sun dried to remove moisture and hence reduce their water activity. Coarse salt is added to improve their organoleptic properties and their preservation. The fruit of a clone of 'Thrubolea' cv. grown on the Greek island of Thassos does not sweeten naturally on the tree, although it is used for a similar final product. *Passuluna*-style olives are shriveled and debittered olives following infestation of the olives by the fungus *Camarosporium dalmaticum* introduced by the parasite of *Bactrocera oleae* eggs, *Prolasiopetera berlesiana*. In France, naturally black ripe 'Tanche' cv. olives are deliberately left on the trees to overripe during cold weather, resulting in shriveling and sweetening of the olives.

15.5.10.1 Suggested cultivars for naturally dried olives

The following cultivars can be used for naturally dried olives: 'Cerasuol', 'Meski', 'Tanche', and 'Thrubolea'.

15.5.11 Olives treated with lye

An ancient method for debittering green ripe olives is treating them with olive wood ash or wood ash and lime (CaO). A past example is from Campania, Italy, where 'Pisciotanna' cv. olives were used. Today, lye solutions (sodium or potassium hydroxide) have largely replaced this method.

15.5.12 Stuffed, seasoned, and marinated table olives

Many Spanish-style green olives are pitted or pitted and stuffed with filling material such as cheese, pimento, anchovy, almond, or onion. Fresh stuffing material can be a source of unwanted organisms, and some may cause allergic reactions. Another form of stuffed olive is Ascolano-style freshly prepared from destoned lye-treated green olives from 'Ascolano Tenera' cv. stuffed with meat filling (beef, pork, chicken, and turkey), then fried in olive oil.

Table olives can also be seasoned by adding to a covering brine various combinations of dried herbs and spices, such as garlic, cumin, oregano, pepper, thyme, rosemary, lavender, fennel, paprika, and citrus peel. Addition of plant extracts, essential oils, or oleoresins (a natural antioxidant of these) give a more uniform flavor with less risk of deterioration. Packed products are then pasteurized or a mixture of benzoic and sorbic acids is added to the packing solution to give a final concentration of 0.5 and 1.0 g/L, respectively, with unpasteurized products.

15.5.13 Picholine-style olives

Traditionally, picholine-style olives originate from Languedoc and Luques in southern France. The process is similar to that for Spanish-style green olives except there is no fermentation step. In principle, green ripe 'Picholine' cv. olives are placed in lye solution until it has penetrated three-quarters of the way through the flesh. The treated olives are washed several times over a few days to remove excess lye, and then placed in 5–6% salt brine for 2 days, then into 7% salt brine, and the pH is adjusted to 4.5. Olives retain an intense green color and are ready to eat within 2 weeks. As fermentable sugars are still present in the olives, packing and pasteurization procedures must be undertaken to avoid subsequent fermentation of the final product.

15.5.13.1 Suggested cultivars for Picholine-style olives

The following cultivars can be used for picholine-style olives: 'Picholine' as well as cvs. used for Spanish-style green olives (see Section 15.5.5.1).

15.5.14 Castelvetrano-style olives

Castelvetrano-style olives are popular in Italy, especially in Sicily. They are a sweet green olive but different from Sicilian-style green olives that undergo a natural fermentation without pretreatment with lye. With Castelvetrano-style olives, large fruited local cultivars 'Nocellara de Belice' and 'Nocellara Etnea' are used. After washing raw green olives, depending on their ripeness they are placed in a 2–3% lye solution in a suitable plastic barrel or drum. After one hour, coarse salt (3–4 kg/100 L barrel) is added and agitated to mix and dissolve the salt. The barrel is sealed and further agitated over a day or so to ensure mixing and to form the processing brine. The olives are ready to eat after two weeks. Under these conditions, the olives quickly lose their bitterness while retaining a bright green color. When the barrel is opened, the lye/salt brine is drained, and the olives are washed to remove excess lye. Under ambient storage conditions, the Castelvetrano-style olives have a shelf life of only a few months, especially under hot conditions. As this olive style is a seasonal product, either refrigerated storage at 4–7 °C or suitable packaging solution is recommended.

Major volatile compounds (at μg/kg olive fruit) associated with this olive style are acetic acid, propionic acid, 2-butanone, ethanol, 2-butanol, 1-propanol, and minor compounds isopentanol, hexanal, nonanal, 3-hydroxy-2-butanone, ethyl acetate, ethylpropanoate, propyl propanoate, propyl acetate, 1-butanol, 1-hexanol, 1-pentanol, 1-propanol, 2-pentanol, isobutanol, 3-pentanol, 4-penten-1-ol, and cis-3-hexen-1-ol (Sabatini & Marsilio, 2008).

15.6 Nutritional, health, and safety aspects of table olives

Table olives are a valuable high-energy foodstuff with significant biological and nutritive value, especially because of their quantity and quality of fat, together with a number of essential and health-promoting nutrients. They are often eaten as snacks, but traditionally they have been an important part of the diet of persons living in and around the Mediterranean Basin. The benefits of table olive products to health have been predominantly based on evidence from studies on consumption of virgin olive oil (VOO), monounsaturated fats, and minor components, especially polyphenols and often in the context of the Mediterranean diet.

15.6.1 Mediterranean diet (as per Crete)

The Mediterranean diet is associated with a lower incidence of chronic degenerative diseases and longer life expectancy. It is universally accepted that the health benefits are ascribed to the diet for being rich in fruit and vegetables, low in red meat, and with accompanying VOO consumption as the main fat source. Benefits of VOO relate to its healthy fat profile (MUFAs, PUFAs, and relatively low SFAs) and minor components, especially polyphenols. Investigations involving humans (population, clinical, and case studies) and animals (*in vivo* and *in vitro*) have revealed that oleic acid and olive phytonutrients, especially polyphenols, have promising valuable biological actions such as antioxidant, anti-inflammatory, and antibiotic effects (Cicerale *et al.*, 2012).

Epidemiological and clinical studies have shown that those consuming the traditional Mediterranean diet, typical of Cretans up to 1960, live longer, have decreased incidence of degenerative disease, and have reduced morbidity and mortality (Bach-Faig *et al.*, 2011). This style of diet compared to Western diets has been linked to lower incidences of chronic health problems such as obesity, cardiovascular disease, and cancer. A meta-analysis of numerous investigations involving 2–4 million subjects revealed that partaking the Mediterranean diet reduced mortality due to cardiovascular disease (10%), reduced incidence and mortality from cancer (4%), reduced overall mortality (8%), and reduced incidence of neurodegenerative disease (13%) (Sofi *et al.*, 2010).

More recent research has focused on the importance of olives and olive oil as functional products and their contribution in addition to antioxidants in fruit and vegetables. Table olives and olive oil contain abundant antioxidants and other health-promoting minor components. Processed olives that have not been treated with lye contain up to 16 g/kg of polyphenols typified by acteosides, hydroxytyrosol, tyrosol, and phenylpropionic acids. Olive oil, especially EVOO, contains smaller amounts of hydroxytyrosol and tyrosol, but has abundant secoiridoids and lignans. Both olives and olive oil contain substantial amounts of other minor components deemed to be anticancer agents, as well as the peroxidation-resistant lipid, oleic acid.

Population studies have revealed an inverse relationship between fruit and vegetable consumption and the incidence of cardiovascular disease and cancer. These benefits are relevant to the Mediterranean diet as well as other healthy diets rich in plant-derived foods. Diets with substantial quantities of fruits and vegetables, of at least 500 g/day, reduce the risk of chronic medical problems such as cardiovascular diseases, type 2 diabetes, and cancer developing in aging humans (Wang *et al.*, 2014). In primary disease prevention, dietary maneuvers are the favored initial management of conditions such as early type 2 diabetes and high blood cholesterol.

15.6.2 Health benefits of table olives in the Mediterranean diet

Processed olives are a high-energy food with their fat component responsible for up to 80–85% of their energy value. For three different table olive products, prepared from 'Memecik' cv. fruit as Spanish-style olives (green ripe olives), Kalamata-style olives (pink/purple/black), and Greek-style black olives, energy values were approximately 650, 900, and 1050 kJ/100 g flesh, respectively. These values are consistent with commercially available products as per label: Spanish-style green olives ('Manzanilla' cv. 610 kJ/100 g; 'Cerignola' cv. 660 kJ/100 g), Greek-style black ('Kalamon' cv. 1020 kJ/100 g), and California-style black ripe ('Manzanilla' cv. 620 kJ/100 g). Heat-dried olives have nearly double the energy value at 1900 kJ/100 g. A 3 g 'Kalamon' cv. table olive, with a 5:1 flesh-to-stone ratio with 2.5 g flesh/olive, has an energy equivalent ≈ 25 kJ/olive. An intake of 10 of these table olives per day would have an energy equivalent of ≈ 250 kJ/day.

Eating enough food to provide energy (kilojoules) for body functions and activity is essential, but if more energy is consumed than can be used, weight gain and obesity are likely. Australian research suggests that on average, a 70 kg person needs 8700 kJ/day, with lesser amounts for women, sedentary, and bedridden persons. Energy is only one aspect of the diet, but if the diet predominantly consists of *empty calories* (i.e., few nutrients are present), health-related problems are more likely. A well-balanced diet lowers the risk of obesity and numerous medical problems, including, hypertension, heart attacks, strokes, type 2 diabetes, and some cancers. Childhood obesity must be avoided as it can lead to future health problems.

15.6.3 Composition of processed table olives

Composition of table olives varies with cultivar, growing conditions, maturation stage at harvest of the raw olive fruit, processing technology, and final presentation such as stuffed, added olive oil, and/or added herbs and spices. As an approximate guide, flesh content is 10–30% fat, 1–1.5% protein, 1.5–2.5% fiber, 60–80% moisture, and 2–7% ash. High levels of fat are present in commercially available Kalamata-style ('Kalamon' cv.) olives (26%), whereas those from lye-treated 'Manzanilla' cv. were 16%, possibly reflecting the processing method, cultivar, and added oil. Around 75% of fat in table olives is the MUFA oleic acid, with essential PUFAs linoleic acid and α-linolenic acid making up the balance. In a study of 67 commercial table olive products, the fat profiles expressed as g/100 g edible portion were SFAs, 2.07–5.99%; MUFAs, 5.67–19.42%; PUFAs, 0.52–3.87%; and TFAs (trans fatty acids), 0.08–0.44% (López *et al.*, 2006). It should be noted that in some jurisdictions, food products with <0.5 g of trans fats can be labeled as TFA free.

Ash represents residual minerals, so higher values relate to greater salt content of the olives. Commercially available Spanish-style green olives stuffed with anchovies had the highest value of sodium (4000 mg/100 g of edible portion), whereas low-salt products such as California-style black ripe olives had 750 mg/100 g. Table olives contain a number of vitamins, such as B group (thiamine and riboflavin) as well as β-carotene (vitamin A precursor) and vitamin E (α-tocopherol). Ascorbic acid (vitamin C) is present in some processed green ripe olives if packed with this antioxidant to prevent browning. Olive flesh also contains low levels of minerals such as potassium, calcium, magnesium, sulfur, iron, magnesium, zinc, and copper. Iron levels are highest in California-style black ripe olives (0.14 mg/100 g). Most table olive products have negligible or low sugar content, unless they have not undergone fermentation, fermentation is incomplete, or sugar has been added to the packing brine. Heat-dried olives, including UC13A6 cv., retain sugars so they are distinctly sweet to taste (personal observation).

A snapshot of nine commercially available table olives in Greece indicated that the polyphenol content is highly influenced by olive cultivar and processing method. Hydroxytyrosol levels for different olive cultivars ranged between 1 and 560 μg/g flesh, where 'Kalamon' (black Kalamata style) > 'Megaritiki' *tsakistes* (natural green) > 'Agouromanaki' (natural green) > 'Chondrolies' (Greek-style black) > green (Spanish-style

green) > ‘Amfissa’ (Greek-style black) > ‘Throuba Thassos’ (salt-dried black) > ‘Mavrolies’ (Greek-style black) > ‘Megaritiki’ (salt-dried black). On a per fruit basis, Kalamata-style olives and green ‘Megaritiki’ *tsakistes* contained the greatest amount of hydroxytyrosol (1.8−2.0 mg/fruit), followed by Greek-style black ‘Chondrolies’ with a quantity of 1.0 mg/fruit. Oleuropein was found in small amounts in ‘Chondrolies’ and ‘Mavrolies’, but in the case of ‘Throumba Thassos’, which is processed by dry salt in a traditional Greek way, oleuropein was found in significant quantity (1.2 mg/fruit) with much smaller amounts of hydroxytyrosol. Furthermore, with ‘Throumba Thassos’, consuming 20 olives/day delivers 25 mg/day of oleuropein, considered to be a safe amount (Zoidou *et al.*, 2010).

15.6.4 Polyphenols in processed table olives

Generally, antioxidant activity of olive products is closely linked to their polyphenol content (Del Monaco *et al.*, 2015). Health-promoting qualities of olive polyphenols have been reported extensively (Buckland *et al.*, 2012; Charoenprasert & Mitchell, 2012; Bulotta *et al.*, 2014; Covas *et al.*, 2015).

Most kinds of table olives are a good source of polyphenols, especially hydroxytyrosol and oleuropein in dried olives. Indicative content of hydroxytyrosol is 250–760 mg/kg for Kalamata olives, 170–510 mg/kg for Spanish style olives, and 100–340 mg/kg for Greek-style naturally black olives (Blekas *et al.*, 2002). The data suggest that overall hydroxytyrosol levels in commonly available table olives range from 100 to 760 mg/kg (0.5–3.8 mg/5 g portion) with a theoretical median value of ≈ 300 mg/kg (1.5 mg/5 g portion). In contrast, EVOO has around 8 mg/kg oil (Neveu *et al.*, 2010), obviously much less than that in table olives. Exact intake would depend on the bioavailability of hydroxytyrosol from table olives. The bioavailability of two polyphenols, tyrosol and hydroxytyrosol, from olive oil was 40–95% (Vissers *et al.*, 2002). Polyphenol levels in the juice and oil of packed table olives were different (Romero *et al.*, 2004) with hydroxytyrosol and tyrosol predominating in the juice and tyrosol acetate, hydroxytyrosol acetate, hydroxytyrosol, tyrosol, and lignans (1-acetoxypinoresinol and pinoresinol) in oil. Overall turning-color olives in brine had the highest polyphenol levels (≈1200 mg/kg), and oxidized olives (California-style black ripe olives) the lowest (≈200 mg/kg). With the latter, hydroxytyrosol is lost due to oxidation and polymerization during processing (Marsilio *et al.*, 2001; Charoenprasert & Mitchell, 2012). Cultivar differences in polyphenol levels were also observed, with ‘Manzanilla’ > ‘Hojiblanca’ > ‘Gordal’. A tenfold difference in hydroxytyrosol has been observed between cultivars (Marsilio *et al.*, 2001). Furthermore, levels decreased according to presentation, with plain > pitted > stuffed, mainly due to additional washing steps.

It has been recognized that daily consumption of olive polyphenols (5 mg/day) decreases the risk of cardiovascular disease, reducing peroxidation of blood lipids (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2011). The EFSA Panel considered that polyphenols in the olive, *Olea europaea* L. (olive fruit, olive oil, and olive mill waste water), standardized by their content of hydroxytyrosol and its derivatives (e.g., oleuropein complex) could protect low-density lipoprotein (LDL) particles from oxidative damage with beneficial physiological effects. Also in order to bear the claim, the EFSA Panel considered that 5 mg of hydroxytyrosol and its derivatives, in olive oil, should be consumed daily. However, at the time of their report, the EFSA Panel indicated there was insufficient evidence to support the consumption of olive oil polyphenols (and its derivatives) to maintain normal blood high-density lipoprotein (HDL) cholesterol concentrations, to maintain normal blood pressure, and to exert anti-inflammatory effects in the context of diseases such as osteoarthritis and rheumatoid arthritis; to contribute to upper respiratory tract health, because the claimed effect was too general and nonspecific; and to help to maintain a normal function of gastrointestinal tract, because the claimed effect was too general and nonspecific. Effects and actions of polyphenols are listed in Table 15.5.

15.6.5 Selected non-polyphenol minor components in olive fruit

Non-polyphenol minor components of processed olive fruit that are of interest include squalene, amyrins, erythrodiol, uvaol, and phytosterols.

15.6.5.1 Squalene

Olives and olive oils contain useful quantities of squalene that when consumed passes mainly into epithelial tissue, especially into skin sebum. The composition of 30 table olive samples from Italy included squalene

Table 15.5 Some effects and actions of olive polyphenols.

Biological effect/pathology	Olive polyphenol action
Anticancer and chemopreventive	Directly controls cell growth at different stages of carcinogenesis by inducing apoptosis or inhibiting proliferation by diverse mechanisms
Anti-inflammatory	• Reduces inflammation • Cardiovascular diseases (CVD) • Some types of cancer
Antimicrobial and chemotherapeutic	Antibacterial, antifungal, antiviral, antiprotozoal, and antiparasitic
Antioxidant	• RONS (reactive oxygen and nitrogen species) scavenging, reducing power, and metal-chelating activities • Induces endogenous antioxidant enzymes
Autonomic nervous system	Cholinergic and adrenergic effects
Cardiovascular effects	• Antihypertensive action • Platelet and endothelial function against atherosclerosis
Central nervous system	Neuroprotective, analgesic, antinociceptive, and behavioral
Endocrine systems	Antidiabetic, osteoprotective, and other endocrine effects
Immunomodulatory	Modulates immune function
Respiratory	Antioxidant and anti-inflammatory properties against lung diseases

(537–1583 mg/kg), α-tocopherol (21–90 mg/kg), and β-carotene (0.4–2.6 mg/kg) (Sagratini *et al.*, 2013). Even though squalene is an intermediate in cholesterol synthesis in humans, daily consumption does not increase cholesterol levels (Martirosyan *et al.*, 2007).

15.6.5.2 Phytosterols

The recommended dose for adults is 2 g phytosterols daily to reduce LDL cholesterol (by 10%) and reduce cardiovascular disease risk (Expert Panel, 2001). Green table olives contain around 35 mg/100 g edible portion, and black table olives contain 50 mg/100 g edible portion. Therefore, one 4 g edible portion of table olives would contain 1.75–2.0 mg of phytosterols.

15.6.5.3 Tocopherols and tocotrienols

The total content of tocopherols and tochotrienols of the table olives varies between cultivars, with α-tocopherol the most abundant. During storage of raw table olives as well as during processing, α-tocopherol content decreases. Indicative levels for processed olive fruit from five cultivars are total tocopherols 3.5–6 mg/kg fresh weight and α-tocopherol 2.3–5.7 mg/kg fresh weight, with levels for 'Negrinha de freixo' > 'Verdeal transmontane' > 'Madural' > 'Cobrançosa' > 'Santulhana' cvs. (Malheiro *et al.*, 2012).

The effects of processing upon tocopherol and tocotrienol content of four Greek olive cultivars – green ripe 'Konservolia' and 'Chaklidikis' (Spanish-style green) and black ripe 'Konservolia' and 'Kalamon' (modified Greek style) – were investigated. Processing decreased α-tocopherol in 'Chalkidikis' cv. olives and α-tocotrienol in naturally black ripe olives. γ-Tocopherol was unaffected by processing. α-Tocopherol accounted for 61–85% of all tocopherols measured in the pulp, and it was higher in black olives than green ones. Changes in the amounts of α-tocopherol pulp from raw to processed olives were 22 to 24, 9 to 16, 33 to 33, and 35 to 40 μg/g, and final α-tocopherol equivalents were 24, 17, 34, and 45 μg/g for Spanish style 'Konservolia' and 'Chaklidikis' and Greek-style 'Konservolia' and 'Kalamon', respectively (Hassapidou & Manoukas, 1993). In another report, processed green olives had higher α-tocopherol content (3.81 mg/100 g edible portion), followed by avocado (Florida, 2.66; Haas, 1.93; unspecified, 1.81). Canned black ripe olives, assumed to be California-style, had less than half that of processed green olives (1.65 mg/100 g edible portion).

15.7 Quality and safety aspects relating to table olives

Two international documents are a guide for producing quality olives (International Olive Council, 2004, 2005). Physical and organoleptic characteristics are described. Criteria are described for fruit maturation, trade preparations, olive size, packing brine details, and damaged and abnormally colored fruit, together with abnormal fermentation, saltiness, bitterness, acid taste, hardness, and crunchiness. More complex characteristics are used where table olive products are evaluated in competitions.

15.7.1 Salt content of table olives

It is well recognized that high dietary intake of salt is inappropriate for persons with medical conditions such as hypertension and heart and kidney disease. The National Health and Medical Council of Australia has set an adequate sodium intake of 20–40 mmol/day corresponding to 1.15– 2.3 g/day with a suggested daily intake for adults of 1.6 g of sodium, which is equivalent to 4 g of sodium chloride. The average adult intake is around 2–3 times this amount, mainly via processed foods. According to the Mayo Clinic, the average US diet contains around 3.4 g/day of sodium, which is 1.1 g/day more than the upper recommended limit for healthy adults of 2.3 g/day. Persons 50 years of age and older, and those with known heart disease and hypertension, should limit their sodium intake to 1.5 g/day.

Many table olive products contain large amounts of salt, where it is being used as a preservative. Because there are different sizes of table olives, from numerous cultivars and maturation states processed by a myriad of methods, it is difficult to generalize. An indicative amount that can be used is 20 to 40 mg of sodium per olive. The olive industry is cognizant of this problem, and research has been directed toward using minimal quantities of salt during processing, packing processed olives in low-acid salt brines, and heat treating the final product. From consumers' point of view, labels contain more specific information for a particular product. Furthermore, popular salt-dried olives such as the *Thassos* style prepared in the traditional way can contain 10% or more sodium chloride if prepared by the traditional method. New methods are being investigated where drying is undertaken with a combination of heat and lower salt levels. Heat or sundried olives contain little sodium unless salt has been added as a flavor. These olives, however, would retain sugars in concentrated form that may be a problem for some diabetic persons.

To reduce sodium chloride levels in table olives, hence sodium intake, a number of investigations have focused on replacing some sodium chloride with calcium chloride or potassium chloride in table olive fermentation without jeopardizing the process, microbial safety risk, and/or sensory characteristics of the final product (Romeo *et al.*, 2009; Bautista-Gallego *et al.*, 2010, 2011). Sensory evaluation was a decisive factor in the acceptability of the final table olive product. With naturally black ripe 'Konservolia' cv. olives, increasing concentrations of calcium chloride or a combination of calcium chloride with potassium chloride produced a bitter product with low acceptability. The most acceptable combination was 4% sodium chloride and 4% potassium chloride, producing olives with lower sodium content and good sensory attributes (Panagou *et al.*, 2011).

A fermentation brine containing sodium chloride and calcium chloride reduced bacterial and yeast growth, whereas a mixture of sodium chloride and potassium chloride had similar effects as a sodium chloride brine. When 'Kalamon' cv. was processed using sodium chloride–potassium chloride brine, coactivity between calcium lactate and calcium acetate induced high growth rates of *Lactobacillus plantarum* and *Debaryomyces hansenii* that had been used as starter cultures (Tsapatsaris & Kotzekidou, 2004).

15.7.2 Microbiological safety and quality of table olives

On the whole, table olive products are a safe foodstuff, more than ever if they are produced under GAP (Good Agricultural Practice), GHP (Good Hygienic Practice), and GMP (Good Manufacturing Practice), followed by end products that are preserved effectively through physicochemical means, preservatives, and/or heat treatment (hot packing, pasteurization, or sterilization). Traditional olive fermentation processes that occur spontaneously are not fully predictable, especially when under inadequate control, leading to product spoilage or health risk to consumers. The presence of such harmful microorganisms or their metabolites is

Table 15.6 Justification of microbiological criteria.

Microorganism	Threshold	Justification/notes
Salmonellas	Absent in 25 g	Pathogen. Testing is desirable even though risk is minimal due to fact that product is not particularly susceptible to its development.
Staphylococci (coagulase-positive)	$<1.10^2$/g	Pathogen. Minimal risk but good indicator for contamination of human origin during handling of product.
Escherichia coli and fecal coliforms	$<1.10^2$/g	Tracers for fecal contamination. Testing for *Escherichia coli* is best performed on vegetable matter since populations of fecal coliforms are not always synonymous with fecal contamination.
Aerobic mesophilic microorganisms	1.10^6 to 5.10^6/g, depending on the type of product	Indicators for pilot testing (figures reported by professionals, based on good-quality products).
Lactic bacteria	1.10^9/g	Presence in unpasteurized products
Yeasts	$<5.10^5$/g	Presence in unpasteurized products
Molds	$<5.10^2$/g	Presence in unpasteurized products
Sulfite-reducing anaerobes	$<1.10^2$/g as indicative or compulsory, depending on type of trade style	Good indicator for colonies of bacillus
Clostridium perfringens	$<1.10^2$/g as indicative or compulsory, depending on type of trade style	Pathogen. Indicator of anaerobic flora. Threshold values apply to commercial treatments for raw semolina and/or cooked vegetables.
Listeria monocytogenes	$<1.10^2$/g	Pathogen. Widespread in the environment. Organism can survive in fermented olives.

Source: RIRDC (2012).

often linked to postprocessing operations rather than during processing, and hence can be problematic for any table olive product.

In Australia, the RIRDC's *Voluntary Industry Standard for Table Olives in Australia* (RIRDC, 2012) has been developed and prepared by the National Table Olive Committee (NTOC) of the Australian Olive Association Ltd (AOA). It is a voluntary industry standard that establishes an objective basis for the wholesale and retail trade of table olive products in Australia. It is consistent with the IOC Trade Standards Applying to Table Olives but has included microbiological criteria for the wholesale and retail olive trade. Table 15.6 gives acceptable levels of potentially harmful organisms as well as those that are present during fermentation or develop during storage.

15.7.2.1 Foodborne pathogenic bacteria

Organisms such as *Listeria monocytogenes*, *Staphylococcus aureus*, and Enterobacteriaceae species such as *Yersinia enterocolitica* and *Escherichia coli* have been identified in table olives; however, there are no reports of major outbreaks of these microorganisms with table olives. Fecal coliforms are occasionally found in green cracked olives (Franzetti *et al.*, 2011). To overcome microbial problems with olives, heat treatments such as pasteurization and sterilization are used. These treatments may negatively affect color and consistency of olives, which must be accepted to ensure microbial safety and stability. Coliform bacteria are part of the Enterobacteriaceae family and include *Escherichia coli* as well as a number of members of the genera *Enterobacter*, *Klebsiella*, and *Citrobacter*. *Listeria monocytogenes* has been shown to survive in green table olives despite the processed olives having a low pH, high salt, and appropriate heat treatment (Caggia *et al.*, 2004).

The life-threatening pathogenic bacterium *Escherichia coli* O157:H7, accountable for hemorrhagic colitis and hemolytic uremic syndrome, may be linked with Spanish-style green olives. Pathogen numbers during

fermentation decline but do not disappear completely, possibly due to acid production, such as lactic, formic, and acetic acids (Spyropoulou *et al.*, 2001). Furthermore, opportunistic pathogens for humans such as *Enterobacter cloacae* have been identified with spontaneously fermented table olives (Bevilacqua *et al.*, 2010).

15.7.2.2 Clostridial food poisoning

Clostridia are an extensive mixed group of bacteria that can easily contaminate food as they generate resistant spores that can endure mild processing methods. Sulfite-producing clostridia can be used as marker organisms for the deadly toxin producing *Clostridium botulinum* in table olive processing. *Clostridia* with respect to table olives can be managed in two ways: with heat treatment and with strict pH control of fermented olives. With heat sterilization, time and temperature must be sufficient to destroy *Clostridium botulinum* spores. Olives preserved by appropriate salt–acid combinations or natural fermentation are generally free of *Clostridium botulinum* and other toxins as long as pH is controlled below 4.6.

Botulism, a rare paralytic illness resulting from a potent neurotoxin produced by *Clostridium botulinum*, is not a major problem with table olives as long as suitable controls are in place and finished products are stabilized to resist its growth. However, there have been sporadic outbreaks of botulism due to *Clostridium botulinum* with noncommercial or homemade table olives that were not stored at the correct pH (<4.3) and salt levels (6% or more) or had not been adequately heat-treated. Recalls of suspected table olive products are common, but they are mainly related to products with inadequate documentation. Historically, in the USA in 1919, there were 29 deaths associated with improperly sterilized black olives. In Italy, there were 16 cases with no deaths due to consumption of homemade green olives (pH 6.2). In Finland, two reported cases with one death were due to consumption of improperly manufactured stuffed olives with almonds. In France, nine cases were identified with no deaths following consumption of homemade green olive paste that had been incorrectly thermally treated. A minor outbreak occurred during the 2012 Olympic Games. A cluster of botulism among Dutch tourists in Turkey, most likely due to olives, has also been reported. The minimal occurrence of botulism is a reflection of the practices in the broader table olive industry (Swaan *et al.*, 2010).

15.7.2.3 Staphylococcal poisoning

Staphylococcus aureus coagulase-positive bacteria are associated with food poisoning. It multiplies in food and produces a toxin, especially at room temperature. There is generally no indication of contamination such as change in color or odor. The presence of this bacterium in food is generally an indication that it has been introduced as a contaminant after processing from human skin, mouth, or nose of handlers (e.g., hand packing of olives, pitting or stuffing procedures, or preparing olive pastes or tapenades). This organism has a high tolerance to salt, so it can grow in brines even if pH is low and inhibitory olive polyphenols are present. Eating affected food results in gastrointestinal symptoms. To reduce the risk for short-term ready-to-eat olive preparations, they can be stored at 4 °C or less. In a study of 35 olive samples available in the Portuguese market, four samples from traditional producers had *Staphylococcus aureus* organisms (Pereira *et al.*, 2008).

15.7.3 Olive softening and shriveling

Naturally black ripe olives are generally softer than green ripe ones. During processing, further softening can be caused by bacteria (*Bacillus* and *Clostridium* spp.), molds (*Aspergillus*, *Fusarium*, *Geotrichum*, *Paecilomyces*, and *Penicillium* spp.), fermentative yeasts (*Saccharomyces* spp.), and oxidative yeasts (*Rhodtorula* spp.). This problem can be reduced by controlling initial pH with acetic acid and facilitating the lactic process. Shriveled olives result from processing at high salt levels or from excessive gas production, with gas collecting in the olive flesh. Olives are normally shriveled if prepared by a salt- and/or heat-drying method.

15.7.4 Gaseous spoilage

With gassy spoilage, more common in turning-color olives, gas collects under the skin as blisters (fisheye) that can result in wrinkled skin or fissures through the flesh. Acutely affected olives float on the brine surface and are often called “floaters.” This problem is associated with coliform bacteria, but not with *Escherichia*

coli. Other implicated species are *Bacillus polymyxa*, *Bacillus macerans*, *Aeromonas liquefaciens*, and saccharolytic anaerobes of the genus *Clostridium*. All of these cause softening, with clostridia also inducing malodorous butyric fermentation and *zapatera* spoilage. These problems can be avoided with adequate salt and pH levels. Yeasts of the genera *Saccharomyces* and *Hansenula* can also cause gassy spoilage. Some of the *Saccharomyces* species can cause softening as well as gassy spoilage, whereas *Hansenula* species do not. Control is difficult as yeasts are always present. However, excessive yeast growth can be controlled by initial pH correction with acetic acid and facilitation of the lactic process. *Zapatera* spoilage that can occur in brined olives is typified by a foul fecal malodor due to propionic acid production from lactic and acetic acids by *Propionibacterium* ssp. alone or with *Clostridium* spp. During the growth of these bacteria, brine pH increases, thus changing the environment for growth of other spoilage or pathogenic organisms.

Spoilage microorganisms with amino acid decarboxylase activity can produce biogenic amines through poor sanitation management during table olive processing. These compounds can cause a variety of symptoms, including headache, insomnia, and diarrhea; however, due to low levels in table olives, problems are unlikely. The biogenic amines putrescine, cadaverine, and tyramine have been detected in fermented green table olives (Hornero-Mendez & Garrido-Fernández, 1994) with foul-smelling *zapatera* spoilage.

15.7.5 Malodorous fermentations

Malodorous fermentations often associated with high-brine pH include putrid, butyric, and *zapatera* spoilage, all associated with bacteria of the genus *Clostridium*. Putrid and butyric spoilage occur early in processing if olives are placed in contaminated brine (untreated water, poor-quality salt, or poorly cleaned fermentation tanks). Malodors of putrid spoilage are reminiscent of the smell of decomposing organic matter. Butyric fermentation (*Clostridium butyricum*) produces butyric acid, giving olives rancid characteristics reminiscent of old butter. In contrast, *zapatera* spoilage occurs at the end of lactic fermentation if the salt concentration is poorly controlled. *Propionicbacterium zeae*, *Propionicbacterium pentosaceum*, and clostridia (*Clostridium sporogenes* and *Clostridium lituseburense*) are involved. Propionic acid generated during fermentation contributes to the malodor together with other volatile substances such as formic, succinic, propionic, butyric, valeric, isovaleric, and caproic acids as well as ethanol, methanol, propanol, acetaldehyde, 2-butanol, and, characteristic to *zapatera* olives, cyclohexane carboxylic acid. Biogenic amines have also been identified in *zapatera* olives. Generally, the only effective method of preventing malodorous spoilage is by controlling brine pH (4.5–4.8) and salt levels (8–10%).

15.7.6 Mold spoilage

Molds associated with table olives include the *Penicillium*, *Aspergillus*, *Verticillium*, and *Geotrichium* genera. Mold growth during processing (fermentation) or after packaging can imbue a musty taste and aroma to table olives. Responsible molds include *Penicillium* (*P. crustosum*, *P. digitatum*, *P. roqueforti*, *P. simplicissimum*, *P. aurantiogriseum*, *P. expansum*, *P. herquei*, and *P. viridicatum*), *Aspergillus niger*, and *Alternaria alternata* (Marsilio & Spotti, 1987). Poorly controlled anaerobic fermentations, where the headspace above the brine is large and allows air to pass through, are ideal for aerobic mold growth of the *Aspergillus*, *Penicillium*, and *Fusarium* genera, and for the production of secondary metabolites such as mycotoxins (aflatoxin and mycotoxin). These compounds can be harmful to health and have been implicated in carcinogenesis. Cracked olives (Franzetti *et al.*, 2011) and Greek-style naturally black olives (Ghitakou *et al.*, 2006; Gourama & Bullerman, 1988) have been implicated. With green olives, aflatoxin B1 (0.4–0.55 μg/kg) was detected in 25% of the olive samples, whereas ochratoxin A (0.2–0.39 μg/kg) was present in 58% of the samples. With Greek-style naturally black olives in two studies, aflatoxin B1 was present. In another study of 12 commercial olive samples, all samples contained ochratoxin A, 80% of samples contained citrinin, and 100% contained aflatoxin B1 (El Adlouni *et al.*, 2006). Fortunately, mycotoxin levels in table olives are relatively low and unlikely to cause health problems unless they exceed toxic levels through poor manufacturing practice. However, it is possible that salt-drying olives by the traditional method may not prevent mold growth, and if mycotoxin levels increase, the product may be unsafe to consume. Therefore, blanching raw olives for a short period before salting may overcome this problem. Sorbic acid (6000 ppm) can retard mold growth, but above 25 °C it will only delay its growth.

Table 15.7 Physicochemical characteristics of packing brine or juice after osmotic balance.

Preparation	Minimum sodium chloride content (%)			Maximum pH limit			Minimum lactic acidity (% lactic acid)		
	SCC, MAT	PR, R	P, S	SCC, MAT	PR, R	P, S	SCC, MAT	PR, R	P, S
Treated olives[a]	5	4	GMP	4.0	4.0	4.3	0.5	0.4	GMP
Natural olives	6	6	GMP	4.3	4.3	4.3	0.3	0.3	GMP
Dehydrated and/ or shriveled olives	10	10	GMP	GMP	GMP	GMP	GMP	GMP	GMP
Olives darkened by oxidation	GMP	GMP	GMP	GMP	GMP	GMP	GMP	GMP	GMP

[a]"Treated olives" covers all olives processed using sodium hydroxide as the debittering agent; "natural olives" covers olives processed by fermentation or soaking with water or weak brine, *without* using sodium hydroxide; and "dehydrated and/or shriveled olives" covers olives that have their moisture reduced by air drying, heat drying, or drying with coarse salt. For "Olives darkened by oxidation," information is nonspecific. All such products should be sterilized with a salt content determined by GMP (generally low, e.g., 2–3%) and pH 7 or less.

Note: Where possible, aromatized olives and olive salad mixtures should meet the above requirements, especially pasteurization. Table olive products that are made according to traditional methods and that do not comply with the above physicochemical characteristics must achieve equivalent safety and quality characteristics before sale. SCC = specific chemical characteristics; MAT = modified atmosphere (e.g., carbon dioxide/nitrogen); PR = addition of preservatives (e.g., sodium benzoate, potassium sorbate); R = refrigeration 0 °C to 4 °C; P = pasteurization, indicative organism: propionic bacteria at 62.4 °C and *z* curve of 5.25; S = sterilization at conditions that will kill *Clostridium botulinum* spores; GMP = good manufacturing practice.

Adapted from International Olive Council (2004) and RIRDC (2012).

15.7.7 Preservation of table olive products

Accepting the importance of GAP, GHP, and GMP, most olives are preserved through a balance of pH, acid, and salt levels, with differences between olives prepared by different methods (see Table 15.7). Outside of specified levels, many olives are preserved by pasteurization, sterilization, adding preservatives, or storing at low temperatures. For pasteurization, the indicative organism is *Propionibacterium*, whereas for sterilization the conditions used must kill its spores. The latter method is used for olives darkened by oxidation that are packed in solutions that are at near-neutral pH. If these are packed in acid brine, they can be pasteurized. Heat treatments inactivate all or most bacterial spoilage organisms as well as (to some extent) lowering the quality of olive products.

To overcome loss of product quality, the use of high-pressure cold pasteurization has been investigated. High-hydrostatic-pressure (HHP) treatments (singly or in combination with natural antimicrobials) were tested for stabilization of 'Manzanilla Aloreña' seasoned olives stored at 25 °C. HHP was highly effective in controlling yeast populations at ≥300 MPa, and no viable yeasts were detected in samples treated at 400 MPa for up to three months. Low levels of endospore-forming bacteria were always detected after HHP treatments. Addition of nisin to the brines reduced bacterial counts but had no effect on yeasts. Thyme oil had almost no effect on yeast concentrations, but rosemary oil reduced yeast viable counts progressively during storage. Essential oils in combination with HHP (400 MPa, 5 min) significantly reduced the concentrations of aerobic mesophilic bacteria. Low-salt brined olives purged with N_2 or supplemented with ascorbic acid were preserved for up to five months without spoilage, suggesting that the NaCl content in brines of packed olives could be reduced considerably by HHP (Abriouel *et al.*, 2014).

Green fermented olives (cv. 'Halkidiki') were subjected to different treatments of HHP processing at different pressures from 400 to 500 MPa (400, 450, and 500 MPa) for 15 or 30 min. Total viable counts, LAB and yeasts/molds, and the physicochemical characteristics of the product (pH, color, and firmness) were monitored right after the treatment and after seven days of storage at 20 °C to allow for recovery of injured cells. Treatments were found insufficient, but treatment at 500 MPa for 30 min was effective for up to five months. After five months of storage at 20 °C, no microbiota were detected. Thus, HHP treatment may

introduce a reliable nonthermal pasteurization method to extend the microbiological shelf life of fermented table olives.

15.7.7.1 Hot packing olives

Many table olive products are hot packed. This involves the use of an adequate thermal process either in the final container (e.g., retort or steam tunnel) or prior to packaging but with the product filled at a temperature that will control nonsporing bacteria, yeasts, and most vegetative and sporing forms of molds. A guide from Australia (New South Wales Food Authority, 2015), indicates that for acid foods, processing at around 100 °C is adequate and pressure equipment is not required. In most cases when hot filling, the product temperature should not be less than 85 °C and preferably between 90 and 95 °C. The container is closed, and then inverted or turned on its side for two or more minutes prior to cooling. Where possible, foods are processed below pH 4.0, with extended processing times recommended for products above that pH.

15.8 Antibiotic aspects of olive polyphenols

Polyphenols, as found in olives, can exert antimicrobial and antiviral activity (Fleming *et al.*, 1973; Federici & Bongi, 1983; Bisingnano *et al.*, 1999). In addition, oleuropein has been reported to repel insects (Lo Scalzo *et al.*, 1994) and protect against pathogens (Uccella, 2001). Olive leaves have traditionally been used as folk medicine to resolve fevers due to a variety of pathogens, including malaria (Solar-Rivas *et al.*, 2000). Oleuropein and its derivatives, through fermentation studies, have been recognized as inhibitors of LAB. Numerous investigators have also shown that oleuropein has antibiotic activity and could have potential as an alternative food preservative. Evidence using *in vitro* studies now suggests that oleuropein and its hydrolysis products may play a role as antipathogenic agents. Investigators have shown that some polyphenols from olive trees may inhibit the growth of the bacteria *Lactobacillus plantarum* and *Leuconostoc mesenteroides* and fungi-like *phytophthora* (Del Rio *et al.*, 2003). It is possible that oleuropein and its hydrolysis products could inhibit the growth of *Salmonella enteritis*, *Bacillus cereus*, *Escherichia coli*, fungi, viruses, and protozoa but not yeasts. In a study using dry olive flesh powder, it had antibacterial (*Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Klebsiella pneumonia* 700603, *Enterobacter cloacae*, *Citrobacter freundii*, *Proteus mirabilis*, and *Salmonella typhimurium*) and antifungal (*Cladosporium herbarum*, *Alternaria alternata*, *Aspergillus fumigates*, and *Aspergillus flavus*) properties.

Recent investigations using olive products, leaves, and fruit, as well as pure compounds, revealed that polyphenols (hydroxytyrosol and oleuropein) and aliphatic aldehydes inhibited or slowed down the growth rate of a number of microorganisms such as *Haemophilus influenza*, *Salmonella typhi*, *Vibrio parahaemoliticus*, and *Staphylococcus aureus*. Additionally, oleuropein inhibited growth of *Mycoplasma hominis*, *Mycoplasma fermentans*, *Mycoplasma pneumoniae*, and *Mycoplasma pirum*. Although the exact mechanism remains tentative, oleuropein may hinder amino acid synthesis required by pathogens for survival. Nonetheless, *in vivo* investigations are still needed to substantiate if the *in vitro* studies can be replicated in the body. Aliphatic aldehydes including hexanal, nonanal, (E)-2-hexenal, (E)-2-octenal, and (E)-2-nonenal have also been demonstrated to be active against fungi such as *Tricophyton mentagrophytes*, *Microsporum canis*, and *Candida* species.

15.9 Probiotic capability of table olive products

Table olives processed by fermentation have additional benefits, and are associated with potentially probiotic bacteria. Today, health-conscious consumers search for foodstuffs of plant origin with functional characteristics, including fermented foods such as olives. Relative to these are probiotics, live microorganisms that when consumed in sufficient amounts are beneficial to health. When consumed, the probiotic bacteria pass into the gastrointestinal tract, where they have a number of effects. These include promoting intestinal competence and motility, changing favorably the intestinal microbial equilibrium, inhibiting the growth of detrimental bacteria, and increasing resistance to infection. Factors to consider regarding the effectiveness of probiotic action are the individual's food habits, differences in gut microorganisms, and the particular host–microbial relationship.

Table olives as carriers of probiotic bacteria raise a number of challenges – cost-effectiveness, acceptance by consumers, and technical issues. Useful bacteria, once isolated and identified from native microbiota, could be cultured and introduced as starter cultures into brines at the beginning of fermentation so that they could dominate the microbial population. The ultimate test of effectiveness would be whether the combination of table olive and probiotic bacteria considerably improves consumers' health (Lavermicocca *et al*., 2005; De Bellis *et al*., 2010; Argyri *et al*., 2014; Blana *et al*., 2014, 2016; Rodríguez-Gómez *et al.*, 2014).

15.10 Conclusion

There are several table olive processing methods and many cultivars used internationally. Most types of table olives offer a significant number of micronutrients and polyphenols to the human diet. It seems that even though there are thousands of table cultivars, some of them are considered as unique. If new cultivars are developed and total industrialization of olive products occurs, it will be a tragedy for losing such an ancient foodstuff that has sustained millions of people over the past. Challenges to table olives in the future will fall into a number of categories, including mechanized harvesting, raw olive quality, production methods, and health issues. It would appear that diversity will always be the strength of table olives. Currently, the focus on table research has moved from the spontaneous fermentation by natural skin flora to the addition of selected starter cultures of LAB that have probiotic potential, and this will lead to the preparation and use of table olives as a real, functional food.

References

Abriouel, H., Benomar, N., Gálvez, A., & Pulido, R.P. (2014) Preservation of Manzanilla Aloreña cracked green table olives by high hydrostatic pressure treatments singly or in combination with natural antimicrobials. LWT – Food Science and Technology 56, 427–431.

Alves, M., Gonçalves, T., & Quintas, C. (2012) Microbial quality and yeast population dynamics in cracked green table olives' fermentations. Food Control 23, 363–368.

Argyri, A.A., Panagou, E.Z., Nychas, G.E., & Tassou, C.C. (2014) Nonthermal pasteurization of fermented green table olives by means of high hydrostatic pressure processing. BioMed Research International, 515623.

Arroyo-López, F.N., Querol, A., Bautista-Gallego, J., & Garrido-Fernández, A. (2008) Role of yeasts in table olive production. International Journal of Food Microbiology 128, 189–196.

Bach-Faig, A., Berry, E.M., Lairon, D., Reguant, J., Trichopoulou, A., Dernini, S., Medina, F.X., Battino, M., Belahsen, R., Miranda, G., *et al.* (2011) Mediterranean diet pyramid today: science and cultural updates. Public Health Nutrition 14, 2274–2284.

Bautista-Gallego, J., Arroyo-López, F.N., Durán-Quintana, M. C., & Garrido-Fernández, A. (2010) Fermentation profiles of Manzanilla-Aloreña cracked green table olives in different chloride salt mixtures. Food Microbiology 27, 403–412.

Bautista-Gallego, J., Arroyo López, F.N., Romero Gil, V., Rodríguez Gómez, F., García García, P., & Garrido Fernández, A. (2011) Chloride salt mixtures affect Gordal cv. green Spanish-style table olive fermentation. Food Microbiology 28, 1316–1325.

Bevilacqua, A.L., Cannarsi, M., Gallo, M., Sinigaglia, M., & Corbo, M.R. (2010) Characterization and implications of Enterobacter cloacae strains, isolated from Italian table olives "Bella di Cerignola." Journal of Food Science 75, M53–60.

Blana, V.A., Grounta, A., Tassou, C.C., Nychas, G.J.E., & Panagou, E.Z. (2014) Inoculated fermentation of green olives with potential probiotic Lactobacillus pentosus and Lactobacillus plantarum starter cultures isolated from industrially fermented olives. Food Microbiology 38, 208–218.

Blana, V.A., Polymeneas, N., Tassou, C.C., & Panagou, E.Z. (2016) Survival of potential probiotic lactic acid bacteria on fermented green table olives during packaging in polyethylene pouches at 4 and 20°C. Food Microbiology 53, 71–75.

Blekas, G., Vassilakis, C., Harizanis, C., Tsimidou, M., & Boskou, D.G. (2002) Biophenols in table olives. Journal of Agriculture and Food Chemistry 50, 3688–3692.

Bravo-Abad, F., & Inigo, R.M. (1988) Bacterial population and changes in chemical composition of the olive in the course of lactic fermentation. Alimenteria 189, 87–89.

Buckland, G., Travier, N., Barricarte, A., Ardanaz, E., Moreno-Iribas, C., Sanchez, M.J., Molina-Montes, E., Chirlaque, M.D., Huerta, J.M., Navarro, C., *et al.* (2012) Olive oil intake and CHD in the European prospective investigation into cancer and nutrition Spanish cohort. British Journal of Nutrition 108, 2075–2082.
Bulotta, S., Celano, M., Lepore, S.M., Montalcini, T., Pujia, A., & Russo, D. (2014) Beneficial effects of the olive oil phenolic components oleuropein and hydroxytyrosol: focus on protection against cardiovascular and metabolic diseases. Journal of Translational Medicine 12, 219.
Caggia, C., Randazzo, C.L., Salvo, M., Romeo, F., & Giudici, P. (2004) Occurrence of *Listeria monocytogenes* in green table olives. Journal of Food Protection 10, 2189–2194.
Charoenprasert, S., & Mitchell, A. (2012) Factors influencing phenolic compounds in table olives (*Olea europaea*) Journal of Agriculture and Food Chemistry 60, 7081–7095.
Charoenprasert, S., & Mitchell, A. (2014) Influence of California-style black ripe olive processing on the formation of acrylamide. Journal of Agriculture and Food Chemistry 62, 8716–8721.
Chimi, H., & Atouati, Y. (1999) Determination of the optimal stage for harvesting Moroccan picholine olives by monitoring change in total polyphenols. Olivae 54, 56–60.
Cicerale, S., Lucas, L.J., & Keast, R.S. (2012) Antimicrobial, antioxidant and anti-inflammatory phenolic activities in extra virgin olive oil. Current Opinion in Biotechnology 23, 129–135.
Covas, M.I., de la Torre, R., & Fito, M. (2015) Virgin olive oil: a key food for cardiovascular risk protection. British Journal of Nutrition 113 (Suppl. S2), S19–S28.
Crisosto, C.H., Mitcham, E.J., & Kader, A.A. (2015, December 8) Olive: recommendations for maintaining postharvest. Quality Department of Plant Sciences, University of California, Davis. Available from: http://ucanr.org/sites/postharvest/PFfruits/olive
De Bellis, P., Valerio, F., Sisto, A., Lonigro, S.L., & Lavermicocca, P. (2010) Probiotic table olive: microbial populations adhering on olive surface in fermentation sets inoculated with the probiotic strain *Lactobacillus paracasei* IMPC2.1 in an industrial plant. International Journal of Food Microbiology 140, 6–13.
Del Monaco, G., Officioso, A., D'Angelo, S., la Cara, F., Ionata, E., Marcolongo, L., Squillaci, G., Maurelli, L., & Morana, A. (2015) Characterization of extra virgin olive oils produced with typical Italian varieties by their phenolic profile. Food Chemistry 184, 220–228.
EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (2011) EFSA Journal 9, 2033.
El Adlouni, C., Tozlovanu, M., Naman, F., Faid, M., & Pfohl-Leszkowicz, A. (2006) Preliminary data on the presence of mycotoxins (ochratoxin A, citrinin and aflatoxin B1) in black table olives "Greek style" of Moroccan origin. Molecular Nutrition & Food Research 50, 507–512.
Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). (2001) Executive summary of the Third Report of the National Cholesterol Education Program (NCEP). Journal of the American Medical Association 285, 2486–2497.
Franzetti, L., Scarpellini, M., Vecchio, A., & Planeta D. (2011) Microbiological and safety evaluation of green table olives marketed in Italy. Annals of Microbiology 61, 843–851.
Garrido Fernández, A., Fernández Díaz, M.J., & Adams, R.M. (1997) Table olives: production and processing. Chapman & Hall, London.
Georgiadou, E.C., Ntourou, T., Goulas, V., Manganaris, G.A., Kalaitzis, P., & Fotopoulos, V. (2015) Temporal analysis reveals a key role for VTE5 in vitamin E biosynthesis in olive fruit during on-tree development. Frontiers in Plant Science 6, 871.
Ghitakou, S., Koutras, K., Kanellou, E., & Markaki, P. (2006) Study of aflatoxin B1 and ochratoxin A production by natural microflora and *Aspergillus parasiticus* in black and green olives of Greek origin. Food Microbiology 23, 612–621.
Goulas, V., Charisiadis, P., Gerothanassis, I.P., Manganaris, G.A. (2012) Classification, biotransformation and antioxidant activity of olive fruit biophenols: a review. Current Bioactive Compounds 8, 232–239.
Gourama, H., & Bullerman, L.B. (1988) Mycotoxin production by molds isolated from "Greek-style" black olives. International Journal of Food Microbiology 6, 81–90.
Hassapidou, M.N., & Manoukas, A.G. (1993) Tocopherol and tocotrienol compositions of raw table olive fruit. Journal of the Science of Food and Agriculture 161, 277–280.
Hornero-Mendez, D., & Garrido-Fernández, A. (1994) Biogenic amines in table olives: analysis by high-performance liquid chromatography. Analyst 119, 2037–2041.
International Olive Council (IOC). (2005) Quality management guide for the table olive industry. t.ot/doc. no. 14. IOC, Madrid, Spain.
International Oil Council (IOC). (2004) Trade standard applying to table olives. coi/ot/nc no. 1. IOC, Madrid, Spain.
Kailis, S., & Harris, D. (2007) Producing table olives. Landlinks Press, Melbourne, Australia.
Kiai, H., & Hafedi, A. (2014) Chemical composition changes in four green olive cultivars during spontaneous fermentation. LWT – Food Science and Technology. DOI:http://dx.doi.org/10.1016/j.lwt.2014.02.011

Kiritsakis, A.K. (Ed.) (1998) Olive oil: from the tree to the table, 2nd ed. Food and Nutrition Press, Trumbull, CT.

Kiritsakis, A., Nanos, G.D., Polymenopoulos, Z., Thomai, T., & Sfakiotakis, E.M. (1998) Effect of fruit storage conditions on olive oil quality. Journal of the American Oil Chemists' Society 75, 721–724.

Lavermicocca, P., Valerio, F., Lonigro, S.L., De Angelis, M., Morelli, L., Callegari, M.L., Rizzello, C.G., & Visconti, A. (2005) Study of adhesion and survival of lactobacilli and bifidobacteria on table olives with the aim of formulating a new probiotic food. Applied and Environmental Microbiology 71, 4233–4240.

Lee, S.M., Kitsawad, K., Sigal, A., Flynn, D., & Guinard, J.X. (2012) Sensory properties and consumer acceptance of imported and domestic sliced black ripe olives. Journal of Food Science 77, S439–448.

López, A., Montaño, A., García, P., & Garrido, A. (2006) Fatty acid profile of table olives and its multivariate characterization using unsupervised (PCA) and supervised (DA) chemometrics. Journal of Agriculture and Food Chemistry 54, 6747–6753.

Malheiro, R., Casal, S., Sousa, A., Guedes de Pinho, P., Peres, A.M., Dias, L.M., Bento, A., & Pereira, J.A. (2012) Effect of cultivar on sensory characteristics, chemical composition, and nutritional value of stoned green table olives. Food and Bioprocess Technology 5, 1733–1742.

Malik, S.A., & Bradford, J.M. (2006) Changes in oleuropein levels during differentiation and development of floral buds in 'Arbequina' olives. Scientia Horticulturae 110, 274–278.

Marsilio, V., Campestre, C., & Lanza, B. (2001) Phenolic compounds change during California-style ripe olive processing. Food Chemistry 74, 55–60.

Marsilio, V., & Spotti, E. (1987) Indagine sull'inquinamento fungino di olive nere da tavola essiccate. Ind. Conserve 62, 287–291.

Martirosyan, D.M., Miroshnichenko, L.A., Kulakova, S.N., Pogojeva, A.V., & Zoloedov, V.I. (2007) Amaranth oil application for coronary heart disease and hypertension. Lipids in Health and Disease, 6, art 1.

Monteleone, E., Caporale, G., Lencioni, L., Favati, F., & Bertuccioli. M. (1995) Optimization of virgin olive oil quality in relation to fruit ripening and storage. In: Developments in food science, vol. 37: Food flavors: generation, analysis and process influence, ed. G. Charalambous. Elsevier, Amsterdam, 397–418.

Nanos, G.D., Agstidou, A., & Sfakiotakis, E.M. (2002) Temperature and propylene effects on ripening of green and black 'Conservolea' olives. HortScience 37, 1079–1081.

Neveu, V., Perez-Jimenez, J., Vos, F., Crespy, V., du Chaffaut, L., Mennen, L., Knox, C., Eisner, R., Cruz, J., Wishart, D., *et al.* (2010) Phenol-explorer: an online comprehensive database on polyphenol contents in foods. Database (Oxf.) 2010(2010), bap024.

New South Wales Food Authority. (2015) Shelf stable acid preserved foods: factors affecting the shelf stability of acid foods: condiments, sauces and salad dressings. Available from NSW/FA/FI035/0811http://www.foodauthority.nsw.gov.au/_Documents/scienceandtechnical/shelf_stable_acid_preserved_foods.pdf

Nikzad, N., Sahari, M.A., Vanak, Z.P., Safafar, H., & Boland-Nazar, S.A. (2013) Effect of the processing steps on compositions of table olive since harvesting time to pasteurization. Recent Patents on Food, Nutrition & Agriculture 5, 154–165.

Pasqualone, A., Nasti, R., Montemurro, C., & Gomes, T. (2014) Effect of natural-style processing on the oxidative and hydrolytic degradation of the lipid fraction of table olives. Food Control 37, 99–103.

Pereira, A.P., Pereira, J.A., Bento, A., & Estevinho, M.L. (2008) Microbiological characterization of table olives commercialized in Portugal in respect to safety aspects. Food and Chemical Toxicology 46, 2895–2902.

RIRDC (Rural Industries Research Corporation, Australia). (2012) The voluntary industry standard for table olives in Australia. RIRDC Publication No. 12/111. Wagga Wagga, RIRDC.

Rodríguez-Gómez, F., Romero-Gil, V., García-García, P., Garrido-Fernandez, A., & Arroyo-Lopez, F.N. (2014) Fortification of table olive packing with the potential probiotic bacteria Lactobacillus pentosus TOMC-LAB2. Frontiers in Microbiology 5, 1–9.

Romeo, F.V., De Luca, S., Piscopo, A., Perri, E., & Poiana, M. (2009) Effects of post-fermentation processing on the stabilisation of naturally fermented green table olives (cv Nocellara Etnea). Food Chemistry 116, 873–878.

Romero, C., Brenes, M., Yousfi, K., Garcia, P., Garcia, A., & Garrido, A. (2004) Effect of cultivar and processing method on the contents of polyphenols in table olives. Journal of Agriculture and Food Chemistry 52, 479–484.

Ryan, D., Antolovich, M., Herlt, T., Prenzler, P.D., Lavee, S., & Robards, K. (2002) Identification of phenolic compounds in tissues of the novel olive cultivar hardy's mammoth. Journal of Agriculture and Food Chemistry 50, 6716–6724.

Sabatini, N., & Marsilio, V. (2008) Volatile compounds in table olives (*Olea europaea* L., Nocellara del Belice cultivar). Food Chemistry 107, 1522–1528.

Sagratini, G., Allegrini, M., Caprioli, Cristalli, G., Giardina, D., Maggi, F., Ricciutelli, M., Sirocchi, V., & Vittori, S. (2013) Simultaneous determination of squalene, α-tocopherol and β-carotene in table olives by solid phase extraction and high-performance liquid chromatography with diode array detection. Food Analytical Methods 6, 54–60.

Salas, J.J., Sanchez, J., Ramli, U.S., Manaf, A.M., Williams, M., & Harwood, J.L. (2002) Biochemistry of lipid metabolism in olive and other oil fruit. Progress in Lipid Research 39, 151–180.

Salvador, M.D., Arand, A.F., Gomez-Alonso, S., & Fregapane, G. (2001) Cornicabra virgin olive oil: a study of five crop seasons: composition, quality and oxidative stability. Food Chemistry 74, 267–274.

Servili, M., Baldioli, M., Selvaggini, R., Macchioni, A., & Montedoro, G. (1999) Phenolic compounds of olive fruit one- and two-dimensional nuclear and magnetic resonance characterization of Nuzhenide and its distribution in the constitutive parts of fruit. Journal of Agriculture and Food Chemistry 47, 12–18.

Sofi, F., Abbate, R., Gensini, G.F., & Casini, A. (2010) Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. American Journal of Clinical Nutrition 92, 1189–1196.

Spyropoulou, K.E., Chorianopoulos, N.G., Skandamis, P.N., Nychas, G. J. E. (2001) Survival of *Escherichia coli* O157:H7 during the fermentation of Spanish-style green table olives (Conservolea variety) supplemented with different carbon sources. International Journal of Food Microbiology 66, 3–11.

Swaan, C.M., van Ouwerkerk, J.M., & Roest, H.J. (2010) Cluster of botulism among Dutch tourists in Turkey. Eurosurveillance 15(14).

Tsapatsaris, S., & Kotzekidou, P. (2004) Application of central composite design and response surface methodology to the fermentation of olive juice by *Lactobacillus plantarum* and *Debaryomyces hansenii*. International Journal of Food Microbiology 95, 157–168.

Vissers, M.N., Zock, P.L., Roodenburg, A.J., Leenen, R., & Katan, M.B. (2002) Olive oil phenols are absorbed in humans. Journal of Nutrition 132, 409–417.

Wang, X., Ouyang, Y., Liu, J., Zhu, M., Zhao, G., Bao, W., & Hu, F.B. (2014) Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies British Journal of Nutrition 349, art. g4490.

Zoidou, E., Melliou, E., Gikas, E., Tsarbopoulos, A., Magiatis, P., & Skaltsounis, A-L. (2010) Identification of Throuba Thassos, a traditional Greek table olive variety, as a nutritional rich source of oleuropein. Journal of Agriculture and Food Chemistry 58, 46–50.

Zullo, B.A., Di Stefano, M.G., Cioccia, G., & Ciafardini, G. (2014) Evaluation of polyphenol decay in the oily fraction of olive fruit during storage using a mild sample handling method. European Journal of Lipid Science and Technology 116, 160–168.

16 Greek-style table olives and their functional value

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16.1 Introduction

The cultivation of the olive tree (*Olea europea* L.) holds a great share of the primary agriculture sector in Mediterranean countries and is practiced mainly for olive oil and table olive production. The world table olive consumption for the 2014–2015 crop season was estimated at 2,578,000 tons, a good part of which is produced and distributed by the European Union (EU) countries. Specifically, table olive production reached 851,000 tons within the EU and 329,000 tons were exported in the same season, with Spain being the leader of European producing countries (515,000 tons) followed by Greece (235,000 tons) and Italy (79,500 tons). Other important non-European producers are Turkey (438,000 tons), Egypt (450,000 tons), Algeria (216,000 tons), Argentina (28,000 tons), Syria (40,000 tons), Morocco (100,000 tons), Peru (80,000 tons), and the USA (45,000 tons) (International Olive Council [IOC], 2014).

Upon harvest, olives are too bitter for direct consumption due to their high phenolic content, and hence they need to be subjected to processing to remove bitterness. The most common processing method of table olives involves the development of lactic acid fermentation, which results in a final product with enhanced sensory attributes and preservation properties that ensure the microbiological safety during storage and marketing. In general, table olive processing may significantly vary depending on regional and national practices and tradition, which is reflected in the different commercial treatments under which table olives are distributed in the international market. The most broadly known commercial preparations are: (a) treated green olives in brine or “Spanish style,” a preparation that involves the use of alkali treatment to remove bitterness, followed by a washing step to remove the excess of alkali and finally placement of the drupes in brine, where lactic acid fermentation takes place (Brenes & de Castro, 1998); (b) natural black olives in brine or “Greek style,” a preparation that involves direct placement of black olives into brine, where they undergo natural fermentation and slow debittering at the same time (Balatsouras, 1990); (c) black oxidized olives or “Californian style,” a method during which green or turning-color olives are blackened by oxidation, debittered by alkali treatment, and finally canned and sterilized (Brenes-Balbuena *et al.*, 1992); and (d) shriveled or “dry-salted” olives, a method during which fully black ripe olives are placed between layers of coarse salt where they gradually lose their bitterness due to dehydration by the salt and finally become shriveled in appearance (Balatsouras, 1990). A flow diagram of the main preparations of table olives is illustrated in Figure 16.1.

In Greece, table olives are processed as “Spanish style” (57.1%), followed by natural black/turning-color olives (38.1%) and to a lesser extent as black oxidized (2.8%) and finally natural black dry-salted olives (0.9%). The most economically important Greek cultivars used for table olive production on an industrial scale are ‘Conservolea’, used in both green and natural black olive fermentation; ‘Halkidiki’, processed mainly by the “Spanish style” method; and ‘Kalamon’, processed as natural black. For dry-salted olives, the main cultivar intended for this preparation is ‘Thrubolea’ and its clone cv. ‘Throuba Thassos’.

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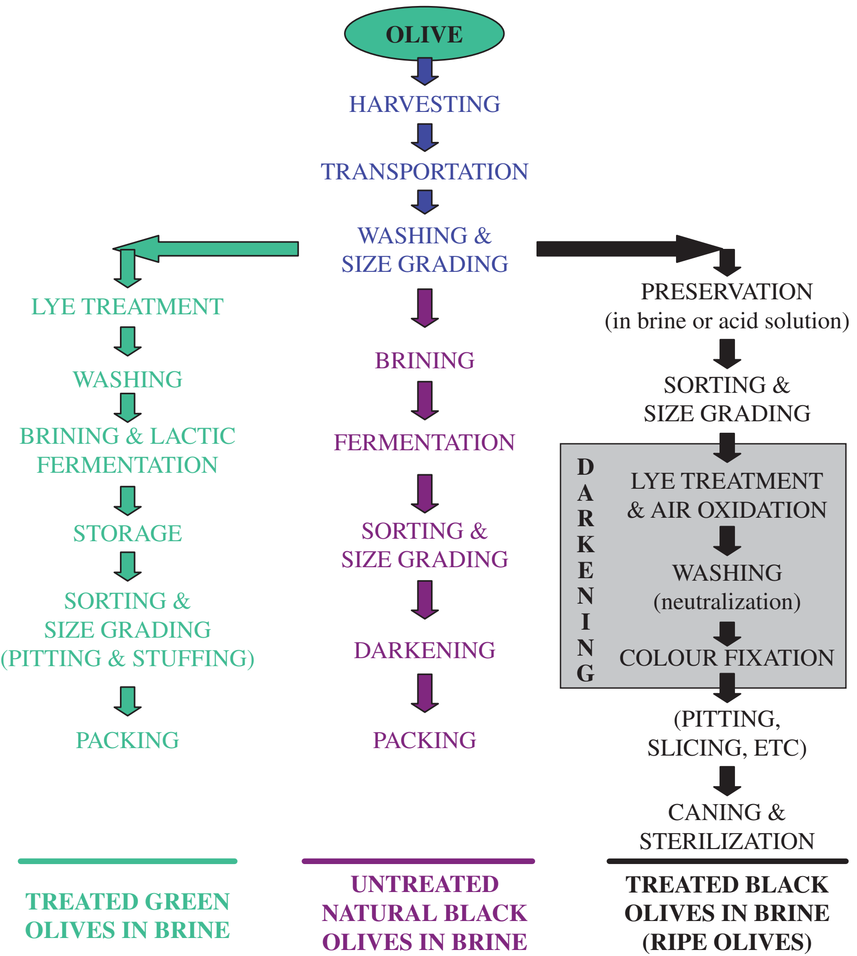

Figure 16.1 Flow diagram of elaboration processes of table olives. TDC olive booklet, Processing technology in olive oil and table olives. Available from http://citeseerx.ist.psu.edu/viewdoc/download;jsessionid=5B8AE3B2B40416D3BA8A6BA3BE6A4A1C?doi=10.1.1.133.3340&rep=rep1&type=pdf.

16.2 Table olives processing in Greece

16.2.1 Natural black olives in brine

One of the most important commercial preparations of table olives in the international market is natural black olives in brine. This particular type is also known as “Greek-style” olives because it is the main type of olive processing traditionally employed in Greece. According to local practices, the olives are harvested at the stage of full ripeness or slightly earlier (when three-fourths of the mesocarp has attained black color) over the period between 15 October and December. It is, however, important to finish the harvest before the incidence of the first winter frost, to avoid irreversible damage of the crop. Special attention should also be given to avoid damage due to bruising or scratching since black olives are harvested at an advanced maturity stage and their texture is softer compared to green olives. Further on, olives are directly placed in brine solution with salt concentration of 10–14% (w/v) or higher, where fermentation starts spontaneously by a mixed population of Gram negatives, Gram positives, and yeasts originating from the olive’s indigenous biota (Balatsouras, 1990). The fast elimination of Gram negatives and some species of Gram-positive

Figure 16.2 Natural black olives cv. 'Conservolea' showing gas pockets.

bacteria during this step of the process is of crucial importance for a proper fermentation since their prolonged survival may result in spoilage phenomena. The formation of "gas pockets" is a common spoilage phenomenon resulting in olive softening; it has mainly been attributed to the action of enterobacteria. These microorganisms release carbon dioxide, which is accumulated in the mesocarp of olives as pockets of gas, while metabolizing sugars. Macroscopically, the phenomenon may appear as gas bubbles on the olive surface or as gas fissures in the mesocarp with a narrow belt on the skin (Figure 16.2), a condition commonly known as "fish eye" or "alambrado" (Lanza, 2013).

In addition, the development of butyric acid fermentation by *Clostridium* species (Gililland & Vaughn, 1946) and pectinolytic activity causing softening of the drupes by *Bacillus* species (Nortje & Vaughn, 1953) have been reported in the literature as the main causes of malodorous (bad flavor) fermentation during the initial stage of olive processing. The traditional practice that employs brining in high salt content may result in the decrease of these microbial groups. However, during brining, salt is progressively taken up by the drupes, and its level in the brine decreases until equilibrium between olives and brine is reached. As the salt content decreases, the survival of undesirable microorganisms may be prolonged and hence the adjustment of salt concentration in the brine at 8.5–9.5% at equilibrium has been proposed in order to minimize the risk of spoilage (Balatsouras, 1990). As these microbial groups decrease, lactic acid bacteria (LAB) and yeasts are the associating microorganisms responsible for the development of the active fermentation process.

The use of salt during brining is a key ingredient in table olive processing because it reduces the water activity (a_w) of the medium, increases the ionic strength of the solution, reduces the solubility of oxygen in water, and finally and most importantly inhibits the growth of spoilage and pathogenic bacteria (Taormina, 2010). The effects of different salt concentrations on the population dynamics and the evolution of pH and titratable acidity have previously been studied for 'Conservolea' natural black olives (Tassou *et al.*, 2002). Olives were brined in 4, 6, and 8% (w/v) NaCl at 18 and 25 °C for a period of six months. Salt concentrations of 4 and 6% (w/v) at both 18 and 25 °C resulted in high LAB population, exceeding 7.0 log CFU/mL while yeasts were maintained at 2–3 log cycles lower. In addition, the attained pH values were maintained at 3.8–3.9, and the total acidity at 0.7 expressed as % (w/v) lactic acid. However, during brining in 8% (w/v) salt, a pronounced delay in LAB growth was observed while yeasts reached higher numbers throughout the process. The prevalence of yeasts over LAB was also reflected by the attained physicochemical characteristics, with final pH values ranging between 4.6 and 4.8 and total acidity at 0.37% (w/v, lactic acid). The inhibitory effect of high salt concentrations on LAB growth dynamics has been observed during natural black fermentation of Turkish cultivars as well (Borcakli *et al.*, 1993; Özay & Borcakli, 1996). During brining in 14% NaCl of black 'Gemlik' and 'Edincik' cultivars, the fermentation process was dominated by yeasts. The absence or very limited growth of LAB resulted in final pH values between 4.2 and 4.4 for the 'Edincik' cultivar and 4.9 and 5.2 for the 'Gemlik' cultivar after 100 and 150 days of brining, respectively. The final values of total acidity reached 0.41 and 0.35% (w/v, lactic acid) for 'Edincik' and 'Gemlik', respectively (Borcakli *et al.*, 1993). Further on, different brining treatments involving brine replacement for 'Gemlik' black olive fermentation were investigated (Özay & Borcakli, 1996). The best LAB growth, reaching 6.0 log CFU/mL, occurred in brines with 6% (w/v) salt concentration followed by brine replacement with fresh brine solution

with the same salt content after 48 days of brining. In the case where the olives were kept in tap water for 24 days and then placed in brine solution with 14% (w/v) NaCl, no LAB population could be enumerated throughout the process. It is evident that under the initial salt concentrations that are traditionally employed (10–14%) by the industry today, yeasts are the prevailing microbiota followed occasionally by LAB. As a result, the fermentation is mainly alcoholic and to a lesser extent lactic, giving a final product with milder taste and less self-preservation characteristics. The titratable acidity is maintained around 0.3–0.5% (w/v, lactic acid) and pH between 4.5 and 4.8 (Balatsouras, 1990). Such values cannot ensure the microbiological stability of black olives during storage and marketing, and thus the whole process has been reconsidered by the industry in the light of new scientific findings to produce a safer product. Today, the Greek table olive industry has reduced the salt level between 6 and 7%, which is kept constant by adding coarse salt on the top of the fermentation vessels during active fermentation. The final product is characterized by lower pH values (3.8–4.0) and higher titratable acidity (0.8–1.0%) that ensures the microbiological stability during storage.

Black olive fermentation in Greece is a natural, spontaneous process that is not always predictable, depending on the availability of nutrients, salt content, pH, aerobic/anaerobic conditions, and temperature, and the presence of interfering microbiota (Nychas *et al.*, 2002). The use of pure starter cultures has been proposed as a way to better control olive fermentation since it decreases the risk of spoilage and accelerates the course of fermentation and acidification of the brine (Hurtado *et al.*, 2012). The application of starter cultures has been extensively used in Spanish-style fermentation, whereas their use in natural black olive fermentation has received little attention. According to Panagou *et al.* (2008), two starters of LAB, namely a commercial strain of *Lactobacillus pentosus* and *Lactobacillus plantarum* BFE 6709 isolated from fermented cassava, were inoculated at the beginning of ‘Conservolea’ natural black olive fermentation. The microbial dynamics and the biochemical profile of fermentation were monitored for a period of 30 days, while the survival of the selected *Lactobacillus* strains was determined during fermentation using molecular analysis. Results showed that both starter cultures were successfully established in the brine resulting in an accelerated fermentation process, reaching population levels that exceeded 7.0 log CFU/mL of brine throughout the process. Yeasts were also present during the process and maintained at levels ranging from 4.0 to 5.0 log CFU/mL of brine. The survival period of Gram-negative bacteria was reduced by 5 days in inoculated fermentations (they were not detected after day 7) compared to the spontaneous process where they were enumerated until day 12, thus minimizing the risk of spoilage. The predominant *Lactobacillus* species in the spontaneous process were *L. pentosus* as determined by repetitive extragenic palindromic-sequence polymerase chain reaction (Rep-PCR) and random amplified polymorphic DNA PCR (RAPD-PCR). More recently, a study on the molecular characterization of LAB species isolated from industrially fermented Greek table olives was undertaken (Doulgeraki *et al.*, 2013), indicating that the main species identified from black olives were *L. pentosus* (47.7%), *L. plantarum* (2.3%), *L. paraplantarum* (2.3%), *Leuconostoc mesenteroides* (45.5%), and *Leuconostoc pseudomesenteroides* (2.3%). In another work, the LAB heterogeneity at strain level was studied from black fermented ‘Conservolea’ olives that were stored in polyethylene pouches under aerobic and modified atmosphere packaging (Doulgeraki *et al.*, 2012). A diverse LAB community consisting of 5 species and 41 strains was revealed using pulsed field gel electrophoresis (PFGE). It is noticeable that the highest heterogeneity regarding strain frequency was observed for *L. pentosus* with 20 strains, followed by *L. plantarum* with 9 strains. The above results indicate that *L. pentosus* is apparently the LAB species that predominates in natural black olive fermentation of Greek olives.

16.2.2 Dry-salted black olives

Dry-salted olives are a traditional preparation of natural black olives processed in Greece and some other countries in the Mediterranean region (e.g., Turkey and some North African countries), resulting in a completely different product from other preparations. The drupes are harvested at full maturation, and the final product is known as “natural black dry-salted olives.” They are prepared by placing the olives between layers of dry salt in containers that allow drainage of the liquid phase of the drupes due to the osmotic pressure exerted by the salt, resulting in gradual debittering of the olives. The final product is shriveled in appearance, has a bitter-sweet taste, and is preserved by the salt that has been absorbed by the olive mesocarp during the dry-salting process. This product is not immersed in brine, and hence it is not subjected to proper fermentation as in the case of green and natural black olives in brine, but it is rather “cured” in dry salt (Harris, 1998).

In Greece, olives of the 'Thassos' variety, a clone of the 'Thrubolea' cultivar grown on the island of Thassos in northern Greece, are used exclusively for the production of natural black dry-salted olives. According to the traditional processing method, olives are harvested from December to January at the stage of full ripeness or overripeness, when the superficial color is completely black. At the time of harvest and due to the advanced stage of ripeness, the drupes are already shriveled on the tree. After harvest, they are subjected to processing by placing them in concrete tanks with coarse dry salt at a proportion of 40% (weight of salt per weight of olives). Due to high osmosis induced by the coarse salt, the water content leaks out of the fruit while the salt is taken up by the olive. It is assumed that along with the water content, other solutes including oleuropein flow out resulting in the progressive debittering of the olives. Not long ago, it has been reported that olive debittering during the dry-salting process is due to enzymatic oxidation, in particular polyphenol oxidase (PPO) activity. The use of salt causes the rupture of the olive tissue, thereby putting the oleuropein component into contact with PPO (Ramírez *et al.*, 2013). The final product is ready for consumption within 30–40 days, and it is characterized by a rich flavor, aroma, and a sweet-bitter taste that is appreciated by local markets. Dry-salted olives are characterized by a long shelf life due to the high salt concentration in the mesocarp, resulting in low water activity levels. The main characteristics of the final dry-salted product are water activity (a_w), 0.75–0.85; pH, 4.5–5.5; oil content, 35–39% (w/w); moisture content, 30–35% (w/w); and reducing sugars, 2.0–2.5% (w/w). From the microbiological point of view, the traditional dry-salting process of 'Thassos' natural black olives showed that the initial microbiota of the drupes consisted of yeasts, LAB, and enterobacteria in populations ranging from 3.7 to 5.7 log CFU/g (Panagou, 2006), but at the end of the process only salt-tolerant yeasts could survive and dominate the surface of the olives. The yeast species has been identified as *Candida famata*, which is the imperfect form of *Debaryomyces hansenii*, a salt-tolerant species that can grow in up to 20–24% salt content (Panagou *et al.*, 2002). Despite the fact that dry-salted olives are preserved in high amounts of salt, fungal growth cannot be avoided, especially by xerophilic fungi such as *Aspergillus* and *Penicillium*, and therefore spoilage frequently occurs. In an attempt to minimize spoilage, olives are dressed with a small amount of extra virgin olive oil to ensure anaerobiosis on the surface of the drupes and then packed in glass jars or plastic bags. For better preservation results, the use of modified atmosphere packaging is advised or the use of a weak acid preservative such as potassium sorbate in the form of dip treatment. The effectiveness of several modified atmospheres has been investigated including 100% CO_2, 100% N_2, and 40% CO_2/30% O_2/30% N_2 during a storage period of six months at chill (4 °C) and room (20 °C) temperatures (Panagou *et al.*, 2002). Results showed that all modified atmospheres were able to prevent spoilage due to fungal growth at both storage temperatures compared to olive samples stored in air (control treatment). The predominant microbiota of olives comprised yeasts, whereas no LAB, Enterobacteriaceae, or *Pseudomonas* spp. were enumerated at all. From the pathogenic bacteria, special attention was given to the presence of *Staphylococcus aureus*, which has the potential to survive at low a_w levels (a_w, 0.86) (Adams & Moss, 2000), but the pathogen was not detected in any sample. The most effective atmosphere was 100% CO_2, which maintained the lowest population of yeasts throughout storage in combination with low storage temperature.

It should be mentioned that for dry-salted olives, the trade standard of the IOC (2004) applying to table olives defines that the minimum amount of salt content in the mesocarp should be 10% (w/w) to ensure the safety of the product, although this concentration is under reconsideration in the revised trade standard for table olives by the IOC as the product is excessively salty. Consequently, the international market for dry-salted olives is rather limited due to modern trends in nutrition that tend to minimize salt consumption in the diet. In order to totally avoid the use of salt and subsequent packaging in a protective modified atmosphere for the microbiological safety of the product, the use of hot air dehydration under mild temperature would be an interesting alternative. This aspect was addressed in a study (Mantzouridou & Tsimidou, 2011) in which 'Thassos' natural black olives were dried in a tunnel under a stream of hot air (40 °C) for 24 h with a resulting a_w level at the end of the process of 0.896. No salt was employed in any processing step, and hence these olives could be considered as a no-salt product. The olives were subsequently packaged under vacuum and 100% N_2 for a period of six months at 4 and 20 °C. Microbiological analysis showed the absence of undesirable microorganisms such as enterobacteria, *S. aureus*, *Bacillus*, and *Clostridium*. At 4 °C the dominant microbiota consisted of yeasts, whereas at 20 °C a mixed community of mesophilic bacteria and yeasts prevailed on the olives. Again, as in the previous case, no fungal growth was observed in any package under vacuum or modified atmosphere with the exception of olives packaged in air (control treatment), where growth of *Penicillium* and *Aspergillus* was identified.

16.3 Functional value of Greek table olives

Table olives are fruits with high nutritional and functional value. The olive's mesocarp hosts the majority of nutrient components, which are represented by a high level of water, lipids, and a low level of sugars and proteins. The lipid content in mature olive drupes predominantly consists of oleic acid (C18:1), followed by palmitic (C16:0), linoleic (C18:2), and stearic acid (C18:0). The monounsaturated oleic acid represents about 70–85% of the total fatty acid composition in the mesocarp. The polyunsaturated linoleic acid is present at 5–10%, while traces of linolenic acid (C18:3) have also been reported (Ajana *et al.*, 1998). Linoleic acid is an omega-6 fatty acid used for the biosynthesis of arachidonic acid, and it is essential for the proper function of human health, and other health benefits such as blood pressure decrease, prevention of oxidation stress, anti-inflammatory properties, and bone loss prevention have also been linked with oleic acid intake (Puel *et al.*, 2007; Terés *et al.*, 2008). Carbohydrates, mainly glucose, fructose, and mannitol, act as precursors for the fatty acid synthesis. The remaining sugar content further serves as a carbon source to support the growth of desired microorganisms during olive fermentation (Garrido Fernández *et al.*, 1997). More complex sugars are present in the pit (lignin) and in the mesocarp (hemicellulose, cellulose, and pectin), playing substantial roles in the structural characteristics of the mesocarp (Kailis & Harris, 2007). The protein content, although low, is of high quality due to the presence of essential amino acids for adults (threonine, valine, leucine, isoleucine, phenylalanine, and lysine) and for children (arginine, histidine, and tyrosine) (Lanza *et al.*, 2010). Regarding Greek olive varieties, the amino acid composition of unfermented olives of cv. 'Koroneiki', 'Throumbolea', and 'Megaritiki' has previously been reported (Manoukas *et al.*, 1973) (Table 16.1). All common amino acids were present in all three varieties; specifically, arginine, alanine, aspartic acid, glutamic acid, and glycine constituted approximately 60% of the free amino acids. Moreover, all essential amino acids were present (apart from tryptophan) in varying concentrations depending on the cultivar.

Apart from their nutritional value, olives also contain secondary compounds, such as phenolics, tocopherols, and triterpenes, which are frequently attributed with functional properties. A number of studies focusing on the content of such components in table olives of Greek olive cultivars are summarized in Table 16.2 and are discussed in detail in this chapter.

Phenolics are a major category of components with important biological properties present in olive drupes. Apart from their contribution to sensory and aromatic characteristics of olives, they are also regarded as natural antioxidants due to their reducing properties as hydrogen- or electron-donating agents (Rice-Evans *et al.*, 1997). They are present in the olive's mesocarp, leaves, and seeds, and their content is influenced by cultivar, maturation stage, and processing method. The phenolic fraction of olives is composed of phenolic alcohols, phenolic acids, flavonoids, and secoiridoids. Hydroxytyrosol, tyrosol, and their glucoside forms comprise the group of phenolic alcohols (Charoenprasert & Mitchell, 2012). Phenolic acids are the simplest forms of phenolic compounds found in the olive fruit, and these are mainly hydroxycinnamic acids (caffeic, *p*-coumaric, and ferulic acids), hydroxybenzoic acids (syringic and vanillic acids), and the more complex verbascoside, which is an ester of caffeic acid and hydroxytyrosol (Rice-Evans *et al.*, 1997; Charoenprasert & Mitchell, 2012). The most frequently reported flavonoids in olives are luteolin 7-O-glucoside, luteolin 4-O-glucoside, apigenin 7-O-glucoside, rutin, and apigenin 7-O-glucoside (Vinha *et al.*, 2005; Benincasa *et al.*, 2015). From the group of secoiridoids, oleuropein, ligstroside, oleuroside, and their aglycon forms are the most important ones found in the drupes (Alagna *et al.*, 2012). The qualitative and quantitative phenolic content of raw olives belonging to different Mediterranean olive cultivars was investigated by a high-performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR)-based approach (Bianco & Uccella, 2000; Bastoni *et al.*, 2001). Specifically, olive drupes originating from Spain (green, cherry, and black cv. 'Hojiblanca'), Portugal (green, cherry, and black cv. 'Douro'), Greece (black cv. 'Thassos' and 'Conservolia'), and Italy (black cv. 'Carolea', 'Taggiasca', and 'Cassanese') were comparatively analyzed. The Greek olive cultivars contained phenolic compounds such as hydroxytyrosol, tyrosol, caffeic acid, and *p*-coumaric acid in amounts similar to the Spanish, Portugese, and Italian cultivars, while hydroxycaffeic acid was found in the 'Thassos' and 'Conservolea' olives.

Table olives produced in Greece have been evaluated as sources of biophenols in a previous study (Blekas *et al.*, 2002). The total phenolic content as well as the content of individual phenols in the mesocarp were determined for 25 samples of commercially available table olives in retail outlets. All samples included the most representative Greek cultivars. namely, cv. 'Halkidiki' and 'Conservolea' processed as Spanish-style green and Greek-style natural black olives in brine, cv. 'Kalamon' processed as Greek-style natural

Table 16.1 Content of representative compounds with functional properties (amino acids, tocopherols, and triterpenic acids) from different Greek table olive cultivars.

Functional compound			**Studied cultivar**	
Amino acids[a]		**'Koroneiki'**	**'Throumbolea'**	**'Megaritiki'**
Arginine		10.17 ± 1.33	9.38 ± 0.30	9.71 ± 0.89
Histidine		2.74 ± 0.91	2.99 ± 0.38	2.34 ± 0.34
Lysine		5.13 ± 0.31	1.85 ± 0.10	7.02 ± 0.57
Methionine		1.29 ± 0.30	1.52 ± 0.37	1.16 ± 0.11
Cystine		Traces	Traces	Traces
Phenylalanine		3.00 ± 0.10	2.76 ± 0.39	3.14 ± 0.20
Tyrosine		2.42 ± 0.35	2.52 ± 0.37	2.22 ± 0.11
Leucine		5.25 ± 1.77	4.71 ± 0.27	5.55 ± 0.37
Isoleucine		3.04 ± 0.83	2.96 ± 0.24	3.28 ± 0.31
Threonine		2.60 ± 0.59	2.51 ± 0.24	3.30 ± 0.29
Valine		4.07 ± 1.11	3.79 ± 0.23	4.03 ± 0.30
Alanine		5.45 ± 0.30	6.49 ± 0.40	3.60 ± 0.16
Aspartic acid		10.99 ± 2.32	11.11 ± 1.73	12.28 ± 0.78
Glutamic acid		10.72 ± 1.61	12.54 ± 0.98	11.85 ± 0.57
Glycine		6.54 ± 1.66	8.79 ± 1.17	3.50 ± 0.38
Proline		4.31 ± 0.62	7.44 ± 1.60	2.70 ± 0.38
Serine		3.05 ± 0.82	3.48 ± 0.53	4.30 ± 0.39
Tocopherols[b]	**'Conservolea' (green)**	**'Conservolea' (black)**	**'Halkidiki'**	**'Kalamon'**
α-tocopherol	143.01 ± 5.0	170.8 ± 11.7	81.3 ± 10.3	187.9 ± 15.4
β-tocopherol	Traces	Traces	Traces	Traces
γ-tocopherol	Traces	45.0 ± 13.9	25.6 ± 2.4	42.9 ± 4.1
α-tocotrienol	Traces	Traces	Traces	Traces
α-tocopherol equivalent	43.1 ± 5.0	175.3 ± 10.8	83.8 ± 10.3	192.1 ± 16.01
Triterpenic acids[c]		**'Conservolea' (treated green)**	**'Conservolea' (natural green)**	**'Kalamon'**
Maslinic acid		552 ± 16	1349 ± 123	1260 ± 58
Oleanolic acid		331 ± 33	536 ± 82	706 ± 48

[a]Content of amino acids of unfermented olives expressed as g/16 g of nitrogen ± standard error of the mean. *Source*: Manoukas *et al.*, Journal of Agriculture and Food Chemistry 21, 215–217, 1973.
[b]Content of tocopherols expressed as μg/g of lipids ± standard deviation. *Source*: Hassapidou *et al.*, Food Chemistry 50, 111–114, 1994.
[c]Content of triterpenic acids expressed as mg/kg of olive flesh. *Source*: Alexandraki *et al.*, LWT – Food Science and Technology 58, 609–613, 2014.

black olives in brine, and cv. 'Thassos' processed as natural black olives in dry salt. The highest total phenol content, expressed as mg/kg caffeic acid, was observed mainly in 'Kalamon' and 'Conservolea' natural black olives, exceeding 1000 mg/kg, while considerable amounts were also found in 'Thassos' dry-salted olives and in 'Halkidiki' and 'Conservolea' Spanish-style green olives in brine. High levels of hydroxytyrosol were determined primarily in 'Kalamon' olives (250.0–760.0 mg/kg), followed by 'Halkidiki' and 'Conservolea' Spanish-style green olives (170.0–510.0 mg/kg), and finally in 'Conservolea' natural black olives, whereas 'Thassos' dry-salted olives contained the lowest amount of this compound (60.0–80.0 mg/kg). The same trend was observed for tyrosol but in overall lower concentrations than hydroxytyrosol. Luteolin on the other hand was recovered in all natural black olive samples ranging between 20.0–75.0 mg/kg and in much lower amounts in Spanish-style green olive samples (2.0–12.0 mg/kg). The high

Table 16.2 Summary of studies focused on different functional characteristics of processed olive drupes belonging to Greek cultivars.

Cultivar	Functional Compound characteristic	Authors
cv. 'Agouromanaki'	Determination of phenolic compounds	Zoidou *et al.* (2010)
cv. 'Conservolea'	Determination of phenolic compounds	Blekas *et al.* (2002), Zoidou *et al.* (2010)
	Determination of tocopherols and tocotrienols	Hassapidou *et al.* (1994)
	Determination of triterpenic acids	Alexandraki *et al.* (2014)
	Determination of dietary fiber	Jiménez *et al.* (2000)
	Olives fermented with LAB cultures with *in vitro* probiotic properties	Grounta *et al.* (2014)
cv. 'Halkidiki'	Determination of phenolic compounds	Blekas *et al.* (2002)
	Determination of tocopherols and tocotrienols	Hassapidou *et al.* (1994)
	Olives fermented with LAB cultures with *in vitro* probiotic properties	Argyri *et al.* (2014), Blana *et al.* (2014)
cv. 'Kalamon'	Determination of phenolic compounds	Blekas *et al.* (2002), Romero *et al.* (2004), Zoidou *et al.* (2010)
	Enrichment of table olives with phenolic compounds	Lalas *et al.* (2011)
	Determination of tocopherols and tocotrienols	Hassapidou *et al.* (1994)
	Determination of triterpenic acids	Romero *et al.* (2010), Alexandraki *et al.* (2014)
cv. 'Koroneiki'	Determination of triterpenic acids	Romero *et al.* (2010)
cv. 'Megaritiki'	Determination of phenolic compounds	Zoidou *et al.* (2010)
cv. 'Thassos'	Determination of phenolic compounds	Blekas *et al.* (2002), Romero *et al.* (2004), Zoidou *et al.* (2010)
	Determination of dietary fiber	Jiménez *et al.* (2000)

amounts of hydroxytyrosol make table olives a great source of this compound, which is nutritionally very important besides virgin olive oil as the only other edible source of hydroxytyrosol. Moreover, the phenolic content of 'Kalamon' and 'Thassos' natural black olives together with other Spanish-style green, turning-color, and black ripe table olives of Spanish cultivars has also been studied in the aqueous (juice) and lipid phase (oil) of olive pulp (Romero *et al.*, 2004). The highest hydroxytyrosol content in the olive juice was determined in turning-color olives (9306.0 ± 1162.0 μM/L) followed by 'Manzanilla' Spanish-style green olives (7566.0 ± 214.0 μM/L) and 'Kalamon' black olives (5417.0 ± 1188.0 μM/L), whereas 'Thassos' dry-salted black olives together with 'Gordal' and 'Hojiblanca' Spanish-style green olives shared the same concentrations (3500.0–4000.0 μM/L). Tyrosol was highest in 'Kalamon' olives (1716.0 ± 226.0 μM/L) followed by turning-color, 'Manzanilla' Spanish-style green and 'Thassos' olives (800.0–1400.0 μM/L). Salidroside and verbascoside were also recovered mainly in 'Kalamon' olives (510.0 ± 115.0 and 4.0 ± 1.0 μM/L, respectively), 'Thassos' olives (500.0 ± 216.0 and 2.0 ± 1.0 μM/L, respectively), and turning-color olives (277.0 ± 128.0 and 3.0 ± 1.0 μM/L, respectively). In another work (Boskou *et al.*, 2006), the phenolic compounds were investigated in the mesocarp and kernel of five different cultivars of Greek table olives: black 'Kalamon' olives in brine, green *tsakistes* (cracked) olives in brine, black 'Crete' olives in brine, black 'Amfissas' olives in brine, and wrinkled 'Thrubes Crete' olives. Hydroxytyrosol was present in a greater abundance in all samples, followed by tyrosol. Small amounts of cinnamic acid, caffeic acid, *p*-coumaric acid, and other phenolic compounds were also detected. In the same study, the total antioxidant activity of the olives was also evaluated by scavenging the DPPH radical. Results indicated that the antioxidant capacity of the olives was in descending order of *tsakistes* > 'Amfissas' > 'Kalamon' > 'Crete' > 'Thrubes Crete'. The authors of the study concluded that consumption of table olives is considered to offer a high intake of antioxidants, while in order to meet the daily demands of polyphenols, a quantity of about 5–10 table olives was proposed. More recently, nine commercial types of Greek table olives were examined for

their content of oleuropein and hydroxytyrosol (Zoidou *et al.*, 2010). All tested samples were found to be rich in hydroxytyrosol content, with 'Kalamon' olives and 'Megaritiki' *tsakistes* (cracked olives) possessing the highest concentration of this component. Oleuropein, on the other hand, in most of the analyzed samples either was not detected or was detected in very low levels, apart from 'Throuba Thassos' that was identified as a rich source of oleuropein. The use of sodium hydroxide solution (lye treatment) during the production of Spanish-style green olives hydrolyzes ester bonds turning oleuropein to hydroxytyrosol. In the case of Greek-style processing, during the long fermentation period, diffusion of constituents and lactic acid hydrolysis of oleuropein to hydroxytyrosol take place. On the other hand, 'Thassos' olives are subjected to a special dry-salting process resulting in dehydration rather than fermentation. As a result, a part of the oleuropein component is not hydrolyzed, and this justifies the fact that 'Thassos' olives have a smaller hydroxytyrosol content (Blekas *et al.*, 2002; Romero *et al.*, 2004) but are very rich in oleuropein (Zoidou *et al.*, 2010). However, in the work of Zoidou *et al.* (2010), oleuropein was not detected in dry-salted black olives of 'Megaritiki' cultivar, and this was attributed to the different olive cultivar or the use of excessive washing of the olives before commercialization. More recently, the phenolic content of 'Kalamon' table olives was fortified with phenols extracted from the olive leaves (Lalas *et al.*, 2011). The oleuropein content of treated table olives was 93.0 ± 2.8 mg/kg compared to the untreated olives (16.7 ± 1.2 mg/kg), whereas the corresponding content of hydroxytyrosol was 855.0 ± 20.2 and 408.0 ± 15.9 mg/kg for treated and untreated olives, respectively. Sensory evaluation showed that although the bitterness of the treated samples was increased, the overall acceptability was equally good for both samples.

The bioavailability of olive phenols of cv. 'Kalamon' and their antioxidant efficacy in healthy volunteers have been investigated (Kountouri *et al.*, 2007) using eight male healthy individuals between 30 and 40 years of age who volunteered for the study. The phenolic content of 'Kalamon' black olives was previously determined, and then biological fluids (blood and urine) from the volunteers were collected after administration of 20 olives. The collected fluid samples were subjected to gas chromatography–mass spectrometry analysis for individual phenolics determination, while the total phenol content and total antioxidant potential in plasma were also estimated. Significant increases of tyrosol and hydroxytyrol content in plasma were observed after olive consumption, indicating that olive phenols are bioavailable. Other phenolics previously detected in olive samples, including vanillin, phloretic acid, vanillic acid, protocatechuic acid, syringic acid, ferrulic acid, quercetic acid, and caffeic acid, were also determined in both plasma and urine, especially in their conjugated form as glucoronides. Hydroxytyrosol has been attributed with biological activities such as prevention of bone loss (Hagiwara *et al.*, 2011) and rheumatoid arthritis (Silva *et al.*, 2015), and protection of human peripheral blood mononuclear cells (PBMCs) against oxidative stress and DNA damage (Ilavarasi *et al.*, 2011). Protection against oxidative stress is regarded as an anticancer property since oxidative stress is involved in many stages of tumor genesis. It has also been presented as a potent inhibitor of key steps of angiogenesis in bovine aorta endothelial cells (BAECs), human microvascular endothelial cells (HMECs), and human umbilical vein endothelial cells ECRF-24 in *in vitro* assays (Fortes *et al.*, 2012), while a novel ester of hydroxytyrosol has been reported for its effective antitumor activity on the human colorectal adenocarcinoma HT-29 cell line (Bernini *et al.*, 2011). In a recent study (Chandramohan *et al.*, 2015), tyrosol administration restored the levels of plasma glucose, plasma insulin, blood glycosylated hemoglobin (HbA1c), and blood total hemoglobin (Hb); a homeostasis model assessment of insulin resistance (HOMA-IR); and the activities of key enzymes involved in the metabolism of glucose, glycogen, and antioxidants in the tissues of streptozotocin (STZ)-induced diabetic rats. Furthermore, the cardioprotective and neuroprotective roles of oleuropein as well as its antitumor, antidiabetic, and anti-obesity activity have previously been noted (Hassen *et al.*, 2014).

Tocopherols and tocotrienols, members of the vitamin E family, are compounds with antioxidant and anti-inflammatory properties (Singh *et al.*, 2005), that are present in processed olive drupe. Changes in tocopherol (*a*-, β-, and γ-tocopherol) and tocotrienol (α-tocotrienol) content of the olive cultivars 'Halkidiki' (green olives), 'Conservolea' (green and black olives), and 'Kalamon' (black olives) were evaluated at the raw stage and after two processing stages (Hassapidou *et al.*, 1994) (Table 16.1). The first processing stage included a debittering treatment (lye treatment) for the two green olive varieties, 'Halkidiki' and 'Conservolea', or a 7-day washing scheme for the two black olive varieties, 'Kalamon' and 'Conservolea'. The second processing stage included the final fermented product of all varieties. Results showed that at the raw stage, α-tocopherol was found in all cultivars at 130.0–175.0 μg/g lipids, the content of which was not affected during processing with the exception of 'Halkidiki' green olives where it decreased to 81.3 ± 10.3 μg/g lipids. The content

of β-tocopherol was circa 25.0 μg/g lipids in raw black olives, but it was not measurable after processing, while traces of the same compound were detected in raw and processed green olives. The different processing stages did not affect the content of γ-tocopherol in all olive cultivars, and the same pattern was observed for α-tocotrienol. It is characteristic that α-tocopherol was recovered at an average of 170.0 μg/g black olives of 'Conservolea' and 'Kalamon' cultivars and remained unchanged during processing (Hassapidou *et al.*, 1994). The contribution of tocopherols in human health has been well documented in the literature. They exert cardiovascular protective action by eliminating reactive oxygen species, inhibiting lipid peroxidation, and attenuating the release of proinflammatory cytokines (Singh *et al.*, 2005). Their activity as free radical scavengers further attenuates neuronal damage in chronic cerebral hypoperfusion (Annaházi *et al.*, 2007), provides protection to hippocampal cells against oxidative stress, and reduces the hippocampal and nigral neuron loss in iron-induced neurotoxicity (Bostanci *et al.*, 2010), conditions commonly related with disorders of the nervous system such as Alzheimer's disease. Moreover, γ-tocopherol has been reported to possess anti-inflammatory activity by reducing prostaglandin E_2 synthesis and inhibiting cyclooxygenase-2 activity (Jiang *et al.*, 2000). This anti-inflammatory effect of γ-tocopherol has been suggested to prevent the incidence of colon cancer, since this type of cancer is associated with increased expression of cyclooxygenase-2 and formation of prostaglandin E_2 (Jiang *et al.*, 2000). Tocopherols have also been associated with prostate cancer (PrCA) risk and progression. Specifically, higher prediagnostic circulating concentrations of α- and γ-tocopherols have been linked with substantially lower risk of prostate cancer (Weinstein *et al.*, 2005). In a recent study (Antwi *et al.*, 2015), it was found that the levels of prostate-specific antigen (PSA) in men with PrCA recurrence were decreased at three and six months after consuming a diet with α-tocopherol; thus, greater intake of foods containing α-tocopherol can be beneficial in men with PSA-defined PrCA recurrence.

Greek olive cultivars have also been found to be rich in triterpene compounds. Pentacyclic triterpenic acids, together with triterpenic alcohols and esters, are a class of non-acylglycerol lipid fraction in olives, mostly located in the epicarp constituting the main component of the epicuticular waxes, with maslinic and oleanolic acids being the representative triterpenic acids reported (Lanza & Di Serio, 2015). They are also present in the mesocarp and on the surface of olive leaves, forming a physical barrier that prevents penetration of microbes into the leaf, while oleanolic acid has been found as well in the wood shell and seeds of olives (Romero *et al.*, 2010). Triterpenic acids have previously been determined in table olives for different cultivars and processing methods (Romero *et al.*, 2010). The authors reported that the lye treatment during Spanish-style and black ripe olive processing resulted in the solubilization of maslinic and oleanolic acids into the lye and washing solutions. On the contrary, these compounds were never found in the brines of natural black olive processing. In the same study, raw olive drupes of 17 different olive cultivars including the Greek cultivars 'Koroneiki', 'Conservolea', and 'Kalamon' were studied for their concentration in triterpenic acids expressed as mg/kg of olive flesh. All Greek cultivars were rich in triterpenic acids, with 'Conservolea' and 'Koroneiki' being the richest in maslinic acid, and 'Kalamon' and 'Koroneiki' in oleanolic acid among all tested samples. Furthermore, processed 'Kalamon' natural black olives were the richest in maslinic and oleanolic acids at 1318.0 ± 401.0 and 841.4 ± 162.9 mg/kg, respectively. More recently, the changes of triterpenic acids throughout natural and treated Greek table olive fermentation were investigated (Alexandraki *et al.*, 2014) (Table 16.1). Olives of the 'Conservolea' cultivar were processed according to the Spanish-style method and as natural green olives, while olives of the 'Kalamon' cultivar were subjected to natural black olive fermentation. In the case of the Spanish-style method, the concentrations of maslinic and oleanolic acids in raw olives were 1230.0 ± 108.0 and 541.0 ± 44.0 mg/kg, respectively, whereas in the final product the concentrations were decreased to 552.0 ± 16.0 and 331.0 ± 33.0 mg/kg, respectively, confirming the findings of the previous study (Romero *et al.*, 2010) on the solubilization of triterpenic acids into the lye and washing solutions during Spanish-style processing. The natural processes, on the other hand, did not affect the triterpenic acid content in the olives throughout the fermentation process. In these cases, the concentration of maslinic acid in the final product was 1349.0 ± 123.0 and 1320.0 ± 256.0 mg/kg for the natural green and black olive fermentation, respectively. The corresponding concentrations of oleanolic acid were 536.0 ± 82.0 and 706.0 ± 48.0 mg/kg, respectively. Triterpenic compounds are considered as another group of functional compounds attracting the interest of the scientific community because of a series of health-promoting properties. Administration of maslinic acid has been examined for its potential as a natural therapeutic agent for type 2 diabetes in mice (Liu *et al.*, 2007). Daily doses of 10.0 and 30.0 mg/kg, administered orally for a period of two weeks, showed a significant reduction in the blood glucose levels. At the same time, the hepatic glycogen content was increased, which may contribute to reducing

glucose supply in the blood. The plasma insulin levels in treated mice significantly decreased two weeks after administration, while after an insulin tolerance test the blood glucose levels in treated mice were significantly decreased compared to the controls. In another work, four triterpenic compounds, including maslinic and oleanolic acids, were examined for their antioxidant and antithrombotic properties *in vitro* (Allouche *et al.*, 2010). Maslinic acid significantly retarded the initiation of LDL oxidation and decreased thrombin formation in a dose-dependent manner. It was also found that it possessed peroxyl radical scavenging activity and acted as a copper chelator. Tsai *et al.* (2008) also reported the antioxidative and anti-inflammatory protection of oleanolic and ursolic acids in the PC12 cell line (a rat adrenal gland pheochromocytoma cell line used as a model for studying the survival of neuronal cells or antioxidant properties). PC12 cells were pretreated with 20 and 40 μM of oleanolic and ursolic acids and then exposed to H_2O_2 and MPP^+ to induce oxidative and inflammatory injury. The pretreatment with triterpenes significantly decreased H_2O_2- or MPP^+-induced cell death, reduced LDH leakage, and attenuated the release of proinflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin-6. In cancer-related research, maslinic acid was investigated as a potent agent against gastrointestinal cancers, causing apoptosis and declined invasion and migration in colon (Juan *et al.*, 2008), liver (Lin *et al.*, 2011), and esophagus and stomach (Lin et al., 2014) cancer cell lines. The antitumor activity of oleanolic acid has been reported in hepatocellular (Wang *et al.*, 2013), breast (Bishayee *et al.*, 2013), colon (Janakiram *et al.*, 2008), and prostate (Gao *et al.*, 2011) carcinoma in rats and mice animal models, while also preclinical and clinical evidence regarding oleanolic acid as a preventive and therapeutic agent in different types of cancer has recently been reviewed (Shanmugam *et al.*, 2014). Treatment with oleanolic acid has recently been examined for its efficacy to protect dopamine neurons from the toxic effects of oxidopamine (6-OHDA) in Parkinsonian rat models (Mabandla *et al.*, 2015). The results obtained in this study showed neuroprotective effects of oleanolic acid 7 days pre-exposure and 1 day post-exposure to 6-OHDA, suggesting that early treatment with this compound may effectively alleviate the degenerative effects of 6-OHDA in Parkinson's disease.

Modern nutrition emphasizes intake of a diet rich in vegetables, fruits, grains, and legumes, and low in saturated fat, while at the same time there is an increasing interest in the production of novel products with beneficial effects on human health. In this sense, research has focused over the last years in the production of food commodities incorporating health-promoting microorganisms, commonly known as probiotics. Probiotics are defined as "live microorganisms, which when consumed in adequate amounts, confer a health benefit to the host" (FAO/WHO, 2001). Intake of probiotics stimulates the immune system, prevents allergies, and alleviates symptoms of lactose intolerance (Kies, 2014; Cuello-Garcia *et al.*, 2015). Many *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Enterococcus*, and *Lactococcus* strains exhibit cholesterol-lowering ability, thus protecting from arterial clogging and risk of stroke and heart attack (Ishimwe *et al.*, 2015). Strains of *L. rhamnosus*, *L. fermentum*, and *L. reuteri* support an important role in the maintenance of urogenital and vaginal health in women and protection against pathogens responsible for bacterial vaginosis (Reid & Bruce 2006; Vujic *et al.*, 2013). Furthermore, their efficacy in protecting against enteric pathogens, reducing the risk of colorectal cancer, and relieving the symptoms of intestinal conditions, such as inflammation and diarrhea, inflammatory bowel diseases, irritable bowel syndrome, colitis, and constipation, has also been reported (Serban, 2014; Faghfoori *et al.*, 2015). Other positive health properties referring to improvement of oral cavity hygiene, wound healing, and angiogenesis (Halper *et al.*, 2003; Anilkumar & Monisha, 2012) have been mentioned. In the food market, the majority of foods containing probiotics are milk-based products such as yogurts, cheeses, and fermented milks. Taking into account, however, the increasing number of individuals facing lactose intolerance and milk proteins allergy, there is an increasing demand for manufacturing nondairy probiotic food products. Table olives, as a plant origin product, may well serve as a tool for this purpose. The first attempt to this approach was published by Lavermicocca *et al.* (2005), where different types of commercial table olives were inoculated with seven probiotic strains of *Lactobacillus* and *Bifidobacteria*. The tested strains successfully adhered and colonized the olive surface with high survival rates during storage, while one of them, namely *L. paracasei* IMPC2.1, was used in a follow-up work (De Bellis *et al.*, 2010) as a starter culture in the fermentation process of 'Bella di Cerignola' green olives, resulting in a final product with functional appeal. While the main source of probiotic isolates is the human gastrointestinal tract, recent studies have been undertaken with focus on the use of novel strains as probiotic and starter cultures originating from the olive's autochthonous microbiota (Bevilaqua *et al.*, 2010; Abriouel *et al.*, 2012; Argyri *et al.*, 2013, 2014; Bautista-Gallego *et al.*, 2013; Rodrıguez-Gomez *et al.*, 2013); Blana *et al.*, 2014; Botta *et al.*, 2014; Peres *et al.*, 2014). Nineteen LAB strains isolated from

Italian 'Bella di Cerignola' table olives have been investigated for their technological and probiotic properties for the selection of multifunctional starter cultures for olive processing (Bevilaqua *et al.*, 2010). All strains were able to grow at a range of pH between 4.0 and 10.0 as well as in media supplemented with 2.5 to 7.5% of NaCl and 0.3% bile salts and survived in MRS broth acidified at pH 2.5. Moreover, they were able to inhibit *E. coli* O157:H7 and compete with *S. aureus*. A collection of 144 LAB strains originating from Spanish 'Aloreña' naturally fermented green olives were studied for their potential as probiotic starter cultures (Abriouel *et al.*, 2012). Fifteen *L. pentosus* strains and one *Leuconostoc pseudomesenteroides* strain from this study exhibited antimicrobial properties and tolerance to low pH and high bile salt concentration. In another work (Bautista-Gallego *et al.*, 2013), lactobacilli isolates from different spontaneous industrial green olive fermentations were selected by *in vitro* phenotypic tests related to probiotic potential. In the same line, four *L. pentosus* strains, previously isolated from diverse table olive processing and selected according to *in vitro* phenotypic tests related to probiotic potential, were used as starters to better control Spanish-style green olive fermentation (Rodríguez-Gómez *et al.*, 2013). Recently, 238 LAB strains, belonging to *L. plantarum*, *L. pentosus*, and *Lc. mesenteroides* species, isolated from 'Nocellara Etnea' table olives, were screened for production of antimicrobial compounds, hemolytic activity, bile salt hydrolysis (BSH) activity, auto-aggregation, bacterial surface hydrophobicity, and survival in a simulated human digestion process through an *in vitro* approach (Botta *et al.*, 2014). The experiments were performed using intestinal epithelial and monocyte/macrophage-derived cell lines of human origin, named respectively H4 clone 1 (H4-1) and TLT. In all tests, the probiotic strains *Lactobacillus rhamnosus* GG and *Lactobacillus casei* Shirota were used in parallel as reference controls. Among the tested strains, *L. plantarum* S11T3E strain has overall shown the best probiotic performance, due to its high resistance to simulated gastric digestion, an increased transepithelial resistance of polarized H4-1 cells, and a significant reduction in *L. monocytogenes* invasion in undifferentiated gut model cells. The latter characteristic was also shown by the *L. pentosus* S3T60C and *L. plantarum* S2T10D strains, whereas the increase of transepithelial resistance was shown by *L. plantarum* S1T10A as well. In another work, the potential probiotic features of LAB strains isolated from cv. 'Galega' fermented olives have been screened (Peres *et al.*, 2014), to eventually develop an improved probiotic food of plant origin. The probiotic features evaluated were the survival to simulated digestion, bile salt hydrolase, proteolytic and antimicrobial activities, ability to autoaggregate and cell surface hydrophobicity, hemolytic capacity, and the ability to hydrolyze mucin. Further on, exopolysaccharide-producing abilities were evaluated as prebiotic properties. From 156 isolated strains, 10 (7 *L. plantarum* and 3 *L. paraplantarum*) were acid salt and bile salt tolerant, and exhibited survival rates up to 48% following simulated digestion. All strains exhibited auto- (4–12%) and co-aggregation features (≥30%), as well as hydrophobicity (5–20%) and exopolysaccharide-producing abilities, while no strain possessed hemolytic capacity or ability to hydrolyze mucin. Antibiotic resistance, oleuropein degradation, proteolytic activity, and antimicrobial activity were strain-dependent features.

Related work for LAB strains isolated from Greek table olives with functional properties has also been published by Greek researchers. In a recent work, Argyri *et al.* (2013) evaluated the probiotic potential of LAB isolated from naturally fermented Greek table olives and selected candidates to be used as probiotic starters for the improvement of the traditional fermentation process and the production of high-added-value functional foods. A total of 71 LAB strains (17 *Lc. mesenteroides*, 1 *Ln. pseudomesenteroides*, 13 *L. plantarum*, 37 *L. pentosus*, 1 *L. paraplantarum*, and 2 *L. paracasei* subsp. *paracasei*) isolated from table olives were screened for their probiotic potential in parallel with *L. rhamnosus* GG and *L. casei* Shirota that were used as reference strains. The *in vitro* tests included survival in simulated gastrointestinal tract conditions, antimicrobial activity (against *L. monocytogenes*, *Salmonella enteritidis*, and *E. coli* O157:H7), Caco-2 surface adhesion, resistance to nine antibiotics, and hemolytic activity. Three *L. pentosus*, 4 *L. plantarum*, and 2 *L. paracasei* subsp. *paracasei* strains demonstrated the highest final population (>8.0 log CFU/mL) after 3 h of exposure at low pH (2.5). The majority of the tested strains were resistant to bile salts even after 4 h of exposure (pH 8.0), while 5 *L. plantarum* and 7 *L. pentosus* strains exhibited partial bile salt hydrolase activity. None of the strains inhibited the growth of the pathogens tested, whereas variable efficiency to adhere to Caco-2 cells was observed. Similar observations were made regarding strains' susceptibility toward different antibiotics. None of the strains exhibited b-hemolytic activity. As a whole, four strains of *L. pentosus*, three strains of *L. plantarum*, and two strains of *L. paracasei* subsp. *paracasei* were found to possess desirable *in vitro* probiotic properties similar to or even better than the reference probiotic strains *L. casei* Shirota and *L. rhamnosus* GG. Two LAB strains of the previous study – namely, *L. pentosus* B281

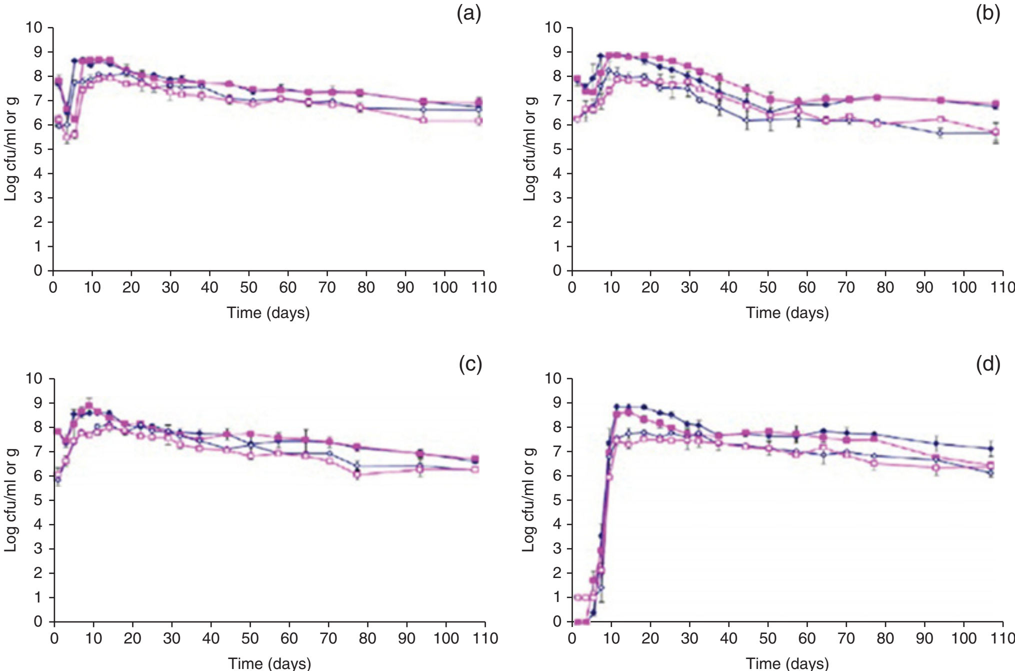

Figure 16.3 Changes in the population of LAB during the fermentation of heat-shocked green table olives cv. 'Halkidiki' by (a) *L. pentosus* B281, (b) *L. plantarum* B282, (c) mixed culture, and (d) indigenous microbiota, in 8% initial NaCl [brine (⧫), olive fruits (◊)] and in 10% initial NaCl [brine (■), olive fruits (□)]. Adapted from Argyri *et al.* (2014).

and *L. plantarum* B282 – were selected and later used as starters during Spanish-style fermentation of green olives cv. 'Halkidiki' (Blana *et al.*, 2014) and heat-shocked green olives of the same cultivar (Argyri *et al.*, 2014) in low (8%) and high (10%) salt brines. In the former work, both probiotic strains successfully colonized the olive surface at populations ranging from 6.0 to 7.0 log CFU/g throughout fermentation. PFGE analysis revealed that *L. pentosus* B281 presented higher colonization in both salt levels at the end of fermentation (81.2% and 93.3% in 8% and 10% NaCl brines, respectively). For *L. plantarum* B282, a high survival rate (83.3%) was observed in 8% NaCl brines, but in 10% NaCl the strain could not colonize the surface of olives. *L. pentosus* B281 also dominated over *L. plantarum* B282 in inoculated fermentations when the two strains were used as combined culture. In the latter work (Argyri *et al.*, 2014), olives were heat-shocked at 80 °C for 10 min prior to brining to reduce the level of the indigenous interfering microbiota on olive drupes and facilitate the dominance of the same probiotic LAB-inoculated cultures. The final population of LAB was maintained above 6 log cycles in both brine and olive flesh (Figure 16.3). Both *L. pentosus* B281 and *L. plantarum* B282 were able to dominate over indigenous LAB, while strain B281 exhibited higher recovery percentages (100 or 94.7% for B281 and 58.8% or 55.0% for B282 in 10% or 8% NaCl, respectively). Similar with the findings of the former study, *L. pentosus* B281 also dominated over *L. plantarum* B282, when the two strains were co-inoculated in olive fermentations. The use of *L. pentosus* B281 was further used as starter culture during natural black olive fermentation of the 'Conservolea' cultivar (Grounta *et al.*, 2014).

The obtained results illustrated that the use of *L. pentosus* B281 resulted in a final product with appropriate physicochemical characteristics and well-appreciated sensory properties. Also, its ability to survive in high numbers on olive drupes forming biofilm together with its *in vitro* probiotic potential make it a good candidate for the production of natural black olives with functional characteristics. Taking into account that the scientific research on the probiotic potential of microorganisms originating from olives is still recent and, in parallel, that the need for novel, health-promoting foods increases, olives should be regarded as a pool of beneficial microorganisms yet unexplored and as a biological tool for delivering their benefits to consumers.

16.4 Conclusion

Greek-style natural black table olives are a good source of bioactive compounds. The natural process in which olives are subjected contributes to better preservation of these compounds in the final fermented product. Naturally processed 'Conservolea' and 'Kalamon' olives are rich sources of triterpenic acids, mainly maslinic and secondarily oleanolic acid, and α- and γ-tocopherols. Hydroxytyrosol is the phenolic compound in the greatest abundance in 'Kalamon', 'Megaritiki', 'Halkidiki', and 'Conservolea' table olives, followed by tyrosol, while dry-salted 'Thassos' olives are characteristically rich in oleuropein content. In addition, with the recent scientific interest regarding table olives as carriers of health-promoting microorganisms, table olives are proposed as a functional product of high added value.

References

Abriouel, H., N. Benomar, A. Cobo, N. Caballero, M.Á. Fernández Fuentes, R. Pérez-Pulido, & A. Gálvez. (2012) Characterization of lactic acid bacteria from naturally-fermented Manzanilla Aloreña green table olives. Food Microbiology 32, 308–316.

Adams, M.R., & M.O. Moss. (2000) Food microbiology, 2nd ed. The Royal Society of Chemistry, Cambridge.

Ajana, H., A. El Antari, & A. Hafidi. (1998) Fatty acids and sterols evolution during the ripening of olives from the Moroccan Picholine cultivar. Grasas y Aceites 49, 405–410.

Alagna, F., R. Mariotti, F. Panara, S. Caporali, S. Urbani, G. Veneziani, S. Esposto, A. Taticchi, A. Rosati, R. Rao, G. Perrotta, M. Servili, & L. Baldoni. (2012) Olive phenolic compounds: metabolic and transcriptional profiling during fruit development. BMC Plant Biology 12, 162.

Alexandraki, V., M. Georgalaki, K. Papadimitriou, R. Anastasiou, G. Zoumpopoulou, I. Chatzipavlidis, M. Papadelli, N. Vallis, K. Moschochoritis, & E. Tsakalidou. (2014) Determination of triterpenic acids in natural and alkaline-treated Greek table olives throughout the fermentation process. LWT – Food Science and Technology 58, 609–613.

Allouche, Y., G. Beltrán, J.J. Gaforio, M. Uceda, & M.D. Mesa. (2010) Antioxidant and antiatherogenic activities of pentacyclic triterpenic diols and acids. Food and Chemical Toxicology 48, 2885–2890.

Anilkumar, K., & A. Monisha. (2012) Role of friendly bacteria in oral health – a short review. Oral Health and Preventive Dentistry 10, 3–8.

Annaházi, A., É. Mracskó, Z. Süle, E. Karg, B. Penke, F. Bari, & E. Farkas. (2007) Pre-treatment and post-treatment with α-tocopherol attenuates hippocampal neuronal damage in experimental cerebral hypoperfusion. European Journal of Pharmacology 571, 120–128.

Antwi, S.O., S.E. Steck, H. Zhang, L. Stumm, J. Zhang, T.G. Hurley, & J.R. Hebert (2015) Plasma carotenoids and tocopherols in relation to prostate-specific antigen (PSA) levels among men with biochemical recurrence of prostate cancer. Cancer Epidemiology. In Press.

Argyri, A.A., A.A. Nisiotou, A. Mallouchos, E.Z. Panagou, & C.C. Tassou. (2014) Performance of two potential probiotic *Lactobacillus* strains from the olive microbiota as starters in the fermentation of heat shocked green olives. International Journal of Food Microbiology 171, 68–76.

Argyri, A.A., G. Zoumpopoulou, K.-A.G. Karatzas, E. Tsakalidou, G.-J.E. Nychas, E.Z. Panagou, & C.C. Tassou. (2013) Selection of potential probiotic lactic acid bacteria from fermented olives by *in vitro* tests. Food Microbiology 33, 282–291.

Balatsouras, G.D. 1990. Edible olive cultivars, chemical composition of fruit, harvesting, transportation, processing, sorting and packaging, styles of black olives, deterioration, quality standards, chemical analysis, nutritional and biological value of the end product. Instituto Sperimentale per la Elaiotecnica, Pescara, Italy.

Bastoni, L., A. Bianco, F. Piccioni, & N. Uccella. (2001) Biophenolic profile in olives by nuclear magnetic resonance. Food Chemistry 73, 145–151.

Bautista-Gallego J., F.N. Arroyo-López, K. Rantsiou, R. Jiménez-Díaz, A. Garrido-Fernández, & L. Cocolin. (2013) Screening of lactic acid bacteria isolated from fermented table olives with probiotic potential. Food Research International 50, 135–142.

Benincasa, C., S. Muccilli, M. Amenta, E. Perri, & F.V. Romeo. (2015) Phenolic trend and hygienic quality of green table olives fermented with *Lactobacillus plantarum* starter culture. Food Chemistry 186, 271–276.

Bevilaqua, A., C. Altieri, M.R. Corbo, M. Sinigaglia, & L.I.I. Ouoba. (2010) Characterization of lactic acid bacteria isolated from Italian Bella di Cerignola table olives: selection of potential multifunctional starter cultures. Journal of Food Science 75, M536–M544.

Bianco, A., & N. Uccella. (2000) Biophenolic components of olives. Food Research International 33, 475–485.

Bishayee, A., A. Mandal, R.J. Thoppil, A.S. Darvesh, & D. Bhatia. (2013) Chemopreventive effect of a novel oleanane triterpenoid in a chemically induced rodent model of breast cancer. International Journal of Cancer 133, 1054–1063.

Blana, V.A., A. Grounta, C.C. Tassou, G.-J.E. Nychas, & E.Z. Panagou. (2014) Inoculated fermentation of green olives with potential probiotic *Lactobacillus pentosus* and *Lactobacillus plantarum* starter cultures isolated from industrially fermented olives. Food Microbiology 38, 208–218.

Blekas, G., C. Vassilakis, C. Harizanis, M. Tsimidou, & D.G. Boskou. (2002) Biophenols in table olives. Journal of Agricultural and Food Chemistry 50, 3688–3692.

Borcakli, M., G. Özay, I. Alperden, E. Özsan, & Y. Erdek. (1993) Changes in chemical and microbiological composition of two cultivars of olive during fermentation. Grasas y Aceites 44, 253–258.

Bostanci, M.Ö., O. Bas, & F. Bagirici. (2010) Alpha-tocopherol decreases iron-induced hippocampal and nigral neuron loss. Cellular and Molecular Neurobiology 30, 389–394.

Botta, C., T. Langerholc, A. Cencic, & L. Cocolin. (2014) *In vitro* selection and characterization of new probiotic candidates from table olive microbiota. PLoS One 9 (4), e99457.

Brenes, M., & A. de Castro. (1998) Transformation of oleuropein and its hydrolysis products during Spanish-style green olive processing. Journal of the Science of Food and Agriculture 77, 353–358.

Brenes-Balbuena, M., P. García-García, & A. Garrido-Fernandez. (1992) Phenolic compounds related to the black colour formed during the processing of ripe olives. Journal of Agricultural and Food Chemistry 40, 1192–1196.

Chandramohan, R., L. Pari, A. Rathinam, & B.A. Sheikh. (2015) Tyrosol, a phenolic compound, ameliorates hyperglycemia by regulating key enzymes of carbohydrate metabolism in streptozotocin induced diabetic rats. Chemico-Biological Interactions 229, 44–54.

Charoenprasert, S., & A. Mitchell. (2012) Factors influencing phenolic compounds in table olives (*Olea europaea*). Journal of Agricultural and Food Chemistry 60, 7081–7095.

Cuello-Garcia, C.A., Brozek, J.L., Fiocchi, A., Pawankar, R., Yepes-Nunez, J.J., Terracciano, L., Gandhi, S., Agarwal, A., Zhang, Y., & H.J. Schunemann. (2015) Probiotics for the prevention of allergy: a systematic review and meta-analysis of randomized controlled trials. Journal of Allergy and Clinical Immunology. DOI:10.1016/j.jaci.2015.04.031

De Bellis, P., F. Valerio, A. Sisto, S.L. Lonigro, & P. Lavermicocca. (2010) Probiotic table olives: Microbial populations adhering on olive surface in fermentation sets inoculated with the probiotic strain *Lactobacillus paracasei* IMPC2.1 in an industrial plant. International Journal of Food Microbiology 140, 6–13.

Doulgeraki, A.I., O. Hondrodimou, V. Iliopoulos, & E.Z. Panagou. (2012) Lactic acid bacteria and yeast heterogeneity during aerobic and modified atmosphere packaging storage of natural black Conservolea olives in polyethylene pouches. Food Control 26, 49–57.

Doulgeraki, A.I., P. Pramateftaki, A.A. Argyri, G.-J.N. Nychas, C.C. Tassou, & E.Z. Panagou. (2013) Molecular characterization of lactic acid bacteria isolated from industrially fermented Greek table olives. LWT: Food Science and Technology 50, 353–356.

Faghfoori, Z., B.P. Gargari, A.S. Gharamaleki, H. Bagherpour, & A.Y. Khosroushahi. (2015) Cellular and molecular mechanisms of probiotics effects on colorectal cancer. Journal of Functional Foods 18, 463–472.

FAO/WHO. (2001) Probiotics in food. Health and nutritional properties and guidelines for evaluation. Report of joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Cordoba, Argentina, October 1–4.

Fortes, C., J.A. García-Vilas, A.R. Quesada, & M.A. Medina. (2012) Evaluation of the anti-angiogenic potential of hydroxytyrosol and tyrosol, two bio-active phenolic compounds of extra virgin olive oil, in endothelial cell cultures. Food Chemistry 134, 134–140.

Gao, X., D. Deeb, Y. Liu, A.S. Arbab, G.W. Divine, S.A. Dulchavsky, & S.C. Gautam. (2011) Prevention of prostate cancer with oleanane synthetic triterpenoid CDDO-Me in the TRAMP mouse model of prostate cancer. Cancers (Basel) 3, 3353–3369.

Garrido-Fernández, A., Fernández-Díez, M.J., & M.R. Adams. (1997) Table olives: production and processing. Chapman & Hall, London.

Gililland, J.R., & R.H. Vaughn. (1942) Characteristics of butyric acid bacteria from olives. Journal of Bacteriology 46, 315–322.

Grounta, A., V. Iliopoulos, A.I. Doulgeraki, G-J.E. Nychas, & E.Z. Panagou. (2014) Biofilm formation of *Lactobacillus pentosus* B281 and *Pichia membranifaciens* M3A during fermentation on naturally black Conservolea olives. In: Proceedings of the 24th International ICFMH conference, Nantes, France, 179.

Hagiwara, K., T. Goto, M. Araki, H. Miyazaki, & H. Hagiwara. (2011) Olive polyphenol hydroxytyrosol prevents bone loss. European Journal of Pharmacology 662, 78–84.

Halper, J., L.S. Leshin, & W.I. Li. (2003) Wound healing and angiogenic properties of supernatants from *Lactobacillus* cultures. Experimental Biology and Medicine (Meywood) 228, 1329–1337.

Harris, L.J. (1998) The microbiology of vegetable fermentation. In: Microbiology of fermented food. ed. B.J.B. Wood. Springer US, New York, 45–72.
Hassapidou, M.N., G.D. Balatsouras, & A.G. Manoukas. (1994) Effect of processing upon the tocopherol and tocotrienol composition of table olives. Food Chemistry 50, 111–114.
Hassen, I., H. Casabianca, & K. Hosni. (2014) Biological activities of the natural antioxidant oleuropein: Exceeding the expectation: a mini review. Journal of Functional Foods. http://dx.doi.org./10.1016/j.jff.2014.09.001
Hurtado, A., C. Reguant, A. Bordons, & N. Rozès. (2012) Lactic acid bacteria from fermented table olives. Food Microbiology 31, 1–8.
Ilavarasi, K., P.V. Kiruthiga, S.K. Pandian, & K.P. Devi. (2011) Hydroxytyrosol, the phenolic compound of olive oil protects human PBMC against oxidative stress and DNA damage mediated by 2,3,7,8-TCDD. Chemosphere 84, 888–893.
International Olive Council (IOC). (2004) Trade standard applying to table olives. COI/OT/NC no. 1, Madrid, Spain. http://www.internationaloliveoil.org./estaticos/view/222-standards
International Olive Council (IOC). (2014) World table olive figures. http://www.internationaloliveoil.org/estaticos/view/132-world-table-olive-figures
Ishimwe, N., E.B. Daliri, B.H. Lee, F. Fang, & G. Du. (2015) The perspective on cholesterol-lowering mechanisms of probiotics. Molecular Nutrition and Food Research 59, 94–105.
Janakiram, N.B., C. Indranie, S.V. Malisetty, P. Jagan, V.E. Steele, & C.V. Rao. (2008) Chemoprevention of colon carcinogenesis by oleanolic acid and its analog in male F344 rats and modulation of COX-2 and apoptosis in human colon HT-29 cancer cells. Pharmaceutical Research 25, 2151–2157.
Jiang, Q., I. Elson-Schwab, C. Courtemanche, & B.N. Ames. (2000) c-Tocopherol and its major metabolite, in contrast to a-tocopherol, inhibit cyclooxygenase activity in macrophages and epithelial cells. Proceedings of the National Academy of Sciences of the USA 97,11494–11499.
Juan, M.E., M. Planas, V. Ruiz-Gutierrez, H. Daniel, & U. Wenzel. (2008) Antiproliferative and apoptosis-inducing effects of maslinic and oleanolic acids, two pentacyclic triterpenes from olives, on HT-29 colon cancer cells. British Journal of Nutrition 100, 36–43.
Kailis, S., & D. Harris. (2007) Producing table olives. Landlinks Press, Collingwood, VIC, Australia.
Kanavouras, A, M. Gazouli, L. Tzouvelekis, & C. Petrakis. (2005) Evaluation of black olives in different brines. Grasas y Aceites 56, 106–115.
Kies, A.K. (2014) 9-Authorised EU health claims related to the management of lactose intolerance: reduced lactose content, dietary lactase supplements and live yogurt cultures. In: Foods, nutrients and food ingredients with authorised EU health claims, ed. M.J. Sadler. Woodhead Publishing, Cambridge, 177–211.
Kountouri, A.M., A. Mylona, A.C. Kaliora, & N.K. Andrikopoulos. (2007) Bioavailability of the phenolic compounds of the fruits (drupes) of *Olea europea* (olives): impact on plasmantioxidant status in humans. Phytomedicine 14, 659–667.
Lalas, S., V. Athanasiadis, O. Gortzi, M. Bounitsi, I. Giovanoudis, J. Tsaknis, & F. Bogiatzis. (2011) Enrichment of table olives with polyphenols extracted from olive leaves. Food Chemistry 127, 1521–1525.
Lanza, B. (2013) Abnormal fermentations in table-olive processing: microbial origin and sensory evaluation. Frontiers in Microbiology 4, 1–7.
Lanza, B., & M.G. Di Serio. (2015) SEM characterization of olives (*Olea europea* L.) fruit epicuticular waxes and epicarp. Scientia Horticulturae 191, 49–56.
Lanza, B., M.G. Di Serio, E. Iannucci, F. Russi, & P. Marfisi. (2010) Nutritional, textural and sensorial characterisation of Italian table olives (*Olea europea* L. cv. "Intosso d' Abruzzo"). International Journal of Food Science and Technology 45, 67–74.
Lavermicocca, P., F. Valerio, S.L. Lonigro, M. De Angelis, L. Morelli, M.L. Callegari, C.G. Rizzello, & A. Visconti. (2005) Study of adhesion and survival of lactobacilli and bifidobacteria on table olives with the aim of formulating a new probiotic food. Applied and Environmental Microbiology 71, 4233–4240.
Lin, C.C., C.Y. Huang, M.C. Mong, C.Y. Chan, & M.C. Yin. (2011) Antiangiogenic potential of three triterpenic acids in human liver cancer cells. Journal of Agricultural and Food Chemistry 59, 755–762.
Lin, C., S. Yan, & M. Yin. (2014) Inhibitory effects of maslinic acid upon human esophagus, stomach and pancreatic cancer cells. Journal of Functional Foods 11, 581–588.
Liu, J., H. Sun, W. Duan, D. Mu, & L. Zhang. (2007) Maslinic acid reduces blood glucose in KK–A^y mice. Biological and Pharmaceutical Bulletin 30, 2075–2078.
Mabandla, M.V., M. Nyoka, & W.M.U. Daniels. (2015) Early use of oleanolic acid provides protection against 6-hydroxydopamine induced dopamine neurodegeneration. Brain Research 1622, 64–71.
Manoukas, A.G., B. Mazomenos, & M.A. Patrinou. (1973) Amino acid compositions of three cultivars of olive fruit. Journal of Agricultural and Food Chemistry 21, 215–217.
Mantzouridou, F., & M.Z. Tsimidou. (2011) Microbiological quality and biophenol content of hot air-dried Thassos cv. table olives upon storage. European Journal of Lipid Science and Technology 113, 786–795.

Nortje, B., & R.H. Vaughn. (1953) The pectolytic activity of species of the genus *Bacillus*: qualitative studies with *Bacillus subtilis* and *Bacillus pumilus* in relation to the softening of olives and pickles. Journal of Food Science 18, 57–59.

Nychas, G.-J. E., E.Z. Panagou, M.L. Parker, K.W. Waldron, & C.C. Tassou. (2002) Microbial colonization of naturally black olives during fermentation and associated biochemical activities in the cover brine. Letters in Applied Microbiology 34, 173–177.

Özay, G., & M. Borcakli. (1996) Effect of brine replacement and salt concentration on the fermentation of naturally black olives. Food Research International 28, 553–559.

Panagou, E.Z. (2006) Greek dry-salted olives: monitoring the dry-salting process and subsequent physico-chemical and microbiological profile during storage under different packing conditions at 4 and 20 °C. LWT 39: 322–329.

Panagou, E.Z., U. Schillinger, C.M.A.P. Franz, & G-J.N. Nychas. (2008) Microbiological and biochemical profile of cv. Conservolea naturally black olives during controlled fermentation with selected strains of lactic acid bacteria. Food Microbiology 25,348–358.

Panagou, E.Z., C.C. Tassou, & K.Z. Katsaboxakis. (2002) Microbiological, physicochemical and organoleptic changes in dry-salted olives of Thassos cultivar stored under different modified atmospheres at 4 and 20 °C. International Journal of Food Science and Technology 37, 635–641.

Peres, C.M., M. Alves, A. Hernandez-Mendoza, L. Moreira, S. Silva, M.R. Bronze, L. Vilas-Boas, C. Peres, & F. Xavier Malcata. (2014) Novel isolates of lactobacilli from fermented Portugese olive as potential probiotics. LWT – Food Science and Technology 59, 234–246.

Puel, C., J. Mardon, S. Kati-Coulibaly, M.-J. Davicco, P. Lebecque, C. Obled, E. Rock, M.-N. Horcajada, A. Agalias, L.A. Skaltsounis, & V. Coxam. (2007) Black Lucques olives prevented bone loss caused by ovariectomy and talc granulomatosis in rats. British Journal of Nutrition 97, 1012–1020.

Ramírez, E., P. García-García, A. de Castro, C. Romero, & M. Brenes. (2013) Debittering of black dry-salted olives. European Journal of Lipid Science and Technology 115, 1319–1324.

Reid, G., & A. Bruce. (2006) Probiotics to prevent urinary tract infections: the rationale and evidence. World Journal of Urology 24, 28–32.

Rice-Evans, C.A., N. J Miller, & G. Papanga. (1997) Antioxidant properties of phenolic compounds. Trends in plant science 2, 152–159.

Rodríguez-Gómez, F., J. Bautista-Gallego, F.N. Arroyo-López, V. Romero-Gil, R. Jiménez-Díaz, A. Garrido-Fernández, & P. García-García. (2013) Table olive fermentation with multifunctional *Lactobacillus pentosus* strains. Food Control 34, 96–105.

Romero, C., M. Brenes, K. Yousfi, P. García, A. García, & A. Garrido. (2004) Effect of cultivar and processing method on the contents of polyphenols in table olives. Journal of Agricultural and Food Chemistry 52, 479–484.

Romero, C., A. García, E. Medina, M. V. Ruíz-Méndez, A. De Castro, & M. Brenes. (2010) Triterpenic acids in table olives. Food Chemistry 118, 670–674.

Serban, D.E. (2014) Gastrointestinal cancers: influence of gut microbiota, probiotics and prebiotics. Cancer Letters 345, 258–270.

Shanmugam, M.K., X. Dai, A.P. Kumar, B.K.H. Tan, G. Sethi, & A. Bishayee. (2014) Oleanolic acid and its synthetic derivatives for the prevention and therapy of cancer: preclinical and clinical evidence. Cancer Letters 346, 206–216.

Silva, S., B. Sepodes, J. Rocha, R. Direito, A. Fernandes, D. Brites, M. Freitas, E. Fernandes, M.R. Bronze, & M.E. Figueira. (2015) Protective effects of hydroxytyrosol-supplemented refined olive oil in animal models of acute inflammation and rheumatoid arthritis. Journal of Nutritional Biochemistry 26, 360–368.

Singh, U., S. Devaraj, & I. Jialal. (2005) Vitamin E, oxidative stress, and inflammation. Annual Review of Nutrition 25, 151–174.

Taormina, P.J. (2010) Implications of salt and sodium reduction on microbial food safety. Critical Reviews in Food Science and Nutrition 50, 209–227.

Tassou, C.C., E.Z. Panagou, & K.Z. Katsaboxakis. (2002) Microbiological and physicochemical changes of naturally black olives fermented at different temperatures and NaCl levels in the brines. Food Microbiology 19, 605–615.

Terés, S., G. Barceló-Coblijn, M. Benet, R. Álvarez, R. Bressani, J. E. Halver, & P. V. Escribá. (2008) Oleic acid content is responsible for the reduction in blood pressure induced by olive oil. PNAS 105, 13811–13816.

Tsai, S.-J., & M.C. Yin. (2008) Antioxidative and anti-inflammatory protection of oleanolic acid and ursolic acid in PC12 cells. Journal of Food Science 73, H174–H178.

Vinha, A. F., F. Ferreres, B. M. Silva, P. Valentão, A. Gonçalves, J. A. Pereira, M. B. Oliveira, R. M. Seabra, & P. B. Andrade. (2005) Phenolic profiles of Portuguese olive fruits (*Olea europaea* L.): Influences of cultivar and geographical origin. Food Chemistry 89, 561–568.

Vujic, G., A.J. Knez, V.D. Stefanovic, & V.K. Vrbanovic. (2013) Efficacy of orally applied probiotic capsules for bacterial vaginosis and other vaginal infections: a double blind, randomnized, placebo controlled study. European Journal of Obstetrics and Gynecology and Reproductive Biology 168, 75–79.

Wang, X., H. Bai, X. Zhang, J. Liu, P. Cao, N. Liao, W. Zhang, Z. Wang, & C. Hai. (2013) Inhibitory effect of oleanolic acid on hepatocellular carcinoma via ERK-p53-mediated cell cycle arrest and mitochondrial-dependent apoptosis. Carcinogenesis 34, 1323–1330.

Weinstein, S.J., M.E. Wright, P. Pietinen, I. King, c. Tan, P.R. Taylor, J. Virtamo, & D. Albanes. (2005) Serum α-tocopherol and γ-tocopherol in relation to prostate cancer risk in a prospective study. Journal of the National Cancer Institute 97, 396–399.

World Health Organization (WHO). (2007) Reducing salt intake in populations. Report of a WHO forum and technical meeting. Geneva, Switzerland, WHO Document Publication Services.

Zoidou, E., E. Melliou, E. Gikas, A. Tsarbopoulos, P. Magiatis, & A.-L. Skaltsounis. (2010) Identification of Throuba Thassos, a traditional Greek table olive variety, as a nutritional rich source of oleuropein. Journal of Agricultural and Food Chemistry 58, 46–50.

17 Food hazards and quality control in table olive processing with a special reference to functional compounds

Mohamed Rahmani

17.1 Introduction

The olive tree (*Olea europaea* L.) belongs to the family *Oleaceae* and is native to the Mediterranean basin and parts of Asia. Nowadays, it is widely cultivated in many other parts of the world for production of olive oil and table olives. The Trade Standard Applying to Table Olives (IOC, 2004) defines table olives as "the product obtained from suitable olive cultivars, processed to remove their natural bitterness, and preserved (by natural fermentation, heat treatment or preservatives) with or without brine until consumption."

Table olives are the most important fermented food worldwide. In the 2013–2014 season, world table olive production reached 2,595,500 tons, a good part of which (28.9%) was produced in the European Union, particularly in Spain, Italy, Greece (715,500 tons), and Turkey (410,000 tons) (IOC, 2014). A renewed interest has been shown in the last decade for the functional properties of table olives in relation to optimal nutrition, which resulted in increased consumption. Worldwide, the consumption of table olives went from 1,831,500 tons in 2004–2005 to 2,540,000 tons in 2013–2014, with an average yearly increase of 3.9% (IOC, 2014).

Processed table olives, as associated with the Mediterranean diet, are highly nutritious food that provides monounsaturated and essential fatty acids, fiber, vitamins, minerals, phytosterols, triterpenic acids, and squalene. They are a good source of phenolic compounds (1–3% of fresh pulp weight), whose most abundant part is oleuropein, which is responsible for the characteristic bitter taste of olive fruits (Marsilio, 2006; Therios, 2009). During maturation, oleuropein is partially converted into dimethyloleuropein, which becomes the major phenol in black olives (Therios, 2009).

Several studies have demonstrated the beneficial effects of oleuropein for its hypotensive, hypoglycemic, antibacterial, and antiviral activities (Gonzalez *et al.*, 1992; Hansen *et al.*, 1996; Manna *et al.*, 2002) and for its antioxidant activity and its capacity to lower blood pressure (Zarzuelo *et al.*, 1991; Pereira *et al.*, 2006). Moreover, table olives are a potential source of lactobacilli probiotics, which exhibit beneficial effects on gut health. Emerging evidence is supporting their role as important modulators of immune system responses, including inflammation at mucosal surfaces (Lescheid, 2014; Chávez-Tapia *et al.*, 2015). The use of table olives as a source of probiotics may be preferable for those who are unable to eat dairy due to intolerances or those who require a low-cholesterol diet.

However, and since the main purpose of table olive processing is the removal of bitterness related to oleuropein, the concentrations of the latter and its hydrolysis products in table olives are usually very low. Hence, innovative procedures for table olive processing might help increase contents of oleuropein and its hydrolysis products, without a prejudice for table olives' palatability. The table olive industry needs also to innovate itself in order to meet the consumers' needs in terms of quality and food safety. In this context, this chapter underlines the legal requirements of the table olive sector, and the effects of processing on quality as related to functional compounds and food safety of the products.

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17.1.1 Legal requirements of the table olive sector

17.1.1.1 Layout of the premises and processing equipment

Processing units need to have satisfying requirements for the premises and equipment to be used for table olives' production and packaging. The design of the premises should differentiate clearly between each work area and comply with the principle of forward workflow, to prevent cross-contamination during the different operations.

The equipment required for table olive production include sorters, graders, tanks, pumps, and packaging units. The contact surfaces of equipment employed should be suitable food-grade materials.

17.1.1.2 Quality management system

Product safety is an extremely important issue throughout the food chain. General implementation of procedures based on the HACCP principles has been adopted at the international level as the premier food safety system. Its application in the food sector is required in many countries, and it serves as the foundation of the World Health Organization's Codex Alimentarius Commission's General Principles of Food Hygiene (CAC/RCP 1-1969, as amended and revised; Codex Alimentarius, 2013).

17.1.1.3 Environmental management

The stages of debittering and subsequent washing produce the largest fraction of wastewater in table olive processing, giving a rise to uploads with high COD and BOD_5 that may reach 40 and 20 g/l, respectively (Niaounakis & Halvadakis, 2006).

In accordance with the environmental management standard ISO 14000, processing units must identify and monitor their relevant environmental aspects, develop an environmental policy and program, and improve performance.

17.1.1.4 Product compliance with standards and labels

17.1.1.4.1 Standard quality

The IOC and Codex Standards for table olives define the types, trade preparations, quality factors, and other properties. These standards apply to the fruit of the cultivated olive tree (*Olea europaea sativa*) that has been suitably treated or processed and that is offered for direct consumption as table olives. They also cover olives packed in bulk containers that are intended for repacking into consumer-size containers.

Methods of analysis and control have been set in these standards to prevent fraud and to ensure that table olives meet the requirements for their designation.

17.1.1.4.2 Specific quality

Consumers consider designation of origin labels to have particular characteristics of quality, which are due to the soil/climatic conditions, and specific production methods, among others. The European Union, with the Regulation No. 2081/1992 (replaced by Regulation No. 510/2006), acknowledges and establishes a particular safety regime for products that have specific quality characteristics due to their place of origin (EU, 1992, 2006). Other countries have also specific legislation for protected geographical indications.

Worldwide and by 2010, 21 kinds of table olives and 105 different olive oils protected as geographical indications (GIs) were identified mainly in the European Union (19 table olives and 101 olive oils) (IOC, 2010). Based on high phenol content, natural methods of production, low salt content, and packaging under vacuum, the "Protected Designations of Origin" are vehicles for nutritious and healthy table olives.

17.2 Table olive processing techniques

17.2.1 Raw materials (fresh olives)

Fruits are selected according to their volume, maturation, shape, flesh-to-stone ratio, fine flesh taste, firmness, and ease of detachment of flesh from the stone. Several factors affect the phenolic content of table olives. Among these factors, the role of olive cultivar, stage of maturation (green, turning-color, or black), and processing techniques should be emphasized.

17.2.1.1 Olive cultivar

Although oleuropein and hydroxytyrosol are the major phenolic compounds in green olive cultivars, their content varies greatly among cultivars since the expression of phenolic compounds in olive fruit is predominantly driven by genetic factors. In Portuguese cultivars, for example, the ranges reported for oleuropein and hydroxyrosol were 0.388–21.681 and 1.477–15.763 g/kg (dry weight), respectively (Charoenprasert & Mitchell, 2012).

17.2.1.2 Stage of maturation

Three phases are usually distinguished during the development of olive fruit (Soler-Rivas *et al.*, 2000): a growth phase characterized by accumulation of oleuropein, a green maturation phase characterized by a reduction in the levels of chlorophylls and oleuropein, and a black maturation phase coinciding with the appearance of anthocyanins and a continued fall in oleuropein concentrations.

17.2.2 Processing techniques

Raw olives are bitter and contain small amounts of sugar (2.6–6.0%) compared with other drupes (12% or more). Therefore, they require processing in order to become suitable for consumption. Some olive cultivars represent an exception to this general rule, as illustrated by the Greek 'Throuba Thassos' cultivar, which sweetens on the tree as it ripens (Muccilli, 2010).

Oleuropein is the bitterness-causing chemical component that also has marked bactericidal properties, and its elimination is therefore required to ease subsequent fermentation and make the end product palatable (Marsilio *et al.*, 1996). This secoiridoid is water-soluble, and its elimination is carried out by lye treatment with repeated water rinses, by drying and curing with dry salt, or by fermentation in brine.

The specific processing conditions vary according to traditions of the producing country, the selected degree of fruit maturity during harvest, and the consumer preference. As a result, different kinds of olives are marketed to meet specific consumer demands and can be classified according to olive types, trade treatments, and styles. A complete definition of all trade preparations can be found in the "Trade Standard Applying to Table Olives" (IOC, 2004).

The important commercial types in the international market are: (a) Spanish-style pickled green olives, (b) California-style black ripe olives, (c) Greek naturally ripe olives, and (d) dry-salted-style olives.

17.2.2.1 Spanish-style green olives in brine

The fruits are harvested at the green maturation stage, and the commercial harvest ceases when the skin color becomes straw. The bitterness of the olive is removed by using food-grade sodium hydroxide treatment (2–3%, w/v), which penetrates the olive flesh to destroy the glucoside oleuropein. The penetration of the lye into the flesh is considered sufficient when the chemical reaches about two-thirds of the distance from the exterior to the pit. Lye treatment not only has the effect of removing bitterness but also markedly increases skin permeability, which, in turn, favors the release of nutrients. Excess of alkali is eliminated using one or two water washes, and the fruits are immediately immersed in brines (8–12%, w/v, salt concentration), where they undergo a lactic acid fermentation. Inoculation with the *L. plantarum* starter culture leads to a faster pH decrease in green table olive processing compared to the spontaneous one, and this may help to reduce the risk of spoilage during the first days of fermentation.

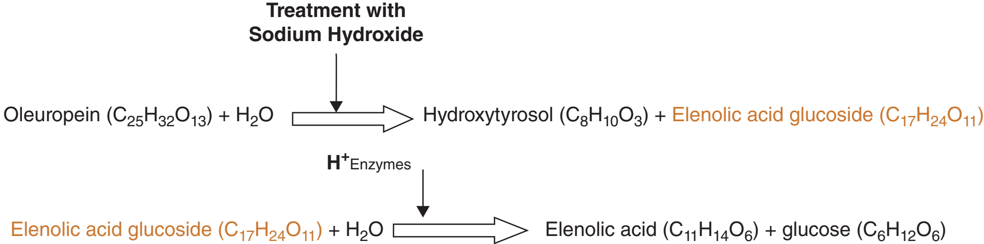

Figure 17.1 Reactions of oleuropein hydrolysis by lye treatment.

As portrayed in Figure 17.1, lye treatment induces oleuropein hydrolysis, which produces elenolic acid and hydroxytyrosol. Commercially available table olive samples contain hydroxytyrosol as the prevailing phenolic compound (Pereira *et al.*, 2006).

By the end of fermentation, olives must have reached the proper pH (≤4.3) so as to permit the packing and consumption of the product. It is essential that sufficient acid is produced to ensure that pathogenic bacteria do not grow. If the final pH value does not comply with the minimum requirements (pH ≤ 4.3) of the trade standard applying to table olives (Codex, 1981), table olives have to be acidified to assure the stability and safety of the product. After the appropriate fermentation, the fruits are stored, graded, sorted, and conditioned (pitted, stuffed, etc.) before packaging and pasteurization.

17.2.2.2 California-style black olives in brine

California-style black olives can be made from either fresh or stored green and turning olives. Storage is carried in brine or in an acidulant solution containing lactic acid, acetic acid, sodium benzoate, and potassium sorbate. No fermentation occurs in this system, unlike in brine storage. Darkening of olives takes place throughout the year, according to demand. It consists of several treatments with dilute NaOH solutions and water washes between them, accompanied with air bubbling to darken olives progressively due to oxidation of ortho-diphenols (Brenes *et al.*, 2004). Usually, three to five lye treatments are generally used; during the first alkaline treatment, the lye penetrates only the skin, while the remaining lyes are permitted to penetrate the pulp progressively until the last one reaches the stone. Once the olives obtain the proper color ring around the outer surface, this is fixed by immersion in an iron lactate or gluconate solution. These olives are usually packed in cans, using light brine, with heat sterilization.

Phenolic compounds are lost during the California-style processing, partly during olive conservation in brine (oleuropein), and partly during the darkening process (hydroxytyrosol) in which oxidation of o-diphenols plays a key role in the color development (Marsilio *et al.*, 2001; Brenes *et al.*, 2004).

17.2.2.3 Greek-style natural black olives in brine

The fruits are placed directly into the brine (8–10%, w/v), without a lye treatment, and oleuropein removal is slow and only partial. In this process, the elimination of the bitterness in olives is achieved enzymatically (ß-glucosidase + esterase), causing the breakdown of oleuropein into elenolic acid and hydroxytyrosol, and equilibrium is reached in 8–12 months. As no lye treatment is used in processing, the olives exhibit a somewhat bitter flavor. When fermentation is completed (pH < 4.6), the olives are graded according to size and color and packaged in fresh brine.

Commercial types of Greek table olives were examined by Zoidou *et al.* (2010) for their polyphenol contents. Hydroxytyrosol was found in all examined table olives, with concentrations ranging from 1.0 to 2.0 mg/fruit. Oleuropein was either absent or present in small quantities.

17.2.2.4 Dry-salted black olives

Although production of this kind of table olives is limited worldwide, it makes a substantial volume in some countries like Turkey, Greece, and North African countries. In Morocco, this preparation accounts for

nearly 20% of the total table olive production, and efforts are being carried out to label these table olives. The following describes the different steps in the Moroccan method used for this preparation.

Olives are harvested when they are fully ripe. In the traditional process, olives are directly treated with dry salt. In the industrial process, olives are subjected to a brief lye treatment (1–3%, w/v) before being drained in air for 2 to 3 days. The bitterness is taken away by the lye treatment, which also serves the purpose of fixing the black color by air oxidation of the olives.

After draining, the olives are packed between layers of dry salt in plastic barrels, with a ratio of olive fruit to dry salt of about 85:15 (%, w/w). Barrels are then turned over longitudinally two to three times a week. Liquid is drawn out of the olives by osmosis and is not allowed to drain away, playing a key role in the preservation of the product. Salt, which acts as a preservative, is also taken up by the olive.

Processing time is around four to six weeks, and the olives are best eaten within three months of processing. The resulting olives are wrinkled in appearance and have a salty bittersweet taste. Addition of olive oil enhances the flavor of the olive; however, oxidation of the oil can give a rancid taste to the olives. Owing to the high salt concentration of the product and its low water activity (0.7–0.9), the prevailing microflora is mainly composed of yeasts and molds (Asehraou *et al.*, 1992; Degirmencioglu *et al.*, 2014). They occur in higher numbers in olives that have undergone an initial lye treatment. The removal of the naturally occurring polyphenols from the olives by the lye reduces the natural inhibiting factors that resist the growth of yeasts and molds.

Natural dry-salted olives, without any lye treatment, retain some oleuropein. According to Zoidou *et al.* (2010), the Greek 'Throumba Thassos' cultivar processed by dry salt showed a high concentration of oleuropein (1.2 mg/fruit), which makes it a nutritionally rich source of this phenolic compound.

17.3 New trends in table olive processing and quality control, with a special reference to functional products

Improvement of the functional properties of table olives requires a new approach to control processing and storage. The following relates to innovative trials aiming to preserve oleuropein and enhance probiotic flora in table olives.

17.3.1 Debittering methods

Many trials were carried out to find substitutes for the chemical debittering of olives, such as preserving the phenolic fraction and/or reducing time of debittering, and the volume and pollutant charge of wastewater.

- Postharvest storage of olives under a CO_2 atmosphere and its effects on table olive quality were investigated by Dortoglou *et al.* (2006). After 12 days of storage, development of color and flavor and reduction in olive bitterness were noted. According to the authors, this method appears to provide natural debittering and needs further investigation for the development of table olive processing that will enable fast olive debittering with minimal environmental impact.
- Debittering with water instead of alkali helps preserve biophenols in the finished products. After 60 days of fermentation, the rates of biophenol degradation varied between 61.6 and 84.0% in water-debittered olives, whereas these rates varied between 98.7 and 99.1% in the alkali-debittered olives (Valencic *et al.*, 2010).
- Debittering by oleuropeinolytic *Lactobacillus* strains prevents the loss of fermentable material during the washing step of lye treatment, enhances the initial growth of lactic acid bacteria, and results in a favored end product with high sensory quality. It also contributes to reducing substantially the amount of wastewater during table olive processing (Idrissi Janati *et al.*, 2004). The proposed mechanism for enzymatic debittering is shown in Figure 17.2.

According to tolerance to olive leaf extract (up to 10%, w/v) and oleuropein (1%, w/v), *L. plantarum*, *L. pentosus*, and *P. pentosaceuscan* can be used as starters in the controlled fermentation of non-debittered green olives (Ciafardini *et al.*, 1994; Ghabbour *et al.*, 2011). *L. plantarum* showed the highest percentage of

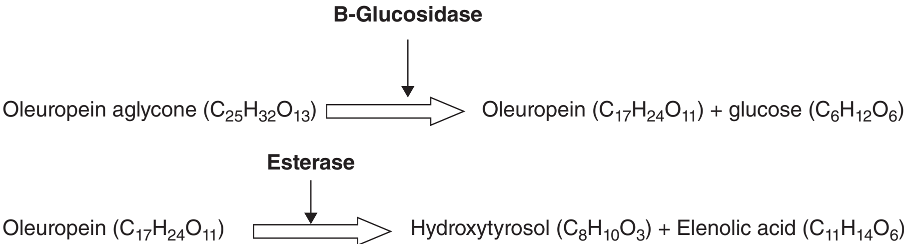

Figure 17.2 Mechanism of oleuropein hydrolysis by enzymatic method.

strains producing β-glucosidase and degrading oleuropein as the sole carbon source, followed by *L. pentosus*, *P. pentosaceus*, and *L. brevis* (Ghabbour *et al.*, 2011). This biotechnological method could replace the use of lye in table olive processing.

17.3.2 Enrichment of table olives with polyphenols

Lalas *et al.* (2011) investigated the enrichment of table olives of 'Kalamon' cultivar with two polyphenols (oleuropein and hydroxytyrosol) from olive leaves. As indicated by their results, treated table olives showed an increase of 457% in the case of oleuropein and 109% in the case of hydroxytyrosol. Such increases in polyphenol content did not affect the overall acceptability of treated olives.

17.3.3 Selection of starter cultures with a probiotic activity

Green table olives, produced according to the Spanish style, are obtained by a fermentation that can be carried out by spontaneous microflora, even if the use of starter cultures is desirable in order to obtain a more controlled process. In this regard, the selection of *Lactobacillus* strains with dual roles of starter and probiotic culture has been considered (De Bellis *et al.*, 2010; Rodríguez-Gómez *et al.*, 2014; Argyri *et al.*, 2015). The approach includes selection of the strain on the basis of its probiotic properties, molecular characterization, compatibility with the table olive environment, and efficacy as starter. *In fine*, the objective is to prepare table olives containing probiotic bacteria in adequate amounts to improve consumers' health, without altering the quality characteristics of fermented olives.

Results indicated that the human strain *L. paracasei* IMPkC2.1 and *L. pentosus* TOMC-LAB2 can be considered as examples of strains used in the dual roles of starter and probiotic culture, which allowed the control of fermentation processes and the realization of a healthy product (De Bellis *et al.*, 2010; Rodríguez-Gómez *et al.*, 2014).

17.4 Food safety requirements for table olives

Processing of table olives should be conducted under good sanitary practices in order to maintain all ingredients and all necessary chemical and microbiological standards. Hazard and Critical Control Point (HACCP) analysis is a compulsory system used by the table olive sector to identify and manage potential hazards during production. The following seven principles must be applied to develop a HACCP plan:

1. Conduct a hazard analysis.
2. Identify the critical control points in the process.
3. Establish critical limits for preventive measures associated with each identifiable control point.
4. Establish critical control point monitoring requirements.
5. Establish corrective actions to be taken when monitoring indicates that critical limits are not met.
6. Establish effective record-keeping procedures.
7. Establish procedures for identifying that the HACCP system is working correctly.

17.4.1 Food hazards in table olives

As reported from market surveys in Spain (López-López *et al.*, 2004), Italy (Caggia *et al.*, 2004; Franzetti *et al.*, 2011; Tofalo *et al.*, 2012), Portugal (Pereira *et al.*, 2008), and Greece (Panagou *et al.*, 2006), proper fermentation conditions together with good hygiene practices during and after processing did not allow the presence of pathogenic bacteria on olive. Inadequate processing and faulty hygienic conditions might generate health hazards for the consumer, as exemplified in this section.

17.4.1.1 Spanish-style green olives in brine

Although green olives are generally fermented foods, inadequate fermentation and/or processing may result in a product with a pH above 4.6, or may allow the growth of microorganisms that raise the pH to a level that is conducive to the growth of pathogenic microorganisms. Therefore, green olives with a pH higher than 4.6 would be a low-acid canned food and would represent a potential health hazard if they are not acidified or properly thermally processed. In such a case, the product can harbor pathogenic bacteria such as *Escherichia coli*, *Yersinia*, *L. monocytogenes*, *Bacillus cereus*, and *C. botulinum*. Formation of biogenic amines (putrescine) might take place post fermentation, and might be related to *zapateria* spoilage (Medina-Pradas & Arroyo-López, 2015).

17.4.1.2 California-style black olives in brine

In case of insufficient sterilization, *C. botulinum* can develop in such products, with the concomitant production of botulin (Medina-Pradas & Arroyo-López, 2015). Acrylamide formation has been reported in these products, probably generating from higher temperatures used for sterilization. However, no clear information is available about its mechanism of formation (Casado & Montano, 2008; Charoenprasert & Mitchell, 2014).

17.4.1.3 Greek-style natural black olives in brine

The presence of biogenic amines (cadaverine and tyramine) has been reported in these products by Tofalo *et al.* (2012). Pathogenic bacteria were reported in these products and included *Staphylococcus* sp., *Yersinia*, *E. coli*, and *L. monocytogenes* (Medina-Pradas & Arroyo-López, 2015).

17.4.1.4 Dry-salted black olives

A faulty drying process applied to black table olives, resulting from insufficient dry salt concentration, may not prevent mold growth, and consequently the subsequent amount of mycotoxins may alter the safety of the product. According to the Codex Standard for table olives, the minimum amount of dry salt to be added is 8% (w/w).

17.4.2 Preventing food hazards in table olives

Table olives with health risks should be withdrawn from the market. Traceability plays an important role in consumer safety by allowing speedy and targeted recalls and withdrawal and answering the requirements of regulations. The processing units should draw up a recall program for the withdrawal from the market of any lots showing health or quality defects during distribution. This program will be based on the information relating to the traceability of the lot. The processing units should also keep and make available, upon request, any health or hygiene complaints concerning its products.

To reduce the risk of foodborne illness and spoilage, good practices in agriculture (GAP), hygiene (GHP), and manufacturing (GMP) should be well adopted by table olive processing units. Table olive safety should be monitored through physicochemical, microbiological, and heat treatment parameters, as detailed in this section.

Table 17.1 Salt and pH levels of brines and packing solutions for trade preparations of table olives.

Type and preparation	Minimum sodium chloride content	Maximum pH limit
Treated olives	5.0%	4.3
Natural olives	6.0%	4.3
Pasteurized treated and natural olives	GMP	4.3
Dehydrated and/or shriveled olives	8.0%	GMP
Darkened by oxidation with alkaline treatment and green ripe olives	GMP	GMP

Source: FICOPAM (2016).
GMP = Good manufacturing practice.

17.4.2.1 Physicochemical parameters

The salt and pH levels stipulated by the Codex Alimentarius (2013) for different table olive products are given in Table 17.1. Control and verification systems should be employed in order to guarantee a safe and hygienic product. A brief analysis of these requirements indicates that the maximum pH limit for treated and natural olives, whether pasteurized or not, is 4.3. The difference lies in the minimum salt content of the brine, which is higher for natural olives (6%). In dry-salted black olives, the minimal concentration of dry salt in olives is 8% (w/w).

17.4.2.2 Microbiological parameters

No official microbiological criteria for table olives are available. However, the Standards of the Codex Alimentarius (2013) prescribe the minimum requirements related to hygiene for table olives. The final product shall be free from microorganisms and parasites in amounts that may represent a hazard to health, and it shall not contain any substance originating from microorganisms in amounts that may represent a hazard to health (Pereira *et al.*, 2008). Some countries, like France and Australia, have adopted national microbiological criteria for table olives.

17.4.2.3 Heat treatments

Pasteurized olives: The cumulative lethal rate during heat processes performed at temperatures below 100 °C should be greater than 15 pasteurization units. Propionic bacteria shall be considered as the reference microorganisms for table olives, for which the equation of the thermal death time is defined by a reference temperature equal to 62.4 °C and a *z* curve of 5.25 (IOC, 2004).

Sterilized olives: These shall have received a processing treatment sufficient in both time and temperature to destroy spores of *C. botulinum*. Cumulative sterility value must be greater than 15 sterilizing units, when the reference temperature is fixed at 121 °C and the *z* curve of the thermal death time according to the temperature is 10 °C (IOC, 2004).

17.5 Conclusion

Table olive consumption tends to portray a worldwide increase, due to beneficial impacts on health of the so-called “functional compounds,” particularly phenolic compounds and probiotic flora. Among phenolic compounds, oleuropein is the main component and is responsible for the olive’s bitterness. Therefore, table olive processing has to remove oleuropein to reduce bitterness and make the end products palatable. There are four main trade preparations of table olives: green or Spanish-style green olives in brine, California-style black olives in brine, Greek naturally black olives in brine, and dry-salted olives. The lye treatment used usually for olive debittering, in the Spanish and California styles, results in high amounts of wastewater

and diminishes the nutritional value of the end products, consecutive to the loss of water-soluble components (reducing sugars, organic acids) and the degradation of oleuropein. Dry-salted olives retain the highest amounts of oleuropein, since they are not treated with lye and are not fermented in brine.

Greek-style processed table olives show a somewhat intermediate behavior, with appreciable amounts of hydroxytyrosol. Several studies have been carried out to find substitutes for the lye treatment, in order to reduce the production cost and improve the nutritional value of table olives. Use of oleuropeinolytic *Lactobacillus* strains, as starters to perform the biological elaboration of table olives with improved sensory and nutritional characteristics, is offering good perspectives.

References

Argyri, A.A., Nisiotou, A.A., Pramateftaki, P., Doulgeraki, A.I., Panagou, E.Z., & Tassou, C.C. (2015) Preservation of green table olives fermented with lactic acid bacteria with probiotic potential under modified atmosphere packaging. LWT – Food Science and Technology, 62, 783–790.

Asehraou, A., Faid, M., & Jana, M. (1992) Physico-chemical properties and the microflora of moroccan black table olives. Grasas y Aceites, 43, 130–133.

Brenes, M., Romero, C., & De Castro, A. (2004) Combined fermentation and evaporation processes for treatment of washwaters of the Spanish-style green olive processing. Journal of Chemical Technology & Biotechnology 79, 253–259.

Caggia, C., Randazzo, C.L., Salvo, M., Romeo, F., & Giudici, P. (2004) Occurrence of *Listeria monocytogenes* in green table olives. Journal of Food Protection 10, 2189–2194.

Casado, F.J., & Montaño, A. (2008) Influence of processing conditions on acrylamide content in black ripe olive. Journal of Agriculture and Food Chemistry 56, 2021–2027.

Charoenprasert, S., & Mitchell, A. (2014) Influence of California-style black ripe olive processing on the formation of acrylamide. Journal of Agriculture and Food Chemistry 62, 8716–8721.

Charoenprasert, S., & Mitchell, A. (2012) Factors influencing phenolic compounds in table olives (*Olea europaea*). Journal of Agriculture and Food Chemistry 60, 7081–7095.

Chávez-Tapia, N.C., González-Rodríguez, L., Jeong, M.S., López-Ramírez, Y., Barbero-Becerra, V., Juárez-Hernández, E., Romero-Flores, J.L., Arrese, M., Méndez-Sánchez, N., & Uribe, M. (2015) Current evidence on the use of probiotics in liver diseases. Journal of Functional Foods 17, 137–151.

Ciafardini, G., Marsilio V., Lanza, B., & Pozzi, N. (1994) Hydrolysis of oleuropein by *Lactobacillus plantarum* strains associated with olive fermentation. Applied and Environmental Microbiology 60, 4142–4147.

Codex Alimentarius. (2013) Codex standard for table olives, Codex Stan 66-1981, Rev. 1987, 2013.

De Bellis, P., Valerio, F., Sisto, A., Lonigro, S.L., & Lavermicocca, P. (2010) Probiotic table olives: microbial populations adhering on olive surface in fermentation sets inoculated with the probiotic strain *Lactobacillus paracasei* IMPC2.1 in an industrial plant. International Journal of Food Microbiology 140, 6–13.

Degirmencioglu, N., Gurbuz, O., Degirmencioglu, A., & Yildiz, S. (2014) Effect of pretreatments on microbial growth and sensory properties of dry-salted olives. Journal of Food Protection 77, 1527–1537.

Dourtoglou, V.G., Mamalos, A., & Makris, D.P. (2006) Storage of olives (*Olea europaea*) under CO_2 atmosphere: Effect on anthocyanins, phenolics, sensory attributes and in vitro antioxidant properties. Food Chemistry 99, 342–349.

European Union (EU) (1992) Council Regulation (EEC) No 2081/92 of 14 July 1992 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. EU, Brussels.

European Union (EU) (2006) Council Regulation (EC) No 510/2006 of 20 March 2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. EU, Brussels.

Fédération des Industries de la Conserve des Produits Agricoles du Maroc (FICOPAM). (2016) Good manufacturing practice. Personal communication.

Franzetti, L., Scarpellini, M., Vecchio, A., & Planeta, D. (2011) Microbiological and safety evaluation of green table olives marketed in Italy. Annals of Microbiology 61, 843–851.

Ghabbour, N., Lamzira, Z., Thonart, P., Cidalia, P., Markaouid, M., & Asehraou, A. (2011) Selection of oleuropein-degrading lactic acid bacteria strains isolated from fermenting Moroccan green olives. Grasas y Aceites 62, 84–89.

Gonzalez, M., Zarzuelo, A., Gamez, M.J., Utrilla, M.P., Jimenez, J., & Osuna, I. (1992) Hypoglycemic activity of olive leaf. Planta Medica 58, 513–515.

Hansen, K., Andersen, A., Christensen, B.S., Brooegger, S., Rosendal, J.S., Nyman, U., & Wagner, S.U. (1996) Isolation of an angiotensin converting enzyme (ACE) inhibitor from *Olea europaea* and *Olea lancea*. Phytomedicine 2, 319–324.

Idrissi Janati, I., Rahmani, M., & Souizi, A. (2004) Essais de désamérisation biologique des olives de table à échelle industrielle. Olivae (Madrid) 101, 34–37.

International Olive Council (IOC). (2004) Trade standard applying to table olives COI/OT/NC No. 1. IOC, Madrid, Spain.

International Olive Council (IOC). (2010) Technical-legal study on geographical designations for olive oil and table olives. Annexe VI: list of potential GIs in IOC members. IOC, Madrid, Spain. Available from: http://www.internationaloliveoil.org/estaticos/view/388-geographical-indications

International Olive Council (IOC). (2014) [Statistical data related to "world table olive production"]. IOC, Madrid, Spain.

Lalas, S., Athanasiadis, V., Gortzi, O., Bounitsi, M., Giovanoudis, I., Tsaknis, J., & Bogiatzis, F. (2011) Enrichment of table olives with polyphenols extracted from olive leaves. Food Chemistry 127, 1521–1525

Lescheid, D.W. (2014) Probiotics as regulators of inflammation: a review. Functional Foods in Health and Disease 4, 299 –311.

López-López, A., García-García, P., Duran-Quintana, M.C., & Garrido-Fernández, A. (2004) Physicochemical and microbiological profile of packed table olives. Journal of Food Protection 67, 2320–2325.

Manna, C., D'Angelo, S., Migliardi, V., Loffredi, E. Mazzoni, O., Morrica, P., Galletti, P., & Zappia, V. (2002) Protective effect of the phenolic fraction from virgin olive oils against oxidative stress in human cells. Journal of Agriculture and Food Chemistry 50, 6521–6526.

Marsilio, V. (2006) The use of LAB starters during table olive fermentation. In: 2nd International Seminar Olivebioteq, 5–10 November, Marsala-Mazara del Vallo, Italy, special seminars and invited lectures, 221–233.

Marsilio, V., Campestre, C., & Lanza, B. (2001) Phenolic compounds change during California-style ripe olive processing. Food Chemistry 74, 55–60.

Marsilio, V., Lanza, B., & Pozzi, N. (1996) Progress in table olive debittering: Degradation *in vitro* of oleuropein and its derivatives by *Lactobacillus plantarum*. Journal of the American Oils Chemists' Society 73, 593–597.

Medina-Pradas, E., & Arroyo-López, F. (2015) Presence of toxic microbial metabolites in table olives. Frontiers in Microbiology 6(art. 873), 1–6.

Mucilli, S. (2010) Isolation and characterization of yeasts isolated from naturally fermented olive brine with bioprotective function. Ph.D. thesis, Catania University, School of Agriculture, Italy.

Niaounakis, M., & Halvadakis, C.P. (2006) Table olives. In: Olive processing waste management: literature review and patent survey, 2nd ed. Waste Management Series 5. Elsevier, Amsterdam, 295–319.

Panagou, E.Z., Tassou, C.C., & Skandamis, P.N. (2006) Physicochemical, microbiological, and organoleptic profiles of Greek table olives from retail outlets. Journal of Food Protection 69, 1732–1738.

Pereira, A.P., Pereira, J.A., Bento, A., & Estevinho, M.L. (2008) Microbiological characterization of table olives commercialized in Portugal in respect to safety aspects. Food and Chemical Toxicology, 46, 2895–2902.

Pereira, A.P., Pereira, J.A. Ferreira, I.C.F.R., Valentao, P., Andrade, P.B., Seabra, R., Estevinho, L., & Bento, A. (2006) Table olives from Portugal: phenolic compounds, antioxidant potential, and antimicrobial activity. Journal of Agricultural and Food Chemistry 54, 8425–8431.

Rodríguez-Gómez, F., Romero-Gil, V., García-García, P., Garrido-Fernández, A., & Arroyo-López, F.N. (2014) Fortification of table olive packing with the potential probiotic bacteria *Lactobacillus pentosus* TOMC-LAB2. Frontiers in Microbiology 5(art. 467), 1–9.

Soler-Rivas, C., Espin, J.C., & Wichers, H.J. (2000) Oleuropein and related compounds. Journal of the Science of Food and Agriculture 80, 1013–1023.

Therios, I. (2009) Structure and composition of the olive fruit. In: Olives. Crop Production Science in Horticulture Series 18. CABI, Reading, UK, 25–31.

Tofalo, R., Schirone, M., Perpetuini, G., Angelozz, G., Suzzi, G., & Corseti, A., (2012) Microbiological and chemical profiles of naturally fermented table olives and brines from different Italian cultivars. Antonie Van Leeuwenhoek 102, 121–131.

Valencic, V., Mavsar, D., Bucar-Miklavcic, M., Butinar, B., Cadez, N., Golob, T., Raspor, P., & Mozina, S.S. (2010) The impact of production technology on the growth of indigenous microflora and quality of table olives from Slovenian Istria. Food Technology and Biotechnology 48, 404–410.

Zarzuelo, A., Duarte, J., Jimenez, J., Gonzales, M., & Utrilla, M.P. (1991) Vasodilator effect of olive leaf. Planta Medica 57, 417–419.

Zoidou, E., Melliou, E., Gikas, E., Tsarbopoulos, A., Magiatis, P., & Skaltsounis, A.L. (2010) Identification of Throuba Thassos, a traditional Greek table olive variety, as a nutritional rich source of oleuropein. Journal of Agriculture and Food Chemistry 58, 46–50.

18 Improving the quality of processed olives: acrylamide in Californian table olives

Charoenprasert Suthawan and Alyson E. Mitchell

18.1 Introduction

Raw olive fruit is very bitter due to high concentrations of phenolic compounds, in particular the secoiridoid oleuropein and its related derivatives. To make olive fruit edible, it must first be cured (processed) to reduce the content of oleuropein and related derivatives. There are numerous methods used worldwide to cure olives. In general, Greek methods use ripe olives that are either gradually fermented in brine, dry cured with salt, sun cured on the tree, or cured in oil. Spanish methods use unripe olives that are typically cured briefly in lye (NaOH), fermented, and pasteurized, while American methods use unripe olives cured over several days in lye with air oxidation followed by sterilization. These processing methods rely on the leaching of bitter constituents into surrounding medium (e.g., brine) and/or the base-catalyzed hydrolysis of oleuropein/ligstroside (lye) into non-bitter products such as glucose, hydroxytyrosol, and elenolic acid.

Table olives and especially olive oil are key components of the Mediterranean diet, a diet linked to the reduction of certain chronic ailments including cardiovascular and Alzheimer's disease (Han *et al.*, 2009; Pérez-López *et al.*, 2009; Frisardi *et al.*, 2010). However, depending upon the processing method used to cure olives, olives will contain different levels of biologically active phenolic compounds (e.g., hydroxytyrosol). One of the most popular styles of table olive is the California-style black ripe olive (CBRO), created by Freda Ehmann in the 1890s. Unlike Spanish and Greek-style olives, CBROs contain relatively low levels of phenolics and high concentrations of acrylamide (226–1925 μg/kg). Acrylamide is classified as a probable human carcinogen (Group 2A) by the International Agency for Research on Cancer (IARC, 1994). The presence of acrylamide in CBRO is a puzzling phenomenon as acrylamide is generally formed through reaction between free amino acids (especially asparagine) and carbonyl compounds derived from reducing sugars (e.g., glucose, fructose, and maltose) via the Maillard reaction at temperatures above 120 °C (Mottram *et al.*, 2002; Tareke *et al.*, 2002; Yaylayan *et al.*, 2003; Zyak *et al.*, 2003; Stadler *et al.*, 2004). Olives contain only trace levels of free asparagine (52.80 to 198.00 μg/kg), and there is no correlation between the concentration of glucose, amino acids, and acrylamide formation in CBROs (Casado & Montano, 2008). Moreover, the processing methods used to make CBROs (e.g., brine storage and successive lye treatments and rinsing) would be expected to remove small hydrophilic precursors such as asparagine and glucose.

Lipid oxidation may contribute to acrylamide formation in CBROs. Umano and Shibamoto (1987) demonstrated that the reactive aldehyde, acrolein, can be formed from lipids (triacylglycerols) when they are heated. Acrolein can be oxidized to acrylic acid. It is possible that acrylamide may form as a result of a reaction between acrylic acid and a nitrogen source (e.g., an amino acid) in lipid-rich foods (Yasuhara *et al.*, 2003). Understanding the mechanism of acrylamide formation in CBROs is critical for developing strategies to decrease levels and improve product quality. Although the relevance of human dietary exposure to acrylamide is still unclear, regulatory agencies such as the World Health Organization (WHO) continue to advocate that food manufactures must reduce acrylamide in foods (see WHO Technical Report Series No. 959;

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Figure 18.1 Conversion of acrylonitrile to acrylamide by nitrile hydratase.

WHO, 2011). Herein, we discuss possible formation mechanisms of acrylamide in CBROs as well as possible mitigation strategies.

18.2 Acrylamide formation in food and potential adverse health effects

Acrylamide, acrylic amide or 2-propenamide (CAS number 79-06-1) is an odorless and colorless crystalline compound. It is soluble in water, acetone, and ethanol and insoluble in nonpolar solvents. Melting and boiling points of acrylamide are 84.5 and 136 °C, respectively. Acrylamide is synthesized on an industrial scale by conversion of acrylonitrile by nitrile hydratase (Figure 18.1). Acrylamide is primarily used for the production of polyacrylamide, which is widely used in water treatment, gel electrophoresis, paper making, oil recovery, ore processing, fabrics, and the synthesis of dyes, among other uses.

Polyacrylamide is considered to be harmless to humans, but the acrylamide monomer is toxic (Arribas-Lorenzo & Molales, 2012). Polyacrylamide normally contains acrylamide monomers as the residual from in its production. Since polyacrylamide is used in many industries, especially in water treatment, the monomeric acrylamide present in polyacrylamide may be released into the environment as a contaminant. Acute health effects of acrylamide are skin and eye irritation. Chronic health effects include neurotoxicity, genotoxicity, carcinogenicity, and reproductive toxicity (Arribas-Lorenzo & Molales, 2012).

18.2.1 Acrylamide in heat-treated foods

In 2002, researchers at the University of Stockholm announced findings of significant levels (μg/kg to mg/kg) of acrylamide in foods cooked at elevated temperatures (Tareke *et al.*, 2000, 2002; Yasuhara *et al.*, 2003; Fredriksson *et al.*, 2004). Following this in 2005, the Swedish National Food Administration announced that foods processed and cooked at high temperatures contain relatively high levels of acrylamide (Swedish National Food Administration, 2002). This was of special concern as levels reported often exceeded drinking water standards of 0.5 mg/kg, set by WHO (2003) for many countries. Early studies demonstrated that acrylamide forms in starch-rich foods cooked at elevated temperatures (>120 °C) from endogenous components within the food (Tareke *et al.*, 2002). Subsequent studies demonstrated that acrylamide forms in these foods as a result of a reaction between amino acids and reducing sugars through the Maillard reaction (Mottram *et al.*, 2002; Bronislaw *et al.*, 2005). Glucose and asparagine are widely recognized to be the predominant precursors of acrylamide in starch-rich foods. Labeling experiments using ^{13}C and ^{15}N isotopes demonstrated that the backbone of acrylamide comes from asparagine and that it is formed as a byproduct of the Maillard reaction (Tareke *et al.*, 2002; Zyzak *et al.*, 2003). The mechanism for the formation of acrylamide from asparagine is shown in Figure 18.2. The first step begins with the formation of a Schiff base as the result of a reaction between the α-amino group of asparagine and a carbonyl source.

The Schiff base forms an oxazolidin-5-one intermediate and the zwitterionic form of the Schiff base (not shown), which decarboxylates to form the azomethine ylide that can react in one of two ways. As shown in pathway A of Figure 18.3, the imine form can hydrolyze to form 3-aminopropionamide that can further degrade via the elimination of ammonia to form acrylamide. Alternatively, as shown in pathway B, the Strecker aldehyde forms from the imine that can lose water to form acrylamide. Decarboxylated Amadori compounds can also be converted to acrylamide. Decarboxylated Amadori compounds may be formed from Amadori compound or the azomethine ylide (Yaylayan *et al.*, 2003; Stadler *et al.*, 2004).

Figure 18.2 Proposed mechanism for the formation of acrylamide from the reaction of asparagine with a carbonyl compound. Adapted from Zyzak *et al.* (2003) and Wedzicha *et al.* (2005).

The mechanism of the formation of acrylamide in lipid-rich foods appears to differ from that of starch-rich foods. Carbonyl compounds in foods may arise also from lipid oxidation, particularly during heating (Frankel, 1998), and these may react with free amino acids to form acrylamide. Yasuhara *et al.* (2003) proposed a possible pathway for the formation of acrylamide from secondary products of lipid oxidation and amino acid degradation, as shown in Figure 18.3. Here, the simple aldehyde acrolein is generated through the thermal degradation and/or oxidation of free fatty acids or glycerol. Acrolein is next oxidized to form acrylic acid. Acrylic acid can then react with ammonia, a degradation product of amino acids that occurs during thermal processing (Figure 18.3).

In model browning studies, Yasuhara *et al.* (2003) demonstrated that acrylamide forms from a mixture of acrolein and ammonia at room temperature (10.3 μg/g) and its formation increases with temperatures up to 180 °C (753 μg/g). Above this temperature, the levels of acrylamide decrease. Levels of acrylamide increased when acrolein was replaced with acrylic acid in the model. Based upon these findings, the authors proposed that the backbone of acrylamide is derived from acrylic acid and/or acrolein, and that the amine group results from ammonia, a product of the degradation of amino acids during high-heat treatment.

Ehling *et al.* (2005) investigated the formation of acrylamide from several complex lipids, including lard, tallow, corn, canola, soybean, cod liver, and sardine oils. Acrylamide formed when the mixtures of each complex lipid were mixed with asparagine and were heated to 180 °C for 30 min. The lipids with a higher degree of unsaturation, such as sardine and cod liver oil, formed relatively higher levels of acrylamide (435.4–642.0 μg/g asparagine) as compared to lipids composed primarily of saturated fats (e.g., beef fat and lard), which formed 36.0–59.6 μg/g asparagine. Based upon this, Ehling *et al.* (2005) proposed that the

HO OH HO Glycerol
O R OH Fatty acids
O H Acrolein
[O]
O OH Acrylic acid
NH_3 Amino acids
O NH_2 Acrylamide

Figure 18.3 Mechanism of formation of acrylamide from acrylic acid with amino acid. *Source*: Yasuhara *et al.* (2003). Reproduced with permission of American Chemical Society.

aldehydes and ketones produced through oxidative degradation of lipids were responsible for the production of acrylamide, and that lipids with doubly allylic methylene groups (a CH_2 group connected to two vinyl groups) will produce the highest levels of acrylamide. According to this hypothesis, the aldehyde and/or ketone compounds generated react with asparagine and lead to the formation of acrylamide. More recently, Capuanoa *et al.* (2010) demonstrated that the level of oil oxidation positively influenced the formation of acrylamide.

Although the generation of acrylamide from the reaction of carbonyl compounds generated from lipid oxidation with asparagine and/or the reaction of acrylic acid with ammonia have been demonstrated in model systems, neither mechanism has been demonstrated in a whole food. Furthermore, some lipid-rich foods, such as olives, have only trace levels of free asparagine (Casado & Montaño, 2008). However, the predominant amino acids present in olive proteins are glutamic acid and asparagine (Casado *et al.*, 2007). Therefore, protein degradation during olive processing should be considered.

18.2.2 Adverse health effects of acrylamide

18.2.2.1 Genotoxicity

Acrylamide is an α,β-unsaturated aldehyde; it is a good Michael's acceptor and is reactive toward nucleophiles. It readily reacts with functional groups such as thiols (−SH), amines ($-NH_2$), and carboxylates ($-COO^-$) (Konings *et al.*, 2006; Gokmen & Senyuva, 2008; Arribas-Lorenzo & Molales, 2012). These chemical functional groups are typically present in critical biomolecules, such as DNA and proteins. Covalent binding of acrylamide to DNA and proteins can lead to the dysfunction of enzymes and adducts on DNA.

Figure 18.4 Structure of glycidamide.

Acrylamide can also be metabolized into the reactive epoxide, glycidamide (Figure 18.4), by cytochrome P450 2E1 as demonstrated in both experimental animals (Sumner *et al.*, 1992) and humans (Pérez *et al.*, 1999; Gamboa da Costa *et al.*, 2003).

Reaction between glycidamide and acrylamide with DNA can result in DNA adducts. Glycidamide is more reactive than acrylamide toward DNA (Gamboa da Costa *et al.*, 2003; Besaratinia & Pfeifer, 2004; Doerge *et al.*, 2005a, 2005b). The formation of the highly reactive glycidamide is thought to be the primary pathway resulting in the genotoxicity associated with acrylamide (Sumner *et al.*, 1999; Besaratinia & Pfeifer, 2004). Studies in rats and mice indicated that acrylamide is rapidly absorbed after administration via aqueous gavage or through food. Acrylamide is widely distributed in tissues, where it is converted to glycidamide and increases DNA adducts in these tissues. Glycidamide is also rapidly absorbed, is widely distributed in tissues, and increases DNA adducts to a higher degree than acrylamide (Doerge *et al.*, 2005a, 2005b; Konings *et al.*, 2006). The extent of metabolism of acrylamide to glycidamide may result in a higher risk of genotoxicity.

The main DNA adducts of acrylamide and glycidamine identified in tissues are the N7-(2-carbamoyl-2-hydroxyethyl) guanine adduct (N7-GA-Gua), followed by the N3-(2-carbamoyl-2-hydroxyethyl) adenine adduct (N3-GA-Ade) and the N1-(2-carboxy-2-hydroxyethyl)-2'deoxyadenosine adduct (N1-GA-dA) (Gamboa da Costa *et al.*, 2003). The N7-guanine and N3-adenine adducts produce potentially mutagenic abasic sites through spontaneous depurination. The abasic sites of N7-guanine are thought to result in G T transversion. The N3-adenine adduct can undergo spontaneous depurination more rapidly than the N7-guanine adduct. Among the three DNA adducts, the N1-GA-dA has the highest mutagenic potential because of direct interference in the base-pairing region (Gamboa da Costa *et al.*, 2003; Besaratinia & Feifer, 2004).

18.2.2.2 Carcinogenicity

The induction of cancer by a chemical carcinogen involves multiple stages that can conceptually be categorized as initiation, promotion, and progression. The process begins with a chemical carcinogen causing a genetic error, by modifying the molecular structure of DNA. This error can lead to a mutation during DNA synthesis. Generally, this occurs with an adduct forming between a reactive group of the chemical carcinogen (electrophile) and a nucleotide in DNA (nucleophile), and it is termed initiation. The second stage, termed promotion, involves the proliferation of mutated daughter cells through increased cell division or reduced cell apoptosis. The third stage, termed progression, involves the subsequent acquisition of additional genetic changes and clastogenic effects. The final stage results in the transfer from the pre-neoplastic state to neoplastic state (Klaunig, 2008). Acrylamide is clastogenic (causing breakage of chromosomes) and mutagenic in mammalian cells both *in vitro* and *in vivo* (WHO, 2006). The IARC classified acrylamide as a probable human carcinogen (IARC Group 2A) in 1994, based on laboratory animal studies (IARC, 1994). The studies demonstrated that acrylamide promotes cancer development in animals after oral administration. Acrylamide is a multi-organ carcinogen. It increases the incidence of lung and skin tumors in mice and induced mesotheliomas, pheochromocytomas, and uterine, thyroid, mammary gland, brain, and pituitary tumors in rats (WHO, 2006; Klaunig, 2008). The relationship between dietary intake of acrylamide and the incidence of human cancer is still ambiguous (Capuano & Fogliano, 2011).

It is widely accepted that carcinogenicity of acrylamide is related to its conversion to glycidamide, formation of glycidamide-DNA adducts, and consequent mutagenesis (Klaunig, 2008; Capuano & Fogliano, 2011); however, other mechanisms may also play a role in its toxicity. For example, indirect genotoxicity via glutathione depletion has also been proposed. Acrylamide is detoxified via a Michael-type addition reaction between the thiol group of glutathione and acrylamide and is catalyzed by glutathione *S*-transferase. This reaction results in acrylamide–glutathione conjugates and mercapturic acid metabolites, which are excreted

in urine (Fennell *et al.*, 2005; Besaratinia & Pfeifer, 2007). Glutathione is involved in antioxidant mechanisms against oxidative stress by directly scavenging hydroxyl radicals and singlet oxygen, while regenerating vitamins C and E to their active forms (Rahman, 2007). Oxidative stress causes cell injury leading to several chronic diseases, including cancer. Therefore, carcinogenicity of acrylamide may result from its ability to promote depletion of glutathione stores, leading to oxidative stress, cell injury, and consequently cancer. In addition, redox homeostasis normally ensures that cells respond properly to endogenous and exogenous stimuli; thus, it plays an important role in survival of cells. Excessive reactive oxygen resulting from glutathione depletion may obstruct cellular redox balance, consequently affect redox-sensitive transcription factors or directly interfere with expression of oxidative stress-responsive genes, and finally alter cell regulation (Besaratinia & Pfeifer, 2007; Trachootham *et al.*, 2008).

Another mechanism of carcinogenicity of acrylamide may be through the inhibition of mitotic/meiotic motor proteins. Proteins such as kinesin motor protein are involved in the formation of spindle fibers, which have a role in separation of chromosomes during cell division. Acrylamide may bind at the sulfhydryl groups of these proteins, resulting in clastogenic effects and development of cancer (Exon, 2006; Capuano & Fogliano, 2011). Acrylamide may also inactivate proteins or enzymes associated with DNA repair, which finally leads to mutation (Exon, 2006).

18.2.2.3 Neurotoxicity

Neurotoxicity caused by exposure to acrylamide was observed in both humans and laboratory animals (LoPachin & Gavin, 2012). Acrylamide results in damage to both the peripheral nervous system (PNS) and central nervous system (CNS) (LoPachin & Gavin, 2012). At-risk individuals include those associated with the construction industry, mines, tunnels, and manufacturers of flocculator, polyacrylamide, or acrylamide monomer (Pennisi *et al.*, 2013). These workers may be exposed to acrylamide through inhalation or dermal absorption. Neurotoxicity of acrylamide is characterized by numbness of the extremities, sensory and cognitive impairment, and skeletal muscle weakness. According to a WHO (2006) report, chromic expose of laboratory rats to acrylamide produced degeneration at nerve terminals in the brain in areas critical for learning, memory, and cognitive function (i.e., the cerebral cortex, thalamus, and hippocampus). Morphological changes in nerves were also noted. The same expression of neurotoxicity was observed in rats given drinking water containing acrylamide (21 mg/kg body for 40 days) and in rats given acrylamide of 50 mg/kg for 11 days; thus, neurotoxicity of acrylamide is cumulative. The no-observed-effect level (NOEL) of acrylamide for neurotoxicity is 0.2 to 10 mg per kg of body weight, per day, based on experimental studies in rats and mice (WHO, 2005). The estimated daily dietary acrylamide intake is 0.3–2.0 μg/kg of body weight for the general population and 5.1 μg/kg for high-level consumers (the 99th percentile) (WHO, 2005). Therefore, the NOEL of acrylamide for neurotoxicity is much higher than the estimated daily acrylamide intake from dietary exposure. However, since neurotoxicity of acrylamide is cumulative, the effect of continued consumption of foods containing high levels of acrylamide on neurotoxicity should be investigated.

The molecular mechanisms of neurotoxic action of acrylamide are not yet clear. In general, neurotoxicity of acrylamide is associated with cerebellar Purkinje cell injury, degeneration of the distal region of axons, and degeneration of nerve terminals in Pthe NS and CNS (Lehning *et al.*, 2002). LoPachin and Gavin (2012) hypothesized that the electrophilic structure of acrylamide allows it to form adducts with cysteine on presynaptic proteins, leading to inhibition of neurotransmission. Activities of many nerve terminal proteins were thought to be regulated by the ionization of the sulfhydryl group of cysteine to highly reactive thiolate. Therefore, formation of adducts of acrylamide with cysteine may result in neurotoxicity. Another hypothesis for acrylamide neurotoxicity involves the ability of acrylamide to interfere with kinesin-related fast axonal transport (Sickles *et al.*, 2002). Acrylamide may bind to the motor protein kinesin, resulting in the reduction of quantity of vesicles moving in the fast anterograde system. Continued inhibition of kinesin may lead to deficiencies of fast-transported proteins in the distal region of axons and/or axon terminals, consequently resulting in dysfunction of axons.

18.2.2.4 Reproductive toxicity

In February 2011, acrylamide was added to the Proposition 65 list in California as a reproductive toxicant based on studies in laboratory animals. This list is administered by the Office of Environmental Health

Hazard Assessment (OEHHA, 2013). The no-observable-adverse-effect level (NOAEL) for reproductive toxicity is 2–5 mg/kg body weight/day. The NOAELs vary depending on the endpoint of fertility or embryonic death (Exon, 2006). Rats and mice exposed to acrylamide showed reduced fertility, reduced live litter size, abnormal sperm, decreased sperm counts, dominant lethal effects, implantation losses, and lower numbers and survival of pups (Tyl & Friedman, 2003; Exon, 2006). No strong evidence observed indicates the effects of acrylamide on fertility or the reproductive system in females, with the exception of slight reduction in the body weight of offspring (WHO, 2006).

Reproductive toxicity of acrylamide is in all probability related to neurotoxicity. Acrylamide may bind to motor protein kinesin, resulting in degeneration of axons (Sickles *et al.*, 2002). These may affect reproductive performance, mating, and sexual behavior. Reduced hind-limb function was reported to be one of the neurotoxicity effects of acrylamide. Impaired hind-limb function may reduce mounting activity, copulatory activity, and intromission (Exon, 2006). Anesthesia of the penis may also affect mounting and intromission (Tyl & Friedman, 2003). Sperm flagellum also contains kinesin (Miller *et al.*, 1999). Interference with kinesin may affect sperm mobility and consequently fertilization (Tyl & Friedman, 2003). Kinesin-like proteins in the nucleus are involved in the separation of chromosomes during cell division. Acrylamide may interfere with these proteins and result in the dominant lethality. Reaction of acrylamide with spermatid protamine proteins may also cause clastogenesis and dominant lethal mutation, which results in decreased litter size (Tyl & Friedman, 2003).

18.3 Regulation of acrylamide in food

In the United States, the primary agency responsible for regulating acrylamide in drinking water is the Environmental Protection Agency (EPA). This is done under the Safe Drinking Water Act (1974). The EPA set Maximum Contaminant Level Goals (MCLGs), which are enforceable levels. The MCLG for acrylamide is zero. EPA has set this level of protection based on the best available science to prevent potential health problems. As already stated, in 1990, acrylamide was added to the Proposition 65 list of chemicals known to the state of California to cause cancer and/or reproductive toxicity. Proposition 65 prohibits companies that do business in California from knowingly discharging a listed chemical into sources of drinking water. It also requires companies to provide clear and reasonable warning (labels) about significant levels of the listed chemicals in their products and foods. According to Proposition 65, the no-significant-risk level (NSRL) for acrylamide as a carcinogen is 0.20 μg/day, whereas the Maximum Allowable Dose Levels (MADLs) for acrylamide as a reproductive toxic agent is 140 μg/day.

Polyacrylamides are often used in drinking water treatment; therefore, the acrylamide residual may be transferred to water (Arribas-Lorenzo & Molales, 2012). Polyacrylamide may contain acrylamide monomers as an impurity. Due to their potential health effects, the EPA (2009) and the WHO (2011) have limited acrylamide levels in drinking water to 0.5 μg/L or less, whereas the European drinking water quality standards (EU) require levels in drinking water not to exceed 0.1 μg/L (Department of the Environment, 2011).

Based upon the many studies on the health effects of acrylamide, most national authorities continue to encourage food manufacturers to lower and monitor acrylamide levels in their food products. To date, the US Food and Drug Administration (FDA) and European Commission (EC) have not set a maximum allowable level for acrylamide levels in foods. However, the FDA and other national agencies such as the European Food Safety Authority (EFSA) continue to monitor acrylamide levels in foods and study the effect of dietary exposure to acrylamide on human health.

18.4 Acrylamide levels in olive products

Acrylamide is found in CBROs at relatively high concentrations as compared to starch-rich foods of concern. According to a survey on acrylamide levels in foods (FDA, 2002–2004) the levels of acrylamide range from 200 to 2000 μg/kg in CBROs. These levels are similar to levels in French fries (100–1300 μg/kg), potato chips (250–3000 μg/kg), and cereals (<1000 μg/kg), and this makes CBROs one of the eight top foods in terms of acrylamide content per portion (Di Novi & Howard, 2004). Charoenprasert and Mitchell

Table 18.1 Average acrylamide levels in fresh, brine-stored, Spanish-style, Greek-style, California-style black ripe, and California-style green ripe olives.

	Acrylamide in olive (μg/kg)	
Olive samples	**Year 1**	**Year 2**
Fresh	<0.71	<0.71
Brine stored	<1.42	<0.71
Spanish style	<0.71	<0.71
Greek style	<0.71	<0.71
California-style black ripe, brand G	470.97±36.45	460.08±35.50
California-style green ripe, brand G	59.64±6.86	68.87±12.54

Source: Charoenprasert and Mitchell (2014). Reproduced with permission of American Chemical Society.
Note: Average of three measurements. The limit of detection (LOD) and the limit of quantification (LOQ) of the acrylamide analysis method were 0.71 and 1.42 μg/kg, respectively.

(2014) measured acrylamide levels in Greek-style, Spanish-style, California-style black ripe, and California-style green ripe olives (Table 18.1). These olives were obtained from local markets and analyzed over two harvest years (2010 and 2014). Significant amounts of acrylamide were present in the California-style black ripe (470.97±36.45 and 460.08±35.50 μg/kg) and California-style green ripe olives (59.64±6.86 and 68.87±12.54 μg/kg), whereas levels in the Greek-style and Spanish-style olives were below detection limits. In addition, the level of acrylamide in fresh and brine-stored Manzanilla olives was below the limit of detection. Together, these results indicate that formation of acrylamide in table olives can be influenced by the processing methods used to make the table olives.

18.5 Effects of table olive processing methods on acrylamide formation

Casado and Montaño (2008) studied the effect of the CBRO processing method on formation of acrylamide. Free amino acids and acrylamide were quantified in olives before and after sterilization. Acrylamide was found only in the sterilized olives (234.3–1349.0 μg/kg of fresh olives). The major free amino acids found in olives before sterilization include glutamic acid, serine, glutamine, alanine, arginine, valine, and aspartic acid. Asparagine was present in olives before sterilization, but at very low concentrations that ranged from 52.8 to 198.0 μg/kg. No correlation was observed between the pre-sterilization concentration of glucose and amino acids (both total and individual) and the formation of acrylamide. These observations demonstrate that asparagine and glucose are not the main precursors of acrylamide in CBROs and that the mechanism of formation of acrylamide in olives differs from that in starch-based foods.

The temperature used to process olives (e.g., sterilization vs. pasteurization) is critical in the formation of acrylamide (Casado & Montaño 2008; Charoenprasert & Mitchell, 2014). Higher concentrations of acrylamide are found in California-style black ripe olives than in California-style green ripe olives, whereas only trace amounts of acrylamide are found in fresh, fresh brine-stored, Spanish-style, and Greek-style table olives (Table 18.1). California-style black ripe and green ripe processing methods require sterilization at temperatures >110 °C (Garrido Fernandez *et al.*, 1997; Charoenprasert & Mitchell, 2012). In contrast, Spanish- and Greek-style table olive processing methods preserve olives using additives and/or pasteurization at temperatures <65 °C (Garrido Fernandez *et al.*, 1997).

Significantly higher concentrations of acrylamide are found in olives after sterilization as compared to olives that are processed but not sterilized (Table 18.2). Additionally, acrylamide levels have been shown to increase with increasing sterilization time with temperatures between 110 and 125 °C (Casado & Montaño, 2008). These results indicate that the high temperatures used to sterilize CBROs play a key role in the formation of the high levels of acrylamide found in these olives.

Table 18.2 Effect of air oxidation, ferrous gluconate treatment, and sterilization on acrylamide levels in California-style black ripe olives.

Processing method	Acrylamide in olives (μg/kg)
Complete (lye, air oxidation, ferrous gluconate, and sterilization)	569.42±38.34
No air oxidation (lye, ferrous gluconate, and sterilization)	310.27±0.71
No ferrous gluconate (lye, air oxidation, and sterilization)	545.99±28.40
No air oxidation and ferrous treatment (lye and sterilization)	344.35±14.91
No sterilization (lye, air oxidation, and ferrous gluconate)	51.83±12.78

Source: Charoenprasert and Mitchell (2014). Reproduced with permission of American Chemical Society.

California-style black and green ripe processing methods are essentially the same, with the exception that California-style green ripe olives are processed fresh (not brine stored) and are not subject to air oxidation or ferrous gluconate treatment. Air oxidation during lye treatment is required to oxidize and polymerize o-diphenols, mainly hydroxytyrosol and caffeic acid, to form brown-black pigments. The surface color obtained through this process is not stable and fades during the shelf life of the packed product. To prevent color deterioration, iron salts, such as ferrous gluconate, can be used to stabilize color as they form a stable iron (Fe)–phenol complex. The influence of air oxidation and ferrous gluconate treatment on acrylamide formation in CBROs is shown in Table 18.2. Ferrous gluconate treatment had no effect on acrylamide levels, whereas air oxidation during lye treatment increases the formation of acrylamide in the olives significantly. Olives processed without the air oxidation step had lower levels of acrylamide as compared to olives processed with the complete CBRO processing method. These results suggest that air oxidation, during lye treatment, promotes the formation of the precursors leading to higher concentration of acrylamide in the end products (Charoenprasert & Mitchell, 2014).

The lye treatment used to remove oleuropein in olives also has a great impact on acrylamide levels in CBROs. The levels of acrylamide in fresh olives (11,288.29±5.68 μg/kg) or in brine-stored olives (7694.27±336, 37.63 μg/kg), which were directly sterilized, were more than 10 times greater than those found in olives that underwent lye processing for 4 days (569.42±38.34 μg/kg) prior to sterilization (Charoenprasert & Mitchell, 2014). This result indicates that the lye treatment step may result in the elimination of precursors of acrylamide and result in a lower level of acrylamide in CBROs.

Another factor that may influence acrylamide formation in olives is pH. Casado *et al.* (2010) explored the influence of pH on acrylamide formation in extracted olive juice. The pH of the olive juice was adjusted with hydrochloric acid or sodium hydroxide to cover a range of pH levels between 4 and 9 prior to sterilization. The highest levels of acrylamide formed in the olive juice between a pH of 5.5 and 6.0. The formation of acrylamide decreased when the pH of olive juice fell out of this range. As carbon dioxide is widely used in commercial settings to neutralize olives prior to sterilization, Charoenprasert and Mitchell (2014) investigated the effect of carbon dioxide exposure time and pH on acrylamide formation in CBROs (Table 18.3). The initial pH of the lye-treated olives was 9.76, while after neutralization with carbon dioxide, the pH was

Table 18.3 Effect of neutralization with carbon dioxide (pH) on acrylamide formation in California-style black ripe olives.

Neutralization time (hr)	Measured pH	Acrylamide (μg/kg)
0	9.76	338.67±9.23
1	8.61	437.36±4.97
2	8.29	585.04±0.71
3	7.65	591.43±19.88
4	7.11	681.60±11.36

Source: Charoenprasert and Mitchell (2014). Reproduced with permission of American Chemical Society.

Table 18.4 Effect of brine composition on acrylamide formation in California-style black ripe olives.

Brine composition	Acrylamide (μg/kg)
Sample a (complete brine)	685.86±26.98
Sample b (brine without sodium benzoate)	642.55±44.02
Sample c (brine without acetic acid)	563.74±36.21
Sample d (brine without calcium chloride)	303.17±43.31

Source: Charoenprasert and Mitchell (2014). Reproduced with permission of American Chemical Society.

reduced to 7.11. The lowest levels of acrylamide (338.67±9.23 μg/kg) were found in olives that were not neutralized with carbon dioxide, whereas the highest levels of acrylamide (681.60±11.36 μg/kg) were found in olives that had been neutralized for 4 hr. These results indicated that the formation of acrylamide in whole olives, as well as in olive juice, is pH dependent.

Both fresh and brine-stored olives are used to make CBROs. Olives that cannot be processed immediately after harvest are preserved in a brine solution. The concentrations of acrylamide in CBROs is influenced by brine storage time. During brine storage, acrylamide levels increase during the first month and subsequently decline with increasing storage time. Acrylamide levels are highest in olives stored in brine for one month. Charoenprasert and Mitchell (2014) also evaluated the influence of the components typically added to commercial brines (i.e., calcium chloride, sodium benzoate, or acetic acid) on the formation of acrylamide. Higher concentrations of acrylamide are found in olives stored in brine containing calcium chloride, as compared to olives stored in brine without calcium ions added (Table 18.4). This may have resulted from enhanced stability of the cell wall and cell turgor of the olive fruit as calcium forms cross-linkages between pectin molecules. The release of acrylamide and its precursors from olive fruit into the surrounding brine may be impeded due to improved cell wall structure of the olive fruit. However, removing calcium ions from the brine solution may affect texture and consumer acceptance of olive products and should be further studied before being adopted as a method for mitigating acrylamide in CBROs.

18.6 Methods to mitigate acrylamide levels in processed table olives

18.6.1 Reduction of acrylamide in California-style black ripe olives using additives

The influence of various ionic salts (i.e., sodium bisulfite, sodium chloride, sodium dihydrogen phosphate, sodium borate, calcium chloride, magnesium chloride, and ferrous gluconate) on the reduction of acrylamide in olive juice was evaluated by Casado *et al.* (2010). Among the ionic salts tested, sodium bisulfite was the only salt that significantly reduced levels of acrylamide. Sodium bisulfite is a common antioxidant, used in vegetable processing, that has been shown to reduce acrylamide levels in fried potatoes (Ou *et al.*, 2008) and in heated flour (Levine & Smith 2005). The mechanism of reduction of acrylamide by sodium bisulfite is still unclear. However, it could be explained with the reaction between sodium bisulfite and α,β-unsaturated carbonyl compounds. According to this proposed mechanism, sodium bisulfite initially reacts with an α,β-unsaturated carbonyl at the carbonyl group to form a sulfonate. This initial stage is reversible. A second molecule of sulfite is irreversibly added at the β-position to form a disulfonate, leading to the reduction of the levels of α,β-unsaturated carbonyl compound. This disulfonate is in equilibrium with a monosulfonate and its acetal. The inhibition effect of sodium bisulfite is concentration dependent. Lower levels of acrylamide were also found when higher concentrations of sodium bisulfite were added into the olive juice (Casado *et al.*, 2010).

López-López *et al.* (2014) tested the inhibition effect of water-soluble vitamins including B1, B3, B6, B7, and C on acrylamide formation in olive juice. The inhibition effect of the tested water-soluble vitamin is shown in Figure 18.5. Vitamin B1 effectively decreases acrylamide levels formed in the sterilized olive juice. The author suggested that the thiazole ring, which contains nitrogen and sulfur atoms, in vitamin B1

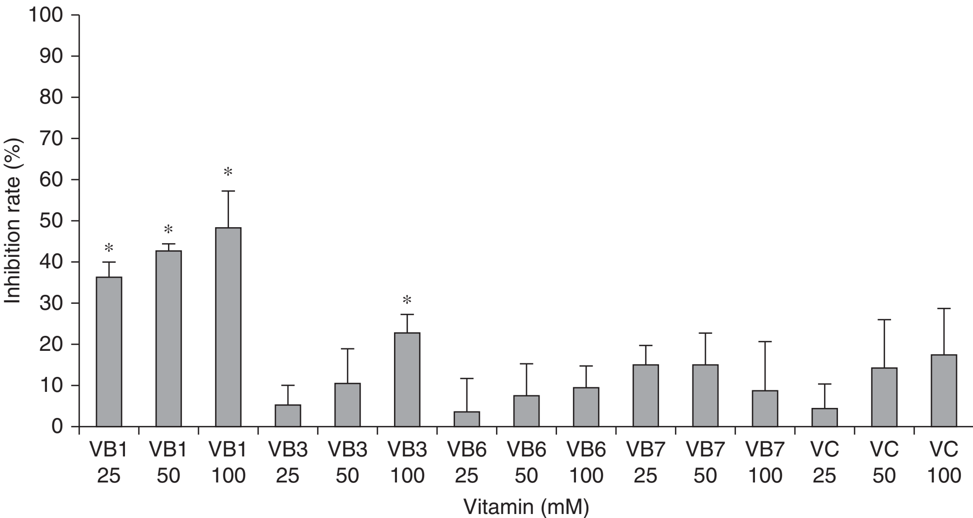

Figure 18.5 Dose–response effect of selected water-soluble vitamins on the acrylamide inhibition (percentage from control, olive juice without additives) in a ripe olive model system. *Source*: López-López *et al.*, (2014).

may play an important role in reduction of acrylamide. Unfortunately, the addition of vitamin B1 resulted in a bad odor in the heated olive juice, greatly limiting its potential use to reduce acrylamide in commercial production.

The effects of protein and nonprotein amino acids on acrylamide levels in the olive juice model system were also studied (Casado *et al.*, 2010; López-López *et al.*, 2014). Cysteine and proline were the most effective at lowering acrylamide as compared to other tested protein amino acids (i.e., Gly, Lys, Arg, Asp, Gln, Glu, Ser, Ala, Tyr, Val, Pro, Trp, and His). Cysteine and proline are unique in that they can form adducts with acrylamide. The rate of adduct formation by cysteine was faster than other amino acids lacking a thiol group (−SH) (Koutsidis *et al.*, 2009). Acrylamide is a reactive α,β-unsaturated aldehyde with an electrophilic carbon. It can undergo nucleophilic addition or Michael addition with compounds containing thiol (SH−) or amine (NH_2−) groups (Mather *et al.*, 2006). The effect of cysteine and proline on the reduction of acrylamide in the olive juice model system is thought to be due to their ability to form acrylamide adducts through Michael addition (Koutsidis *et al.*, 2009). Sarcosine was the most effective nonprotein amino acid to reduce acrylamide levels in olive juice as compared to other nonprotein amino acids tested (i.e., ornithine, taurine, and gamma-aminobutyric acid [GABA]).

A range of additives, including sodium bisulfate, cysteine, N-acetyl-L-cysteine, glutathione, and methionine, were evaluated for their ability to reduce acrylamide in processed CBROs (Casado *et al.*, 2010; López-López *et al.*, 2014). Acrylamide was not detected in olives packed in brine containing 50 mM cysteine, N-acetyl-L-cysteine, or 25 mM glutathione, whereas brine with 50 mM methionine did not reduce acrylamide levels in olives. Cysteine, N-acetyl-L-cysteine, and glutathione contain a thiol group (−SH), whereas the sulfur atom of methionine is methylated and therefore may not react with acrylamide. Unfortunately, the use of these compounds is limited as they negatively impact the sensory quality of olives (e.g., they contain sulfur and produce an unpleasant odor). Brine containing 25 mM of sodium bisulfite and 20 mM of sodium sulfite also decreased acrylamide in sterilized olives (Casado *et al.*, 2010; López-López *et al.*, 2014), but the use of these compounds in olives is prohibited by the European Commission regulations of 2011. Proline and sarcosine at 100 and 200 mM can reduce acrylamide in the end products by approximately 60–80% (López-López et al, 2014), but the use of these amino acids would significantly increase the cost of CBROs.

18.7 Conclusion

The mechanism of formation of acrylamide in CBROs is still unclear, yet it is apparent that acrylamide is formed via a different mechanism than that of starch-rich foods. Several processing factors, including brine

storage, lye treatment, air oxidation, neutralization, and sterilization, influence the levels of acrylamide in finished CBROs. Brine storage for longer periods of >30 days may help reduce acrylamide through increased degradation and/or diffusion of acrylamide precursors into the surrounding medium during storage. Despite the fact that the lye treatment step significantly reduces levels of acrylamide as compared to fresh olives, the air oxidation during this treatment promotes acrylamide formation. This suggests that acrylamide precursors are present in raw olives and that these may be washed away during the lye processing, but additional precursors form during this step via oxidation reactions (e.g., lipids and proteins) that are finally converted into acrylamide during sterilization. Modifications of the technique of oxidizing olives (e.g., shorter exposure time) during lye treatment, or additional washing, may help mitigate acrylamide formation in finished products.

The pH of the final canning solution also plays a key role in acrylamide formation. Higher concentrations of acrylamide are formed at neutral conditions compared to acidic or basic conditions. Therefore, modifying pH may also help to mitigate acrylamide in finished products. Using higher temperatures and shorter times when sterilizing olives may also lower the concentration of acrylamide formed in CBROs. Inclusion of additives (i.e., cysteine, N-acetyl-L-cysteine, glutathione, $NaHSO_3$, Na_2SO_3, proline, and sarcosine) can decrease acrylamide levels in CBROs. However, the use of these additives is not possible as they negatively impact sensory quality and would increase the processing cost of CBROs.

Future studies should focus on identifying precursors and the mechanism of formation of acrylamide in CBROs in order to preserve the functional compounds of olives. The precursors will likely be compounds that are anchored to cell membranes or held in vesicles and/or generated from the oxidation of lipids or degradation of proteins.

References

Arribas-Lorenzo, G., & Molales, F.J. (2012) Dietary exposure to acrylamide from potato crisps to the Spanish population. Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment 26, 289–297.

Besaratinia, A., & Pfeifer, G.P. (2004) Genotoxicity of acrylamide and glycidamide. Journal of the National Cancer Institute 96, 1023–1029.

Besaratinia, A., & Pfeifer, G. (2007) A review of mechanisms of acrylamide carcinogenicity. Carinogenesis 28, 519–528.

Capuano, E., & Fogliano, V. (2011) Acrylamide and 5-hydroxymethylfurfural (HMF): a review on metabolism, toxicity, occurrence in food and mitigation strategies. LWT – Food Science and Technology 44, 793–810.

Capuano, E., Olivieroa, T., Açarb, O.C., Gökmenb, V., & Foglianoa, V. (2010) Lipid oxidation promotes acrylamide formation in fat-rich model systems. Food Research International 43, 1021–1026.

Casado, F.J., & Montaño, A. (2008) Influence of processing conditions on acrylamide content in black ripe olives. Journal of Agricultural and Food Chemistry 56, 2021–2027.

Casado, F.J., Sánchez, A.H., & Montaño, A. (2010) Reduction of acrylamide content of ripe olives by selected additives. Food Chemistry 119, 161–166.

Casado, F.J., Sánchez, A.H., Rejano, L., & Montaño, A. (2007) d-Amino acids formation in sterilized alkali treated olives. Journal of Agricultural and Food Chemistry, 55, 3503–3507.

Charoenprasert, S., & Mitchell, A. (2012) Factors influencing phenolic compounds in table olives (*Olea europaea*). Journal of Agricultural and Food Chemistry 60, 7081–7095.

Charoenprasert, S., & Mitchell, A.E. (2014) Influence of California-style black ripe olive processing on the formation of acrylamide. Journal of Agricultural and Food Chemistry 62, 8716–8721.

Department of the Environment (2011) European and national drinking water quality standards. Northern Ireland Environment Agency. Available from http://www.coralenvironmental.com/wp-content/uploads/European-and-National-Drinking-Water-Quality-Standards.pdf

Di Novi, M., & Howard, D. (2004) The updated exposure assessment for acrylamide. In: Presentation at the JIFSAN Meeting entitled Acrylamide in Food: Update – Scientific Issues, Uncertainties, and Research Strategies, Chicago, IL, April 13. Available from http://www.jifsan.umd.edu/acrylamide204.htm

Doerge, D.R., Young. J.F., McDaniel, L.P., Twaddle, N.C., & Churchwell, M.I. (2005a) Toxicokinetics of acrylamide and glycidamide in B6C3F1 mice. Toxicology and Applied Pharmacology 202, 258–267.

Doerge, D.R., Young. J.F., McDaniel, L.P., Twaddle, N.C., & Churchwell, M.I. (2005b). Toxicokinetics of acrylamide and glycidamide in Fischer 344 rats. Toxicology and Applied Pharmacology 208, 199–209.

Ehling, S, Hengel, M., & Shibamoto, T. (2005) Formation of acrylamide from lipids. In: Chemistry and safety of acrylamide in food, ed. F.M. Mottram. Springer, New York, 223–233.

European Commission (2011) Commission Regulation, No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. Official Journal of the European Union L 295, 1–177.

Exon, J.H. (2006) A review of the toxicology of acrylamide. Journal of Toxicology and Environmental Health B 9, 397–412.

Fennell, T.R., Sumner, S.C., Snyder, R.W., Burgess, J., Spicer, R., Bridson, W.E., & Friedman, M.A. (2005) Metabolism and hemoglobin adduct formation of acrylamide in humans. Toxicological Sciences 85, 447–459.

Frankel, E.N. (1998) Lipid oxidation. The Oily Press, Dundee, Scotland.

Fredriksson, H., Tallving, J., Rosén, J., & Åman, P. (2004) Fermentation reduces free asparagine in dough and acrylamide content in bread. Cereal Chemistry 81, 650–653.

Frisardi, V., Panza, F., Seripa, D., Imbimbo, B.P., Vendemiale, G., Pilotto, A., & Solfrizzi, V. (2010) Nutraceutical properties of Mediterranean diet and cognitive decline: possible underlying mechanisms. Journal of Alzheimer's Disease 22, 715–740.

Gamboa da Costa, G., Churchwell. M.I., Patrice Hamilton, L., Von Tungeln, L.S., Beland, F.A., Matilde Marques, M., & Doerge, D.R. (2003) DNA adduct formation from acrylamide via conversion to glycidamide in adult and neonatal Mice. Chemical Research in Toxicology 16, 1328–1337.

Garrido Fernandez, A., Fernandez Diez, M.J., & Adams, M.R. (1997) Table olives, production and processing. Chapman and Hall, London.

Gokmen, V., & Senyuva, H.Z. (2008) Acrylamide in heated foods. In: Bioactive compounds in foods, ed. J. Gilbert & H.Z. Senyuva. Blackwell, Oxford.

Han, J., Talorete, T.P.N., Yamada, P., & Isoda, H. (2009) Anti-proliferative and apoptotic effects of oleuropein and hydroxytyrosol on human breast cancer MCF-7 cells. Cytotechnology 59, 45–53.

International Agency for Research on Cancer (IARC). 1994. Monographs on the evaluation of carcinogenic risks to humans, vol. 60. Some industrial chemicals. IARC, Lyon, France. Available from http://monographs.iarc.fr/ENG/Monographs/vol60/mono60-16.pdf

Klaunig, J.E. (2008) Acrylamide carcinogenicity. Journal of Agricultural and Food Chemistry 56, 5984–5988.

Konings, E.J.M., Hogervorst, J.G.F., Schouten L.J., & van den Brandt, P.A. (2006) Assessing exposure levels of acrylamide. In: Acrylamide and other hazardous compounds in heat-treated foods, ed. K. Skog & J. Alexander. CRC Press, Boca Raton, FL, 224–225.

Koskinen, M., & Pina, K. (2000) Specific DNA adducts induced by some mono-substituted epoxides in vitro and in vivo. Chemico-Biological Interactions 129, 209–229.

Koutsidis, G., Simons, S.P.J., Thong, Y.H., Haldoupis, Y., Mojica-Lazaro, J., Wedzicha, B.L., & Mottram, A.D. (2009) Investigations on the effect of amino acids on acrylamide, pyrazines, and Michael addition products in model systems. Journal of Agricultural and Food Chemistry 57, 9011–9015.

Lehning, E.J., Balaban, C.D., Ross, J.F., Reid, M.A., & LoPachin, R.M. (2002) Acrylamide neuropathy I. spatiotemporal characteristics of nerve cell damage in rat cerebellum. NeuroToxicology 23, 397–414.

Levine, R.A., & Smith, R.E. (2005) Sources of variability of acrylamide levels in a cracker model. Journal of Agricultural and Food Chemistry 53, 4410–4416.

LoPachin, R.M., & Gavin, T. (2012) Molecular mechanism of acrylamide neurotoxicity: lessons learned from organic chemistry. Environmental Health Perspectives 120, 1650–1657.

López-López, A., Beato, V.M., Sánchez, A.H., García-García, P., & Montaño, A. (2014) Effect of selected amino acids and water-soluble vitamins on acrylamide formation in a ripe olive model system. Journal of Food Engineering 120, 9–16.

Mather, B.D., Viswanathan, K., Miller, K.M., & Long, T.E. (2006) Michael addition reactions macromolecular design for emerging technologies. Progress in Polymer Science 31, 487–531.

Miller, M.G., Mulholland, D.J., & Vogl, A.W. (1999) Rat testis motor proteins associated with spermatid translocation (dynein) and spermatid flagella (Kinesin-II). Biology of Reproduction 60, 1047–1056.

Mottram, D.S., Wedzicha, B.L., & Dodson, A.T. (2002) Acrylamide is formed in the Maillard reaction. Nature 419, 448–449.

Office of Environmental Health Hazard Assessment (OEHHA). (2013) Proposition 65, no significant risk levels (NSRLs) for carcinogens and maximum allowable dose levels (MADLs) for chemicals causing reproductive toxicity. Available from http://www.oehha.ca.gov/prop65/getnsrls.html

Ou, S., Lin, Q., Zhang, Y., Huang, C., Sun, X., & Fu, L. (2008) Reduction of acrylamide formation by selected agents in fried potato crisps on industrial scale. Innovative Food Science and Emerging Technologies 9, 116–121.

Pennisi, M., Malaguarnera, G., Puglisi, V., Vinciguerra, L., Vacante, M., & Malaguarnera, M. (2013) Neurotoxicity of acrylamide in exposed workers. International Journal of Environmental Research and Public Health 10, 3843–3854.

Pérez, H.L., Cheong, H.K., Yang, J.S., & Osterman-Golkar, S. (1999) Simultaneous analysis of hemoglobin adducts of acrylamide and glycidamide by gas chromatography-mass spectrometry. Analytical Biochemistry 274, 59–68.

Pérez-López, F.R., Chedraui, P., Haya, J., & Cuadros, J.L. (2009) Effects of the Mediterranean diet on longevity and age-related morbid conditions. Maturitas 64, 67–79.

Rahman, K. (2007) Studies on free radicals, antioxidants and co-factors. Clinical Intervention in Aging 2, 219–236.

Sickles, D.W., Derek Stone. J., & Friedman. M.A. (2002) Fast axonal transport: a site of acrylamide neurotoxicity? NeuroToxicology 23, 223–251.

Stadler, R.H., Robert, F., Riediker, S., Varga, N., Davidek, T., Devaud, S., Goldmann, T., Hau, J., & Blank, I. (2004) In-depth mechanistic study on the formation of acrylamide and other vinylogous compounds by the Maillard reaction. Journal of Agricultural and Food Chemistry 52, 5550–5558.

Sumner, S.C.J., Fennell, T.R., Moore, T.A., Chanas. B., Gonzalez, F., & Ghanayem, B.A. (1999) Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. Chemical Research in Toxicology 12, 1110–1116.

Sumner, S.C., MacNeela, J.P., & Fennell, T.R. (1992) Characterization and quantitation of urinary metabolites of [1,2,3-13C]acrylamide in rats and mice using 13C nuclear magnetic resonance spectroscopy. Chemical Research in Toxicology 5, 81–89.

Swedish National Food Administration (2002) Information about acrylamide in food. 24 April 2002. SNFA, Stockholm.

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., & Törnqvist, M. (2000) Acrylamide: a cooking carcinogen? Chemical Research in Toxicology 13, 517–522.

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., & Törnqvist, M. (2002) Analysis of acrylamide, a carcinogen formed in heated foodstuffs. Journal of Agricultural and Food Chemistry 50, 4998–5006.

Trachootham, D., Lu, W., Ogasawara, M. A., Rivera-Del Valle, N., & Huang, P. (2008) Redox regulation of cell survival. Antioxidant Redox Signal 10, 1343–1374.

Tyl, R.W., & Friedman, M.A. (2003) Effects of acrylamide on rodent reproductive performance. Reproductive Toxicology 17, 1–13.

Umano, K., & Shibamoto, T. (1987) Analysis of acrolein from heated cooking oils and beef fat. Journal of Agricultural and Food Chemistry 35, 909–912.

U.S. Environment Protection Agency (EPA). (2009) List of contaminants and their maximum contaminant levels. http://water.epa.gov/drink/contaminants/index.cfm#eight. Accessed 01.05.16

U.S. Food and Drug Administration (FDA). (2002–2004) Survey data on acrylamide in food: Individual food products. US Department of Health and Human Services, Center for Food Safety and Nutrition. Available from http://www.fda.gov/food/foodborneillnesscontaminants/chemicalcontaminants/ucm053549.htm#u1102

Wedzicha, B.L., Mottram, D.S., Elmore, J.S., Koutsidis, G., & Dodson, A.T. (2005) Kinetic models as a route to control acrylamide formation in food. Advances in Experimental Medicine and Biology 561, 235–253.

Wedzicha, B.L., Mottram, D.S. Elmore, J.S., Koutsidis, G., & Dodson A.T. (2005) Kinetic Models as a route to control acrylamide formation in food. In: Chemistry and safety of acrylamide in food, ed. M. Friedman & D. Mottram. Springer, New York, 235–253.

World Health Organization (WHO). (2003) Acrylamide in drinking-water: background document for preparation of WHO Guidelines for drinking-water quality. WHO/SDE/WSH/03.04/71. WHO, Geneva.

World Health Organization (WHO). (2005) Summary report of the 64th meeting of the Joint FAO/WHO expert committee on food additive (JECFA), Rome. ILSI Press International Life Sciences Institute, Washington, DC, 1–47.

World Health Organization (WHO). (2006) Joint FAO/WHO expert committee on food additives, 64th meeting Rome, 8–17 February 2005. Available from http://www.who.int/foodsafety/chem/jecfa/publications/reports/en/

World Health Organization (WHO). (2011) Evaluation of certain contaminants in food. Seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives. Technical Report Series No. 959. WHO, Geneva.

Yasuhara, A., Tanaka, Y., Hengel, M., & Shibamoto, T. (2003) Gas chromatographic investigation of acrylamide formation in browning model systems. Journal of Agricultural and Food Chemistry 51, 3999–4003.

Yaylayan, V.A., Wnorowski, A., & Perez Locas, C. (2003) Why aparagine needs carbohydrates to generate acrylamide. Journal of Agricultural and Food Chemistry 51, 1753–1757.

Zyzak, D.V., Sanders, R.A., Stojanovic, M., Tallmadge, D.H., Eberhart, B.L., Ewald, D.K., Gruber, D.C., Morsch, T.R., Strothers, M.A., Rizzi, G.P., & Villagran, M.D. (2003) Acrylamide formation mechanism in heated foods. Journal of Agricultural and Food Chemistry 51, 4782–4787.

19 Antioxidants of olive oil, olive leaves, and their bioactivity

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19.1 Introduction

Antioxidants are compounds that can slow down or prevent the oxidation of fats, oils, and foods containing fatty substances by suspending the induction of, and reducing, autoxidation reactions (Sherwin, 1990; Frankel, 1996; Shahidi & Wanasundara, 1998). The activity of antioxidants is best felt during the initiation period of oxidation. Subsequently, the oxidation moves rapidly, because the concentration of the antioxidant is reduced and more free radicals are present. The hydroperoxide content is gradually increased, and the leftover antioxidant is destroyed very fast. A longer initiation period shows a more powerful activity of antioxidants, which includes phenolic compounds that are able to slow down or prevent the oxidation of fatty substances, by binding the free radicals that are formed during oxidation:

$$ROO^{\cdot} + AnOH \rightarrow ROOH + AnO^{\cdot} \tag{19.1}$$

where *AnOH* represents the phenolic antioxidant, *ROOH* is hydroperoxides, $AnO^{\cdot}$ is the antioxidant radical, and $ROO^{\cdot}$ is the peroxyl radical.

In the reaction in Equation (19.1), the antioxidants offer a hydrogen atom, and the resultant hydroperoxides so produced are decomposed afterward. The effect of antioxidants in inhibiting hydroperoxide decomposition could be critical in preserving oil quality by reducing rancidity due to aldehyde and other carbonyl compound formation (Frankel, 1996). The antioxidant radical can take part in subsequent reactions, as shown here:

$$AnO^{\cdot} + ROO^{\cdot} \rightarrow ROO - AnO \tag{19.2}$$

$$AnO^{\cdot} + ROOH \rightarrow ROO^{\cdot} + AnOH \tag{19.3}$$

$$2AnO^{\cdot} \rightarrow \text{inactive products} \tag{19.4}$$

$$AnO^{\cdot} + RH \rightarrow AnOH + R^{\cdot} \tag{19.5}$$

Phenols with high antioxidant activity (hydroxytyrosol, oleuropein, and caffeic acid) are the ones that favor the reactions in Equations (19.2) and (19.4), more than the reactions in Equations (19.3) and (19.5). As a result, free radicals are inactivated, and the chain reactions are stopped. Tyrosol favors the reactions in Equations (19.3) and (19.5). Under these conditions, radicals that help chain reactions are set free. This explains the limited action of tyrosol as an antioxidant.

Much research has been carried out to help understand the basic processes of oxidation, the antioxidant action (Shahidi, 2000), and the effects of decomposition products of lipid oxidation. Frankel (1996) denoted

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that interfacial phenomena are keys to a better understanding of the antioxidant action in heterogeneous foods and biological systems.

Reactive oxygen species (ROS), such as superoxide anion ($O_2^{\cdot}$), hydroxyl radical ($HO^{\cdot}$), hydrogen peroxide (H_2O_2), free radicals ($R^{\cdot}$, $RO^{\cdot}$, $ROO^{\cdot}$), singlet oxygen ($^1O_2$), and peroxynitrite (ONOO), can be generated from autoxidation and thermal oxidation of lipids as well as from many cellular oxidative pathways (Fang *et al.*, 2002; Briante *et al.*, 2003; Huang *et al.*, 2005). Imbalance between generation and removal of ROS can cause oxidative stress in which excess ROS attacks and virtually damages all biomolecules in the cells, leading to cell death and serious chronic diseases (Scandalios, 2005). To minimize the physiological damage caused by excess ROS, a wide array of enzymatic and non-enzymatic endogenous antioxidant defense systems has evolved to compensate the generation of ROS (Sies, 1993; Fridovich, 1997).

The superoxide dismutase (SOD) catalyzes the dismutation of ($O_2^{\cdot -}$) to oxygen and hydrogen peroxide. Thus, it comprises an important antioxidant defense in nearly all cells exposed to oxygen (Fang *et al.*, 2002, Lee *et al.*, 2009). The dismutation of superoxide by SOD may be written with the following half-reactions:

$$M(n+1)+-SOD+O_2^{\cdot -} \rightarrow Mn+-SOD+O_2$$
$$Mn+-SOD+O_2^{\cdot -}+2H+ \rightarrow M(n+1)+-SOD+H_2O_2$$

where *M* is Cu+, Mn^{++}, Fe^{++}, or Ni^{++}. In these reactions, the oxidation state of the metal cation changes between *n* and *n*+1.

Antioxidants in general, natural or synthetic, prevent oxidation by arresting free radicals, chelating metal ions, or deactivating molecular oxygen (Kiritsakis, 1998; Shahidi & Zhong, 2010).

19.2 Synthetic antioxidants

In the past, oxidation of oils was mainly controlled by the addition of powerful synthetic antioxidants (Monahan & Troy, 1997). However, concerns about their potential toxic, pathogenic, and carcinogenic effects have been raised (Peters *et al.*, 1996; Amarowicz *et al.*, 2000). Shahidi (1998) summarized results of reports on various types of cancer in guinea pigs from the use of synthetic antioxidants, namely butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butylhydroquinine (TBHQ). Thus, the practice of using synthetic antioxidants has changed in recent years to a very large extent. The most powerful synthetic antioxidant, TBHQ, was not allowed in several countries until only a few years ago, and BHA was removed from the generally recognized as safe (GRAS) list of compounds (Goli *et al.*, 2005; Bouaziz *et al.*, 2008).

It is important to note that many recent investigations have focused on the identification of alternative novel antioxidants from natural sources with properties similar to those of the synthetic ones (McCarthy *et al.*, 2001; McBride *et al.*, 2007; Hossain *et al.*, 2008; Hayes *et al.*, 2010; Brahmi *et al.*, 2012; Afaneh *et al.*, 2015).

19.3 Natural antioxidants

Natural foods and food-derived antioxidants such as phenolic phytochemicals and vitamins have received considerable attention because they are known to function as health-promoting agents that act against oxidative damages (Valavanidis *et al.*, 2004; Carrasco-Pancorbo *et al.*, 2005; Perez-Bonilla *et al.*, 2006; Lee *et al.*, 2009; Hayes *et al.*, 2010, 2004). Thus, the presence of phenolic compounds (phenolic acids, polyphenols, and flavonoids) in plants, herbs (especially herbal infusions, frequently used as home medicines), and spices is gaining increasing attention because of their various functions, such as antioxidative, pharmacological, and flavoring properties (Sacchetti *et al.*, 2005). It should be noted, however, that only those natural antioxidants can be legally used as such in foods that have required daily intake (RDI) values, and these include only vitamins C, E, and A as well as selenium. Therefore, other natural sources of antioxidants are used under the disguise of flavorants, such as rosemary and sage, or green tea as a natural ingredient, as well as mixed tocopherols from deodorizer-distillate, mainly from soybean oil processing. Other sources of antioxidants that are used are included as binders, such as flours and other seed components.

19.3.1 Tocopherols

Tocopherols have a fundamental role as oil antioxidants, scavenging radicals in cellular and subcellular membranes and lipoprotein particles. Tocopherols prevent oxidation while at the same time they are oxidized. The antioxidant properties of tocopherols depend not only on their deactivation ability on lipid radicals, but also on the interactions between them. α-Tocopherol behaves as a prooxidant at concentrations higher than 250 μg/g, on the basis of peroxide value, but it is very effective in inhibiting hexanal formation in refined, bleached, and deodorized (RBD) olive oil (Frankel, 1996).

Tocopherols are generally destroyed by heat and the conditions employed during the refining process. For this reason, the concentration of tocopherols in refined olive oil is less than that in virgin olive oil (Kiritsakis, 1998).

19.3.2 Phenols

Compounds with at least one aromatic ring and one or more hydroxyl groups on the ring are characterized as phenols. They work as hydrogen donors and are able to inactivate free radicals. Their antioxidant activity is boosted due to the presence of groups that donate electrons. Some phenols have the tendency to destroy or inactivate free radicals, while others act on hydroperoxyl radicals. Phenols containing more than one phenolic hydroxyl group in their molecule are known as polyphenols and have a better ability to inactivate free radicals than monophenols.

Phenols containing one carboxylic acid group are known as phenolic acids. This class can be divided into two main subgroups: the hydroxybenzoic acids, which are derivatives of benzoic acid, and the hydroxycinnamic acids, which are derivatives of cinnamic acid. A general structure of C6–C1 and C6–C3 characterizes these two groups, respectively. Examples of hydroxybenzoic acids include gallic, vanillic, and syringic acids, while caffeic, ferulic, and sinapic acids are hydroxycinnamic acids. The antioxidant activity of phenolic acids and their derivatives is dictated by the number and position of hydroxyl groups attached to the aromatic ring.

Tyrosol and hydroxytyrosol, which are present in virgin olive oil, are phenolic products of hydrolyzed oleuropein. Hyroxytyrosol is found in high concentrations in olive oil and shows significant antioxidant activity (Montedoro *et al.*, 1992; Tsimidou *et al.*, 1992).

19.3.3 Flavonoids

Flavonoids comprise the largest group of phenolic compounds derived from plants. More than 6000 flavonoids have been reported in the literature, which account for more than half of the naturally occurring phenolics. The distinguished classes of flavonoids are flavanones, flavonols, flavones, flavanols (catechins), isoflavones, flavanonols, and anthocyanidins. The flavonoids, mainly routin and glucosides of apigenin and luteolin, are present in olive leaves.

Apigenin **Luteolin**

Over the last several years, the interest in the biological properties of flavonoids has increased, mainly due to their role in heart diseases. Different flavonoids have been reported for their ability to lower the level of oxidation of low-density lipoproteins, thus resulting in a lower risk of thrombosis. Flavonoids have also demonstrated the ability to lower the oxidation of food lipids (Skerget *et al.*, 2005). In general, flavonoids are considered to be highly effective antioxidants mainly because of their ability to deactivate free radicals

and scavenge and reduce singlet oxygen. Compared to phenolic acids, flavonoids generally display a higher antioxidant activity.

Flavonols, flavans-3-ols, and flavones with catechol structures are the most efficient olive phenolic compound quenchers, because more free hydroxyl groups are present in the flavonoid structure. The ability of phenolic compounds present in olive leaf to scavenge free radicals is mainly influenced by the number and position of free hydroxyl groups in their structure (Benavente-Garcia *et al.*, 2000).

19.3.4 Carotenoids

Carotenoids are a group of pigments found in olive oil and elsewhere. Carotenes and especially β-carotene act as singlet oxygen quenchers (Kiritsakis, 1998). The xanthophyll lutein, found in olive leaf extracts (OLEs), has shown potential *in vitro* and is considered to be useful in the prevention of diseases in which free radicals are implicated.

19.4 Phenols in table olives

The major phenolic compounds present in table olives are simple phenols, polyphenols, acyl glycosides, and flavonoids (Owen *et al.*, 2004). Oleuropein and hydroxytyrosol are the main phenolic components of table olives (Garcia *et al.*, 2008). According to Garrido-Fernandez *et al.* (1997) and Zoidou *et al.* (2010), most of the polyphenols are lost during the preparation of table olives due to degradation, hydrolysis, or dilution in the brine. The amount that remains in the final product, however, exhibits significant antioxidant activity (Soler-Rivas *et al.*, 2000; Zoidou *et al.*, 2010).

The possibility to fortify table olives of the 'Kalamon' cultivar with oleuropein and hydroxytyrosol using polyphenols, extracted from olive leaves, was studied by Lalas *et al.* (2011). OLE used for the treatment of table olives showed a 109% and 457% increase in the latter's hydroxytyrosol and oleuropein contents, respectively. Sensory evaluation of treated table olives showed an increase in bitterness. However, treated and untreated table olives showed equal overall acceptability and preference.

19.5 Phenols and other constituents of olive leaves and other olive tree products

Olive leaves are important by-products of tree pruning and fruit harvesting. They are also one of the olive mills' by-products, and their quantity may vary based on the amount of olive leaves that accompany the olive fruits (Bouaziz *et al.*, 2008). According to Kiritsakis (1998), phenolic compounds of simple or complex structure have been found in olive leaves. Olive leaves contain 6 to 15% oleuropein (main ingredient). Generally, olive leaves, olive fruit, olive oil, and even olive wood contain many phenolic compounds, with the most important ones being oleuropein and hydroxytyrosol (3,4-dihydroxyphenylethanol), accompanied by verbascoside, luteolin-7-glucoside, and rutin (Benavente-Garcia *et al.*, 2000; Hayes *et al.*, 2010). Thus, olive leaves as a rich source of a variety of phenolic compounds have attracted much attention during the last decade, mainly due to their antioxidant contributions to human health (Benavente-Garcia *et al.*, 2000; Skerget *et al.*, 2005; Lee *et al.*, 2009). The concentration of individual flavonoids in mg/kg was quercetin, 62; luteolin, 278; apigenin, 28; and myricetin, 14 (Skerget *et al.*, 2005). Figure 19.1 shows the structures of the most abundant phenolics in OLE.

The demand for olive leaves and OLEs has been increasing for utilization as food additives and supplements. OLEs also seem to be effective stabilizers in vegetable oils under oxidative conditions (Farag *et al.*, 2003; Paiva-Martins *et al.*, 2007; Bouaziz *et al.*, 2008). Thus, the extraction of polyphenols from olive leaves has been studied (Paiva-Martins and Gordon, 2001; Shuichi, 2004; Paiva-Martins *et al.*, 2009), because addition of extract to olive oil increases its shelf life. In addition, OLE, which is a dark brown, bitter-tasting liquid, contains many different compounds often called "olive biophenols." As mentioned, these include oleuropein as the most abundant biophenol, followed by verbascoside, apigenin-7-glucoside, and luteolin-7-glucoside, which serve as natural antioxidants with biological effects (Briante *et al.*, 2002; Kiritsakis *et al.*, 2010). Like

Phenolic compound	Chemical formula
Oleuropein	
Hydroxytyrosol	
Verbascoside	
Apigenin-7-glucoside	
Luteolin-7-glucoside	

Figure 19.1 Chemical structures of the most abundant phenolics in olive leaf extract. *Source*: Benavente-Garcia *et al.* (2000). Reproduced with permission of Elsevier.

with many other natural products, the composition of the leaf extract depends on geographical location, plant nutrition, and cultivar.

Olive leaves are rich in amino acids such as arginine, leucine, proline, glycine, valine, and alanine and low in cysteine, methionine, and lysine. Olive leaves can be considered as a rich source of valuable nutrients, a fact that has also justified their usage as animal feed.

Figure 19.2 The main reaction products obtained from oleuropein hydrolysis by hyperthermophilic β-glucosidase at pH 7.0 and at 60 °C. Rearrangement product of oleuropein aglycon (compound 1) and two forms of elenolic acid (compounds 2 and 3).

19.5.1 Oleuropein

Oleuropein is the main phenolic compound in olive tree leaves and fruits, as well as every other part of the olive tree. It has a myriad of beneficial effects on human health with medicinal potential. Although most of the studies have been performed *in vitro*, some statistical studies on human populations indicate that oleuropein is one of the best-known health promoters (Soler-Rivas *et al.*, 2000; Schroder, 2007). A linear relationship exists between oleuropein content and antioxidant effect of the OLEs (Ranalli *et al.*, 2006; Conde *et al.*, 2008).

Oleuropein is a phenolic secoiridoid compound and has a similar formula to dimethyl oleuropein, as well as the ligstroside, verbascoside, and oleuroside forms of oleuropein. High-performance liquid chromatography (HPLC) analyses showed that oleuropein may contribute up to 14% to the leaf extract, and hydroxytyrosol is the principal product of oleuropein degradation.

Many molecules isolated from olive fruit and olive leaves originate from oleuropein, via aglycon, by the opening of the elenolic acid ring with a final rearrangement into the secoiridoid compound 1, many forms of elenolic acid, and simple phenolic compounds, such as hydroxytyrosol (Figure 19.2) (Briante et al., 2002).

Among these compounds, hydroxytyrosol has significant biological properties (Montedoro *et al.*, 1992; Manna *et al.*, 1999; Ruiz-Gutierrez *et al.*, 2000; Visioli *et al.*, 2000). Thus, several methods have been developed for its purification and isolation from olive mill wastewater (OMWW) as well as from virgin olive oil and olive leaves (Verhe *et al.*, 1992; Capasso *et al.*, 1994, 1996; Ruiz-Gutierrez *et al.*, 2000; Briante *et al.*, 2002; Galanakis, 2015).

19.6 Extraction and activities of phenolics

Lee *et al.* (2009) applied several extraction procedures to obtain OLEs and evaluated their phenolic content and composition. They further reported their antioxidant activities using various radical scavenging assays. Figure 19.3 shows a schematic diagram for fractionation of OLEs using 80% EtOH.

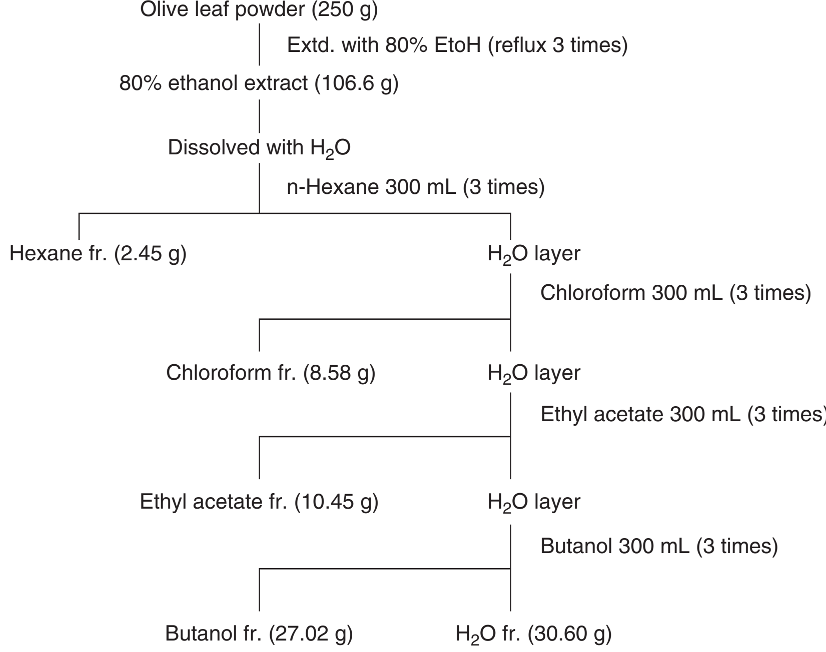

Figure 19.3 Schematic diagram that shows the fractionation of olive leaf extracted in 80% EtOH. *Source*: Lee *et al.* (2009). Reproduced with permission of Elsevier.

Ethanol extraction facilitates obtaining OLE with acceptable yields and important phenolic compounds. According to Gamel and Kiritsakis (1999), the antioxidant effectiveness of olive extracts is significantly affected by the extraction conditions and the polarity of the solvent used for the extraction.

Figure 19.4 shows SOD-like activity of 80% ethanolic extract, butanol, and ethyl acetate fractions that were higher than those of hexane, chloroform, and water fractions (Lee *et al.*, 2009). Although these are results from *in vitro* studies, nevertheless they may also reflect *in vivo* situations (Vissers *et al.*, 2002; Del Boccio *et al.*, 2003; Tan *et al.*, 2003).

Lee *et al.* (2009) tested the peroxide value (PV) of OLEs and other additives in a linoleic acid system and found that the PV of the control increased from 0.5 meq/kg oil to 101.8 meq/kg oil during autoxidation for 84 h and was much higher than in the samples containing extracts. The antioxidant efficiency of the butanol and ethyl acetate fraction was related to the contents of total flavonoids and phenolics, mainly as oleuropein, rutin, and vanillin.

For value-added use of olive leaves, the proper extraction of their bioactive compounds is necessary, and processing cost needs to be reduced as well. Heat treatment can improve extraction kinetics, but at the same time it may also reduce both the phenolic content and the antioxidant capacity of the extracts because polyphenols are sensitive to high temperatures.

Recent studies for industrial applications have proposed some alternatives to conventional extraction, such as supercritical extraction with CO_2, ultrasound-assisted extraction (Knorr *et al.*, 2004; Zhang *et al.*, 2009), microwave-assisted extraction (Hayat *et al.*, 2009), and superheated liquid extraction (Japón-Luján & Luque de Castro, 2006). The ultrasound extraction process is considered one of the most interesting and novel techniques to obtain valuable compounds from plant materials (Vilkhu *et al.*, 2008). The feasibility of using ultrasound to improve the phenolic extraction from olive leaves was assessed by Ahmad-Qasem *et al.* (2013). The extraction kinetics were monitored by measuring the total phenolic content and antioxidant capacity, and were mathematically described by Naik's model. Identification and quantitative analysis of the main polyphenols present were made by HPLC–diode array detection/tandem mass spectrometry (HPLC–DAD/MS–MS).

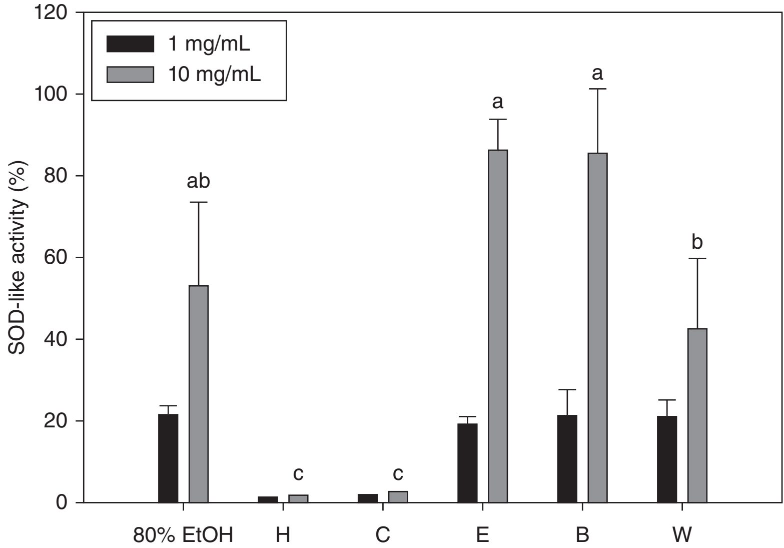

Figure 19.4 Superoxide dismutase (SOD)-like activities of olive leaf extract and fractions. 80% EtOH = 80% ethanol extract; H = hexane fraction; C = chloroform fraction; E = ethylacetate fraction; B = butanol fraction; W = water fraction. For a–c, means in the same column not sharing a common letter are significantly different ($p < 0.05$) by Duncan's multiple test. *Source*: Lee *et al.* (2009). Reproduced with permission of Elsevier.

The improvement of oleuropein extractability was studied by Stamatopoulos *et al.* (2012) by optimizing a steam blanching process as pretreatment of olive leaf extraction. They studied the impact of steam, hot water blanching, and UV-C irradiation as pretreatments on extraction of oleuropein and related biophenols (e.g., hydroxytyrosol, apigenin-7-glucoside, and luteolin-7-glucoside rutin) from olive leaves. Samples blanched with hot water showed significantly higher oleuropein yields and antioxidant activity compared to untreated samples. Oleuropein was found to be the major phenolic compound in olive leaves during this process, as in other studies (Benavente-Garcia *et al.*, 2000; Japón-Luján & Luque de Castro, 2006; Lee *et al.*, 2009; Kiritsakis *et al.*, 2010).

Subcritical water extraction (SWE) has recently emerged as a new possibility for the extraction of bioactive compounds from different sources (Mendiola *et al.*, 2007; Kim *et al.*, 2009; Fabian *et al.*, 2010; Herrero *et al.*, 2010; Monrad *et al.*, 2010; Kumar *et al.*, 2011). This advanced extraction technique leads to, among other things, the formation of compounds, such as 5-hydroxymethylfurfural (HMF). Herrero *et al.* (2010) determined the formation of HMF during SWE and the possible influence of this compound on the overall antioxidant and antiproliferative activities against colon cancer cells. The activity of this formed compound should be taken into consideration as an important bioactive compound obtained from olive leaves.

19.6.1 Factors affecting the presence of phenolic compounds in olive products

Several factors affect the phenolic compounds of olive tree products. These include the cultivar, age of the tree, leaf age, agronomical practices, geographical location, light intensity, sampling parameters, maturity stage of the fruit during harvesting, preservation conditions of the fruit before processing, type of olive oil mill used, and processing conditions (water quantity and temperature) (Kiritsakis, 1998; Gutierez *et al.*, 1999; Benevente-Garcia *et al.*, 2000; Paiva-Martins & Gordon, 2001; Salvador *et al.*, 2001; Torres & Maestri, 2006; Mylonaki *et al.*, 2008).

According to Aguilera *et al.* (2005), the total polyphenol content of olive oils varies between 50 and 1000 mg/kg, depending on the above factors. In the case of maturity stage, as ripening advances, certain metabolic processes may occur that involve changes in the profile of certain compounds, such as

Table 19.1 Mean contents of total phenols and stability of olive oil.

Stage of ripeness	Total phenols (mg caffeic/kg oil)	Stability (h)
Green	336.20	72.80
Spotted	281.50	66.10
Ripe	270.30	61.20

triacylglycerols, fatty acids, tocopherols, chlorophylls, carotenoids, and phenols (Gutierez *et al.*, 1999; Salvador *et al.*, 2001). Table 19.1 summarizes phenol content and oxidative stability of olive oil obtained from olive fruits at different maturity stages. A positive correlation existed between different values of oxidative stability and total polyphenols (Salvador *et al.*, 2001; Fuentes *et al.*, 2013).

Table 19.2 shows the tentative phenolic content derived from two olive cultivars processed in a centrifugal olive oil mill. Table 19.3, on the other hand, shows some of the phenolic compounds of virgin olive oil.

Papoti and Tsimidou (2009) studied the effects of cultivar, leaf age, and collection time on the phenolic content of olive leaves, and reported that the differentiation in the levels of individual components depended more on the harvesting time than on the olive cultivar. Oleuropein content is constantly reduced during the maturation process in the olive fruit, and dimethyloleuropein replaces oleuropein in about the same amounts.

Brahmi *et al.* (2012) determined possible seasonal changes in the total phenol content of olive leaves in two different periods, October and February, and observed a seasonal pattern for the total phenols, o-diphenols, and flavonoids between leaves harvested at the two stages. It seems that environmental factors like air, temperature, total radiation, soil temperature, and photoperiod have a major contribution to the phenol content of olive extracts.

Studies on the effect of copper treatment on olive leaves found that methanolic extracts without copper treatment exhibited better antioxidant properties than leaves treated with different copper formulations, which is in agreement with the higher content of phenols determined in the control sample. The extracts

Table 19.2 Tentative phenolic compounds from two olive cultivars (mg/kg).

	Cultivar	
Parts	'Picual'	'Koroneiki'
Fruit	485	495
Leaves	234	250
Olive oil	85	195
Pit	65	73
Wastewater	193	170

Table 19.3 Some phenolic compounds of virgin olive oil.

Compounds	
Tyrosol	p-Coumaric acid
Hydroxytyrosol	Syringic acid
Π-hydroxybenzoic acid	Gentisic acid
Oleuropein	Sinapic acid
o-Coumaric acid	Sicimic acid
Protocatechuic acid	Cinnamic acid
Vanillic acid	Apigenin
Caffeic acid	Luteolin
Hydrocaffeic acid	Quercetin

obtained from the leaves without any copper treatment scavenged DPPH radicals more effectively than extracts from the other leaves. The use of $Cu(OCl)_2$ decreased the radical scavenging activity significantly.

19.7 Antioxidant and other properties of olive phenolics

In general, phenolics in olive oil, olive leaves, and olive fruit are divided into polar and nonpolar. Phenolics of the nonpolar fraction offer substantial protection to olive oil from oxidation, while the polar fraction is less effective (Frankel, 1996). Phenolic compounds are degraded as a result of their antioxidant activity, and their degradation is proportional to their antioxidant activity.

Olive oil and olive leaves are the primary sources of phenolic compounds such as oleuropein and its derivatives such as hydroxytyrosol and tyrosol, as well as caffeic acid, *p*-coumaric acid, vanillic acid, vanillin, luteolin, diosmetin, rutin, luteolin-7-glucoside, apigenin-7-glucoside, and diosmetin-7-glucoside (Bianco & Uccella, 2000; Tasioula-Margari & Ologeri, 2001; Ryan *et al.*, 2002, 2003; Farag *et al.*, 2003).

The addition of phenolic compounds (tyrosol, hydroxytyrosol, caffeic acid, and oleuropein) to a substrate that contained linolenic acid slowed down the formation of hydroperoxides with conjugated double bonds (conjugated dienes) during oxidation. The prevention of conjugated dienes formation by phenolic compounds was in the order of tyrosol < caffeic acid < oleuropein < hydroxytyrosol. This is explained by the fact that caffeic acid, oleuropein, and hydroxytyrosol are o-diphenols and show greater antioxidant activity than tyrosol, which is a monophenol (Kiritsakis, 1998).

Polyphenols from olive leaves and from OMWW are effective when their concentration is over 200 mg/kg. Polyphenols derived from pomace oil prevented to some extent the oxidation of refined oil when they were added to it (Satue *et al.*, 1995). Also, phenolic ingredients derived from virgin olive oils enhanced the oxidative stability of refined olive oils. The maximum antioxidant activity was noted at addition levels of 100 and 200 mg/kg. Caffeic, ferulic, and vanillic acids showed greater antioxidant activity than *o*- and *p*-coumaric acid. In particular, vanillic acid prevented the formation of hexanal to a great extent. However, the antioxidant activity of phenolic compounds in olive oil is reduced when the initial hydroperoxides concentration is very high.

The demand for whole olive leaves and OLE has increased recently for their use in foodstuffs as additives and as functional food ingredients (Fernandez-Escobar *et al.*, 1999; Delgado-Pertinez *et al.*, 2000; Visioli *et al.*, 2000). Bouaziz *et al.* (2008) denoted that olive leaves' hydrolysate extract has high antioxidant activity and can stabilize refined olive oil and olive pomace oil to a greater extent than common synthetic antioxidants. Oils with added hydrolysate extract showed lower PV by the Rancimat method. Thus, the use of such extracts in the food industry may prolong the shelf life of food products and also contribute to the health benefit of the consumers. Olive leaves are commercialized in several forms, such as intact leaves, powdered leaves, and extracts. Figure 19.5 shows the antioxidant mechanism of hydroxytyrosol.

Comparing the antioxidative activities of OLEs, oleuropein, hydroxytyrosol, and tyrosol with vitamin E and BHT by using kinetic studies, it was observed that olive extracts, oleuropein, and hydroxytyrosol were more effective than BHT or vitamin E in extending the induction period. Hayes *et al.* (2010) examined the effects of OLE on lipid oxidation (thiobarbituric acid-reactive substances [TBARS]) in packed raw beef and noticed that they reduced TBARS significantly ($p < 0.001$). Extracts from olive tree leaves, added in at 250 mg/kg, increased the oxidative stability of virgin olive oil, pomace oil, and corn oil when heated at 180 and 220 °C.

In addition to antioxidant action, OLEs exhibit antimicrobial properties against bacteria, fungi, and mycoplasma (Aziz *et al.*, 1998; Benavente-Garcia *et al.*, 2000; Andrikopoulos *et al.*, 2002; Furneri *et al.*, 2002; Briante *et al.*, 2003; Del-Rio *et al.*, 2003; Somova *et al.*, 2003). They are effective against many pathogenic microorganisms, such as the flu virus, and also against yeasts and bacteria. The activity of a commercial extract derived from olive leaves was investigated against a wide range of microorganisms ($n = 122$) and was found to be most effective against *Campylobacter jejuni*, *Helicobacter pylori*, and *Staphylococcus aureus*, with minimum inhibitory concentrations (MICs) as low as 0.31–0.78% (v/v). However, the extract showed little activity against all other tested organisms ($n = 79$) (Hayes *et al.*, 2010). These antioxidant and antimicrobial actions makes the olive tree resistant to insects and bacterial infestation.

Figure 19.5 Antioxidant mechanism of hydroxytyrosol. *Source*: Bouaziz *et al.* (2008). Reproduced with permission of Elsevier.

19.7.1 Functional activity of olive leaves and olive oil

Phenolics from olive leaves and olive oil function as chemopreventive agents against oxidative damage (Valavanidis *et al.*, 2004; Carrasco-Pancorbo *et al.*, 2005; Perez-Bonilla *et al.*, 2006). Olive leaves can lower blood pressure and increase blood flow in the coronary arteries (Zarzuelo, 1991; Khayyal *et al.*, 2002).

Data on the use of olive leaves against hypertension have been available since the 1950s. There is also a positive effect from minor components, mainly flavonoids, of olive leaves in cardiovascular diseases. Another important activity of olive leaf polyphenols is the prevention of blood clotting and inflammation (Visioli *et al.*, 2000). Several reports have shown that OLE increased blood flow in the coronary arteries (Zarzuelo, 1991), relieved arrhythmia, and prevented intestinal muscle spasms. Generally, OLE exhibits a powerful *in vitro* activity.

OLE, olives, and olive oil may play a role in altering the composition of the gastric flora. It is possible that after ingestion and metabolism of OLE, the biophenol metabolites may have more antimicrobial activity than the unmetabolized components (Hayes *et al.*, 2010).

Olive leaves have traditionally been used by ancient Egyptian and Mediterranean cultures to treat a variety of health problems (Polymerou-Kamilakis, 2004). Historically, olive leaves had been used as a traditional medicine for fever and malaria. Nowadays, olive leaves are recommended for many diseases, such as diabetes, cardiovascular diseases, and viral and microbial infections (Anonymous, 2009). Olive leaves can be used not only therapeutically but also as food additives. In the past, powdered dried olive leaves were used as an ingredient for the preparation of a traditional food called *bsissa* in Tunisia. Lately, OLE has been investigated for use as an additive to food products (Bouaziz *et al.*, 2008; Hayes *et al.*, 2010).

When oleuropein is metabolized in the body with the enzyme β-glucosidase, the resistance of the cells against viruses, bacteria (mainly lactic acid bacteria), and yeasts is strengthened. Beauchamp *et al.* (2005)

denoted that extra virgin olive oil has a component with a similar action to that of the anti-inflammatory drug ibuprofen. This component is called oleocanthal and is the main substance responsible for throat irritation and bitter taste of olive oil. and therefore the best way to find an extra virgin olive oil rich in oleocanthal is to taste it. Oleocanthal of extra virgin olive oil is structurally related to ibuprofen and has the ability to inhibit pain through the system of prostaglandins. Ibuprofen on high doses protects from some forms of cancer. The entire similarity in structure of ibuprofen and oleocanthal present in olive oil enhances the anti-inflammatory action of the latter.

It is believed that the biological activity of oleocanthal is partially responsible for the beneficial health effects of the Mediterranean diet. Virgin olive oil containing oleocanthal is often added as an ingredient in a number of cooked dishes, and therefore it is of great importance to understand how best to preserve the health-promoting benefits of this compound. This is because other olive oil phenolics are generally subjected to degradation upon heating, whereas oleocanthal is heat resistant. When extra virgin olive oil containing 53.9 mg/kg oleocanthal was heated at various temperatures (100, 170, and 240 °C) for various periods (0, 1, 5, 20, 60, and 90 min), oleocanthal was more resistant, compared to other olive oil phenolics, with a maximum loss of 16% as determined by HPLC analysis. However, there was a significant decrease of up to 31% ($p < 0.05$) in the biological activity of oleocanthal as determined by the taste bioassay. The reduction of the biological activity of oleocanthal after heat treatment could be the result of an oleocanthal antagonist formation. This probably decreases the biological activity of oleocanthal (Beauchamp *et al.*, 2005).

HO O O H CHO CHO O OH

Oleocanthal **Ibuprofen**

Olive oil, when used as the basic source of fat, offers major antiatherogenic effects that are not observed to the same extent in other oleic acid–rich oils. This is probably due to the unique microconstituents present in olive oil (Kiritsakis, 1998). Gentile and Uccella (2014), using olive callus cultures, were able to biosynthesize olive biophenols (OBPs) and biophenol-secoiridoids (OBPsecos). They reported that by using a bioreactor system and olive callus cultures, it is possible to produce nutraceutical bioactives that are useful to human health.

19.8 Conclusion

Olive oil contains a variety of phenolic compounds with significant antioxidant and health benefits. Similarly, olive leaves, a by-product of the olive tree cultivation and fruit processing, offer many beneficial effects and potential application for use in food and supplements, with activity against oxidative stress and chemopreventive activity. The minor ingredients, mainly oleocanthal and oleacin, seem to prevent blood clotting and inflammation. Finely chopped olive leaves are also being used as herbal tea.

References

Afaneh, I., Yateem, H., & Al-Rimawi, F. (2015) Effect of olive leaves drying on the content of oleuropein. American Journal of Analytical Chemistry 6, 246–252.

Ahmad-Qasem, H.M., Cánovas, J., Barrajón-Catalán, E., Micol, V., Cárcel, J.A., & García-Pérez, J.V. (2013) Kinetic and compositional study of phenolic extraction from olive leaves (var. Serrana) by using power ultrasound. Innovative Food Science and Emerging Technologies 17, 120–129.

Amarowicz, R., Naczk, M., & Shahidi, F. (2000) Antioxidant activity of various fractions of non-tannin phenolics of canola hulls. Journal of Agriculture and Food Chemistry 48, 2755–2759.

Andrikopoulos, N.K., Antonopoulou, S., & Kaliora, A.C. (2002) Oleuropein inhibits LDL oxidation induced by cooking oil frying by-products and platelet aggregation induced by platelet-activating factor. Lebensmittel-Wissenschaft & Technologie 35, 479–484.

Anonymous. (2009) Olive leaf. Alternative Medicine Review 14, 62–66.

Aziz, N.H., Farag, S.E., Mousa, L.A., & Abo-Zaid, M.A. (1998) Comparative antibacterial and antifungal effects of some phenolic compounds. Microbios 93, 43–54.

Beauchamp, G.K., Keast, R.S, Morel, D., Lin, J., Pika, J., Han, Q., Lee, C.H., Smith, A.B., & Breslin, P.A. (2005) Phytochemistry: ibuprofen-like activity in extra virgin olive oil. Nature 437, 45–46.

Benavente-Garcia, O., Castillo, J., Lorente, J., Ortuno, A., & Del-Rio, J.A. (2000) Antioxidant activity of phenolics extracted from *Olea europaea* L. leaves. Food Chemistry 68, 457–462.

Bianco, A., & Uccella, N. (2000) Biophenolic components of olives. Food Research International 33, 475–485.

Bouaziz, M., Fki, I., Jemai, H., Ayadi, M., & Sayadi, S. (2008) Effect of storage on refined and husk olive oils composition: stabilization by addition of natural antioxidants from Chemlali olive leaves. Food Chemistry 108, 253–262.

Brahmi, F., Mechri, B., Dabbou, S., Dhibi, B., & Hammami, M. (2012) The efficacy of phenolics compounds with different polarities as antioxidants from olive leaves depending on seasonal variations. Industrial Crops and Products 38, 146–152.

Briante, R., Febbraio, F., & Nucci, R. (2003) Antioxidant properties of low molecular weight phenols present in the Mediterranean diet. Journal of Agriculture and Food Chemistry 51, 6975–6981.

Briante, R., La Cara, F., Febbraio, F., Patumi, M., & Nucci, R. (2002) Bioactive derivatives from oleuropein by a biotransformation on *Olea europaea* leaf extracts. Journal of Biotechnology 93, 109–119.

Capasso, R., Evidente, A., & Visca, C. (1994) Production of hydroxytyrosol from olive oil vegetation water. Agrochimica 38, 165–172.

Capasso, R., Evidente, A., Visca, C., Gianfreda, L., Maremonti, M., & Greco, G., Jr. (1996) Production of glucose and bioactive aglycone by chemical and enzymatic hydrolysis of purified oleuropein from *Olea europaea*. Applied Biochemistry and Biotechnology 60, 365–377.

Carrasco-Pancorbo, A., Cerretani, L., Bendini, A., Segura-Carretero, A., Carlo, M.D., Gallina-Toschi, T., Lercker, G., Compagnone, D., & Fernandez-Gutierrez, A. (2005) Evaluation of the antioxidant capacity of individual phenolic compounds in virgin olive oil. Journal of Agriculture and Food Chemistry 53, 8918–8925.

Conde, E., Cara, C., Moure, A., Ruiz, E., Castro, E., & Domínguez, H. (2008) Antioxidant activity of the phenolic compounds released by hydrothermal treatments of olive tree pruning. Food Chemistry 114, 806–812.

Del Boccio, P., Pi Deo, A., De Curtis, A., Celli, N., Iacoviello, L., & Rotilio, D. (2003) Liquid chromatography–tandem mass spectrometry analysis of oleuropein and its metabolite hydroxytyrosol in rat plasma and urine after oral administration. Journal of Chromatography B 785, 47–56.

Delgado-Pertinez, M., Gomez-Cabrera, A., & Garrido, A. (2000) Predicting the nutritive value of the olive leaf (*Olea europaea*): digestibility and chemistry composition and in vitro studies. Animal Feed Science and Technology 87, 187–201.

Del-Rio, J.A., Baidez, A.G., Botia, J.M., & Ortuno, A. (2003) Enhancement of phenolic compounds in olive plants (*Olea europaea* L.) and their influence on resistance against Phytophthora sp. Food Chemistry 83, 75–78.

Fabian, C., Tran-Thi, N.Y., Kasim, N.S., & Ju, Y.H. (2010) Release of phenolic acids from defatted rice bran by subcritical water treatment. Journal of the Science of Food and Agriculture 90, 2576–2581.

Fang, Y.Z., Yang, S., & Wu, G. (2002) Free radicals, antioxidants, and nutrition. Nutrition 18, 872–887.

Farag, R.S., El-Baroty, G.S., & Basuny, A.M. (2003) Safety evaluation of olive phenolic compounds as natural antioxidants. International Journal of Food Sciences and Nutrition 54, 159–174.

Fernandez-Escobar, R., Moreno, R., & Garcia-Creus, M. (1999) Seasonal changes of mineral nutrients in olive leaves during the alternate-bearing cycle. Scientia Horticulturae 82, 25–45.

Frankel, E.N. (1996) Antioxidants in lipid foods and their impact on food quality. Food Chemistry 57, 51–55.

Fridovich, I. (1997) Superoxide anion radical, superoxide dismutase and related matters. Journal of Biological Chemistry 272, 18515–18517

Fuentes, M.M., De Miguel Gordillo, C., Marín Expóxito, J., Sánchez Casas, J., Martínez Cano, M., Martín Vertedor, D., & Franco Baltasar, M.N. (2013) Chemical composition of virgin olive oils according to the ripening in olives. Food Chemistry 141, 2575–2581.

Furneri, P.M., Marino, A., Saija, A., Uccella, N., & Bisignano, G. (2002) In vitro antimycoplasmal activity of oleuropein. International Journal of Antimicrobial Agents 20, 293–296.

Galanakis, M.C. (2015) Separation of functional macromolecules and micromolecules: from ultrafiltration to the border of nanofiltration. Trends in Food Science & Technology 42, 44–63.

Gamel, H.T., &. Kiritsakis, A.K. (1999) Effect of methanol extracts of rosemary and olive vegetable water on the stability of olive oil and sunflower oil. Grasas y Aceites 50, 345–350.

Garcia, A., Romero, C., Medina, E., Garcia, P., De Castro, A., & Brenes, M. (2008) Debittering of olives by polyphenol oxidation. Journal of Agricultural and Food Chemistry 56, 11862–11867.

Garrido-Fernández, A, Fernández-Diez, M.J., & Adams, M.R. (1997) Control methods. In: Table olives: production and processing, ed. A. Garrido-Fernández, M.J. Fernández-Diez, & M.R. Adams. Chapman and Hall, London, 461–479.

Goli, A.H., Barzegar, M., & Sahari, M.A. (2005) Antioxidant activity and total phenolic compounds of pistachio (*Pistachia vera*) hull extracts. Food Chemistry 92, 521–525.

Gutierez, F., Jimenez, B., Ruiz, A., & Albi, M.A. (1999) Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties Picual and Hojiblanca and on the different components involved. Journal of Agricultural and Food Chemistry 47, 121–127.

Hayat, K., Hussain, S., Abbas, S., Farooq, U., Ding, B.M., & Xiam, S.Q. (2009) Optimized microwave-assisted extraction of phenolic acids from citrus mandarin peels and evaluation of antioxidant activity in vitro. Separation and Purification Technology 70, 63–70.

Hayes, J.E., Stepanyan, V., Allen, P., O'Grady, M.N., & Kerry, J.P. (2010) Effect of lutein, sesamol, ellagic acid and olive leaf extract on the quality and shelf-life stability of packaged raw minced beef patties. Meat Science 84, 613–620.

Herrero, M., Plaza, M., Cifuentes, A., & Ibanez, E. (2010) Green processes for the extraction of bioactives from rosemary: chemical and functional characterization via ultra-performance liquid chromatography–tandem mass spectrometry and invitro assays. Journal of Chromatography A 1217, 2512–2520.

Hossain, M.B., Brunton, N.P., Barry-Ryan, C., Martin-Diana, A.B., & Wilkinson, M. (2008) Antioxidant activity of spice extracts and phenolics in comparison to synthetic antioxidants. Rasayan Journal of Chemistry 1, 751–756.

Huang, D., Ou, B., & Priop, R.L. (2005) The chemistry behind antioxidant capacity assays. Journal of Agriculture and Food Chemistry 53, 1841–1856.

Japón-Luján, R., & Luque de Castro, M.D. (2006) Superheated liquid extraction of oleuropein and related biophenols from olive leaves. Journal of Chromatography A 1136, 185–191.

Khayyal, M.T., El-Ghazaly, M.A., Abdallah, D.M., Nassar, N.N., Okpanyi, S.N., & Kreuter, M.H. (2002) Blood pressure lowering effect of an olive leaf extract (*Olea europaea*) in L-NAME induced hypertension in rats. Arzneimittelforschung 52, 797–802.

Kim, J.W., Nagaoca, T., Ishida, Y., Hasegawa, T., Kitagawa, K., & Lee, S.C. (2009) Subcritical water extraction of nutraceutical compounds from citrus pomaces. Separation Science and Technology 44, 2598–2608.

Kiritsakis, A. (1998) Olive oil: from the tree to the table, 2nd ed. Food and Nutrition Press, Trumbull, CT, 227–236.

Kiritsakis, K., Kontominas, M., Kontogiorgis, C., Hadjipavlou-Litina, D., Moustakas, A., & Kiritsakis, A. (2010) Composition and antioxidant activity of olive leaf extracts from Greek olive cultivars. Journal of the American Oils Chemists' Society 87, 369–376.

Knorr, D., Zenker, M., Heinz, V., & Lee, D.U. (2004) Applications and potential of ultrasonics in food processing. Trends in Food Science and Technology 15, 261–266.

Kumar, Y., Dutta, R., Prasad, D., & Misra, K. (2011) Subcritical water extraction of antioxidants compounds from sea buckthorn (*Hippophae rhamnoides*) leaves for the comparative evaluation of antioxidant activity. Food Chemistry 127, 1309–1316.

Lalas, S., Athanasiadis, V., Gortzi, O., Bounitsi, M., Giovanoudis, J., Tsaknis, J., & Bogiatzis, F. (2011) Enrichment of table olives with polyphenols extracted from olive leaves. Food Chemistry 127, 1521–1525.

Lee, O.H., Lee, B.Y., Lee, J., Lee, H.B., Son, J.Y., Park, C.S., Shetty, K., & Kim, Y.C. (2009) Assessment of phenolics-enriched extract and fractions of olive leaves and their antioxidant activities. Bioresource Technology 100, 6107–6113.

Manna, C., Della Ragione, F., Cucciolla, V., Borriello, A., D'Angelo, S., Galletti, P., & Zappia, V. (1999) Biological effects of hydroxytyrosol, a polyphenol from olive oil endowed with antioxidant activity. Advances in Experimental Medicine and Biology 472, 115–130.

McBride, N., Hogan, S., & Kerry, J.P. (2007) Comparative addition of rosemary extract and additives on sensory and antioxidant properties of retail packaged beef. International Journal of Food Science & Technology 42, 1201–1207.

McCarthy, T.L., Kerry, J.P., Kerry, J.F., Lynch, P.B., & Buckley, D.J. (2001) Evaluation of the antioxidant potential of natural food/plant extracts as compared with synthetic antioxidants and vitamin E in raw and cooked pork patties. Meat Science 58, 45–52.

Mendiola, J.A., Herrero, M., Cifuentes, A., & Ibañez, E. (2007) Use of compressed fluids for sample preparation: food applications. Journal of Chromatography A 1152, 234–246.

Monahan, F.J., & Troy, D.J. (1997) Overcoming sensory problems in low fat and low salt products. In: Advances in meat research: production and processing of healthy meat, poultry and fish products, vol. 11, ed. A.M. Pearson & T.R. Dutson. Blackie Academic and Professional, London, 257–281.

Monrad, J.K., Howard, L.R., King, J., Srinivas, K., & Mauromoustakos, A. (2010) Subcritical solvent extraction of procyanidins from dried red grape pomace. Journal of Agriculture and Food Chemistry 58, 4014–4021.

Montedoro, G., Servili, M., Baldioli, M., & Miniati, E. (1992) Simple and hydrolyzable compounds in virgin oil. 1. Their extraction, separation and quantitative and semiquantitative evaluation by HPLC. Journal of Agriculture and Food Chemistry 40, 1571–1576.

Mylonaki, S., Kiassos, E., Makris, D.P., & Kefalas, P. (2008) Optimisation of the extraction of olive (*Olea europaea*) leaf phenolics using water/ethanol-based solvent systems and response surface methodology. Analytical and Bioanalytical Chemistry 392, 977–985.

Owen, R.W., Haubner, R., Würtele, G., Hull, W.E., Spiegelhalder, B., & Bartsch, H. (2004) Olives and olive oil in cancer prevention. European Journal of Cancer Prevention 13, 319–326.

Paiva-Martins, F., Barbosa, S., Pinheiro, V., Mourão, J.L., & Outor-Monteiro, D. (2009) The effect of olive leaves supplementation on the feed digestibility, growth performances of pigs and quality of pork meat. Meat Science 82, 438–443.

Paiva-Martins, F., Correia, R., Felix, S., Ferreira, P., & Gordon, M.H. (2007) Effects of enrichment of refined olive oil with phenolic compounds from olive leaves. Journal of Agricultural and Food Chemistry 55, 4139–4143.

Paiva-Martins, F., & Gordon, M.H. (2001) Isolation and characterization of the antioxidant component 3, 4-dihydroxyphenylethyl 4-formyl-3-formylmethyl-4-hexenoate from olive (*Olea europaea*) leaves. Journal of Agricultural and Food Chemistry 49, 4214–4219.

Papoti, V.T., & Tsimidou, M.Z. (2009) Impact of sampling parameters to the radical scavenging potential of olive (*Olea europaea* L.) leaves. Journal of Agricultural and Food Chemistry 57(9), 3470–3477.

Perez-Bonilla, M., Salido, S., Beek, T.A., Linares-Palomio, P.J., Altarejos, J., Nogueras, M., & Sanchez, A. (2006) Isolation and identification of radical scavengers in olive tree (*Olea europaea*) wood. Journal of Chromatography A 1112, 311–318.

Peters, M.M.C.G., Rivera, M.I., Jones, T.W., Monks, T.J., & Lau, S.S. (1996) Glutathione conjugates of tert-butyl-hydroquinone, a metabolite of the urinary tract tumor promoter 3-tert-butyl-hyroxyanisole, are toxic to kidney and bladder. Cancer Research 56, 1006–1011.

Polymerou-Kamilaki, A. (2004). Ode to the olive tree. Edition supported by the Secretary of Olympic Games, Athens.

Ranalli, A., Contento, S., Lucera, L., Di Febo, M., Marchegiani, D., & Di Fonzo, V. (2006) Factors affecting the contents of iridoid oleuropein in olive leaves (*Olea europaea* L.). Journal of Agricultural and Food Chemistry 54, 434–440.

Ruiz-Gutierrez, V., Juan, M.E., Cert, A., & Planas, J.M. (2000) Determination of hydroxytyrosol in plasma by HPLC. Analytical Chemistry 72, 4458–4461.

Ryan, D., Antolovich, M., Prenzler, P., Robards, K., & Lavee, S. (2002) Biotransformations of phenolic compounds in *Olea europaea* L. Scientia Horticulturae 92, 147–176.

Ryan, D., Prenzler, P.D., Lavee, S., Antolovich, M., & Robards, K. (2003) Quantitative changes in phenolic content during physiological development of the olive (*Olea europaea*) cultivar Hardy's Mammoth. Journal of Agriculture and Food Chemistry 51, 2532–2538.

Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., & Radice, M. (2005) Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chemistry 91, 621–632.

Salvador, M.D., Aranda F., & Fregapane, G. (2001) Influence of fruit ripening on Cornicabra virgin olive oil quality: a study of tour crop seasons. Food Chemistry 73, 45–53.

Satue, M.T., Huang, S.W., & Frankel, E.N. (1995) Effect of natural antioxidants in virgin olive oil on oxidative stability of refined, bleached and deodorized olive oil. Journal of the American Oils Chemists' Society 72, 1131–1137.

Scandalios, J.G. (2005) Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defence. Brazilian Journal of Medical and Biological Research 38, 995–1001.

Schroder, H. (2007) Protective mechanisms of the Mediterranean diet in obesity and type 2 diabetes. Journal of Nutritional Biochemistry 18, 149–160.

Shahidi, F. (2000) Antioxidants in food and food antioxidants. Food/Nahrung 44, 158 –163.

Shahidi, F., & Wanasundara, U.N. (1998) Methods of measuring oxidative rancidity in fats and oils. In: Food lipids: chemistry, nutrition, and biotechnology, 2nd ed., ed. C.C. Akoh & D.B. Min. CRC Press, Boca Raton, FL.

Shahidi, F., & Zhong, Y. (2010) Novel antioxidants in food quality preservation and health promotion. European Journal of Lipid Science and Technology 112(9), 930–940.

Sherwin, E.R. (1990) Antioxidants in food additives. Marcel-Dekker, New York.

Shuichi, H. (2004) Effect of drying temperature on the oleuropein content of olive (*Olea europea* L.) leaves. Food Preservation Science 30, 191–193.

Sies, H. (1993) Strategies of antioxidant defense. European Journal of Biochemistry 215, 213–219.

Skerget, M., Kotnik, P., Hadolin, M., Hra, A.R., Simoni, M., & Knez, Z. (2005) Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chemistry 89, 191–198.

Soler-Rivas, C., Espin, J.C., & Wichers, H.J. (2000) Oleuropein and related compounds. Journal of the Science of Food and Agriculture 80, 1013–1023.

Somova, L.I., Shode, F.O., Ramnanan, P., & Nadar, A. (2003) Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from *Olea europaea*, subspecies Africana leaves. Journal of Ethnopharmacology 84, 299–305.

Stamatopoulos, K., Katsoyannos, E., Chatzilazarou, A., & Konteles, S. (2012) Improvement of oleuropein extractability by optimising steam blanching process as pre-treatment of olive leaf extraction via response surface methodology. Food Chemistry 133, 344–351.

Tan, H.W., Tuck, K.L., Stupans, J., & Hayball, P.J. (2003) Simultaneous determination of oleuropein and hydroxytyrosol in rat plasma using liquid chromatography with fluorescence detection. Journal of Chromatography 785, 187–191.

Tasioula-Margari, M., & Ologeri, O. (2001) Isolation and characterization of virgin olive oil phenolic compounds by HPLC/UV and GC/MS. Journal of Food Science 66, 530–534.

Torres, M.M. & Maestri, D.M. (2006) Chemical composition of Arbequina virgin olive oil in relation to extraction and storage conditions. Journal of the science of Food and Agriculture 86, 2311–2317.

Tsimidou, M., Papadopoulos, G., & Boskou, D. (1992) Determination of phenolic compounds in Virgin olive oil by reversed-phase HPLC with emphasis on UV detection. Food Chemistry 44, 53–60.

Valavanidis, A., Nisiotou, C., Papageorgiou, Y., Kremli, I., Satravelas, N., Zinieris, N., & Zygalaki, H. (2004) Comparison of the radical scavenging potential of polar and lipidic fractions of olive oil and other vegetable oils under normal conditions and after thermal treatment. Journal of Agriculture and Food Chemistry 52, 2358–2365.

Verhe, R., Papadopoulos, G., & Boskou, D. (1992) Preparation of hydroxytyrosol. Bulletin de Liaison Groupe Polyphenols 15, 237–244.

Vilkhu, K., Mawson, R., Simons, L., & Bates, D. (2008) Applications and opportunities for ultrasound assisted extraction in food industry: a review. Innovative Food Science and Emerging Technologies 9, 161–169.

Visioli, F., Galli, C., Bornet, F., Mattei, A., Patelli, R., Galli, G., & Caruso, D. (2000) Olive oil phenolics are dose-dependently absorbed in humans. FEBS Letters 468(2–3), 159–160.

Vissers, M.N., Zock, P.L., Roodenburg, A.J.C., Leenen, R., & Katan, M.B. (2002) Olive oil phenols are absorbed in humans. Journal of Nutrition 132, 409–417.

Zarzuelo, A. (1991) Vasodilator effect of olive leaf. Planta Medica 57, 417–419.

Zhang, H.F., Yang, X.H., Zhao, L.D., & Wang, Y. (2009) Ultrasonic-assisted extraction of epimedin C from fresh leaves of epimedium and extraction mechanism. Innovative Food Science and Emerging Technologies 10, 54–60.

Zoidou, E., Melliou, E., Gikas, E., Tsarbopoulos, A., Magiatis, P., & Skaltsounis, A.L. (2010) Identification of Throuba Thassos, a traditional Greek table olive variety, as a nutritional rich source of oleuropein. Journal of Agricultural and Food Chemistry 58, 46–50.

20 Composition and analysis of functional components of olive leaves

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20.1 Introduction

The olive tree (*Olea europaea* L.) is a Mediterranean historical tree that has been usually cultivated to produce table olives and olive oil, and its leaves have been traditionally used in folk medicine. Recently, several studies have demonstrated the antihypertensive, anticarcinogenic, anti-inflammatory, antimicrobial, and hypocholesterolemic effects of olive leaf (OL) (Pereira *et al.*, 2007; Barrajón-Catalán *et al.*, 2015; Lockyer *et al.*, 2015). Taking into account that the production of OL from pruning has been estimated to be 25 kg per olive tree, in addition to 5% of the weight of harvested olives, which is collected at the oil mill (El & Karakaya, 2009), many researchers and companies are showing an increased interest in the study of this by-product, especially due to its medicinal properties. Some researchers are focusing on characterizing the bioactive compounds in OL by using different analytical techniques. The most suitable techniques to characterize polar and semipolar compounds are high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE), both coupled to mass spectrometry (MS), whereas gas chromatography (GC)-MS is mainly used to characterize nonpolar compounds. Nuclear magnetic resonance (NMR) has also been used to elucidate the structure of some compounds from this matrix.

The composition of OL is complex, and it includes flavonoids and their glycosylated derivatives, secoiridoids and their derivatives, simple phenols, phenolic acids and derivatives, terpenes, fatty acids, minerals, volatile compounds, vitamins, and phytosterols (Table 20.1). However, the heterogeneity of these compounds, the use of diverse analytical techniques, and the development of different analytical methods make it difficult to obtain an overview of the composition of OL. Therefore, this chapter describes the analytical techniques in depth that are used to characterize the bioactive composition of OL, and that make this by-product an important economical resource with which to develop many commercial products, such as enriched food, cosmetics, or nutraceuticals.

20.2 Qualitative and quantitative analysis of olive leaves

20.2.1 Liquid chromatography (LC)

LC, and especially HPLC with different detection systems, is used for the rapid identification, characterization, and quantification of unknown compounds from different matrices. It has become one of the most powerful tools with which to characterize compounds from OL (Table 20.2). The technique is based on the distribution of a compound between a mobile phase (solvent) and a stationary phase (column packing), which depends on the chemical structure of the analyte, and the physicochemical properties of the mobile

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Table 20.1 Main classes of olive leaf compounds.

Family name	Compounds	References
Flavonoids (FV)	Catechin, rutin, luteolin-7-glucoside, apigenin-7-glucoside, diosmetin-7-glucoside, luteolin, luteolin diglucoside, luteolin-7-rutinoside, apigenin-7-rutinoside, taxifolin, diosmin, diosmetin, cirstimaritin, apigenin, quercetin, chryseriol-7-glucoside, eryodictiol	Meirinhos *et al.* (2005), Silva *et al.* (2006), Japón-Luján & De Castro (2008), Mylonaki *et al.* (2008), Kiritsakis *et al.* (2010), Taamalli *et al.* (2012a, 2012b), Quirantes-Piné *et al.* (2013), Talhaoui *et al.* (2014)
Simple phenols (SP) and derivatives	Hydroxytyrosol, tyrosol, hydroxytyrosol hexoside, hydroxytyrosol acetate, tyrosol glucoside, vanillin, acteoside	Silva *et al.* (2006), Japón-Luján & De Castro (2008), Kiritsakis *et al.* (2010), Quirantes-Piné *et al.* (2013), Talhaoui *et al.* (2014)
Phenolic acids (PA) and derivatives	Caffeic acid, vanillic acid, homovanillic acid, syringic acid, gallic acid, ferulic acid, caftaric acid, quinic acid, chlorogenic acid	Quirantes-Piné *et al.* (2013)
Secoiridoids (SC) and derivatives	Secologanoside, oleoside, oleuropein, 6′-O-[2,6-dimetyl-8-hydroxy-2-octenoyloxi], secologanoside, syringaresinol, lucidomoside D, demethyloleuropein, oleuropein, oleuropein aglycon, oleoside methyl ester, oleuroside, oleuropein diglucoside, 2′′-methoxyoleuropein, hydroxyoleuropein, elenolic-7-O-glucoside, ligstroside, elenolic acid, nuzhenide	Silva *et al.* (2006), Japón-Luján & De Castro (2008), Kiritsakis *et al.* (2010), Quirantes-Piné *et al.* (2013), Talhaoui *et al.* (2014)
Lignans (LG)	Pinoresinol, acetoxypinoresinol, syringaresinol	Taamalli *et al.* (2012a, 2012b)
Terpenes (TP) and derivatives	Erythrodiol, uvaol, oleanolic acid, ursolic acid, maslinic acid, squalene	Tabera *et al.* (2004), Priego-Capote *et al.* (2007), Sánchez Ávila *et al.* (2007), Guinda *et al.* (2010)
Fatty acids (FA)	Myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3)	Bahloul *et al.* (2014), Cavalheiro *et al.* (2015)
Minerals	Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Sr, Zn	Paskovic *et al.* (2013), Cavalheiro *et al.* (2015)
Sugars	D-(−)-arabinose, D-(+)-xylose, D-(+)-glucose, D-(+)-mannose, D-(−)-galactose, D-(−)-fructose, sedoheptulose, 1,6-anhidro-β-D-glucose, D-(+)-sucrose, D-(+)-lactose, D-(+)-raffinose, maltotriose, L-rhamnose, D-(+)-galacturonic acid, D-glucuronic acid, xylitol, L-(−)-arabitol, adonitol (ribitol), D-mannitol, D-(+)-chiro-inositol, myo-inositol, galactinol	Gómez-González *et al.* (2010)

Table 20.1 (*Continued*)

Family name	**Compounds**	**References**
Volatile compounds (VC)	Furfural, (e,z)-2,4-hexadienal, hexenol, hexanol, heptenal, heptanal, (e,e)-2,4-hexadienal, 2-acetylfurane, a-pinene, benzaldehyde, 3-ethenylpyridine, hexanoic acid, phenol, 3-octanone, 6-methyl-5-hepten-2-one, octanal, (e,e)-2,4-heptadienal, (e,z)-2,4-heptadienal, benzyl alcohol, phenylacetaldehyde, (E)-2-octenal, 1-octanol, cis-linalool oxide, trans-linalool oxide, linalool, nonanal, phenylethyl alcohol, methyl nicotinate, 4-ketoisophorone, (E,Z)-2,6-nonadienal, (E)-2-nonenal, 1-nonanol, trans-linalool oxide (pyranoid), p-cymen-8-ol, a-terpineol, methyl salicylate, (Z)-4-decenal, decanal, 2-ethylbenzaldehyde, benzothiazole, geraniol, (E)-2-decenal, salicylic alcohol, 1-tridecene, p-menth-1-en-7-al, (E,Z)-2,4-decadienal, 4-vinylguaiacol, (E,E)-2,4-decadienal, eugenol, (E)-b-damascenone, cis-a-bergamotene, (Z,E)-2,6-dodecadienal, trans-a-bergamotene, (E)-isoeugenol, (E)-geranylacetone, (E)-b-ionone, caryophyllene oxide	Brahmi *et al.* (2012), Konoz *et al.* (2013)
Vitamins	β-carotene, α-tocopherol	Tabera *et al.* (2004)
Phytosterols (PT)	β-sitosterol, cholesterol, brassicasterol, 24-methylene cholesterol, campesterol, campestanol, stigmasterol, clerosterol, sitostanol, avenasterol	Tabera *et al.* (2004), Orozco-Solano *et al.* (2010)

phase and the stationary phase (Bączek *et al.*, 2005). Therefore, LC allows the analysis of a large amount of polar and semipolar compounds, which range from phenolic acids and their derivatives (Quirantes-Piné *et al.*, 2013) to tocopherols (Tabera *et al.*, 2004), in a relatively short period of time (from 20 min to 2 h). The most widely used column-packing materials for modern LC are bonded phases, most commonly on a silica support (Whelan *et al.*, 2005). Many types of bonded stationary phases for reverse-phase LC are available today, but the most common column used for analyzing OL compounds is C18 (Table 20.2). Additionally, polar mobile phases, such as mixtures of acetonitrile or methanol and acidified water with different proportions of acetic or formic acid in gradient elution, have been shown to be the most effective solvents to obtain a complete metabolite profile. Indeed, it has been demonstrated that a weakly acidic mobile phase suppresses ionization of most polar compounds, such as phenolic acids, and as a result enhances the separation on a reverse-phase column (Harnly *et al.*, 2007).

Many types of detectors are available to be coupled to HPLC; however, to analyze phenolic compounds, the most widely used are UV-visible (UV-Vis) detectors, such as the diode array detector (DAD), and mass spectrometry (MS). The DAD allows the simultaneous detection of a wide range of wavelengths across the complete UV-Vis spectra. Its robustness makes DAD the best method to quantify compounds and can also help to distinguish subclasses of phenolic compounds (Harnly *et al.*, 2007). LC-MS, in combination or not with DAD and usually with electrospray ionization (ESI), has been increasingly used to characterize

Table 20.2 Some HPLC procedures for the determination of bioactive compounds in olive leaves.

Compounds	Extraction procedure	Stationary phase	Mobile phase and flow rate	Detector	Run time (min)	OL variety	References
Flavonoids	**Solid–liquid extraction** Solvent: MeOH:H_2O (80:20, v/v) (~13 mL/g) Extraction time: 30 min	XTerraR RP 18 (3.5 μm, 4.6 × 150 mm) with a XTerraR RP 18 precolumn (3.5 μm, 4.6 × 10 mm)	A: water with acetic acid (0.1%) B: acetonitrile with acetic acid (0.1%) Flow rate: 1 mL/min	ESI-IT-MS/MS	80	'Koroneiki', 'Megaritiki', 'Kalamon'	Kiritsakis *et al.* (2010)
	Solid–liquid extraction Solvent: MeOH:H_2O (60:40, v/v) (200 mL/g) Extraction time: two cycles of 30 min and 10 min Temperature: 45 °C	Reversed-phase Spherisorb ODS2 (5 μm, 4.6 × 250 mm)	A: water with formic acid (19:1) B: methanol Flow rate: 0.9 mL/min	DAD (λ = 350 nm)	60	'Macedo de Cavaleiros', 'Mirandela', 'Valpaços', 'Mogadouro', 'Figueira de Castelo Rodrigo', 'Fundão'	Meirinhos *et al.* (2005)
	MAE Solvent: EtOH:H_2O (80:20, v/v) Irradiation power: 200 W Extraction time: 8 min	Zorbax Eclipse XDB C18 (5 μm, 4.6 × 150 mm) and a Kromasil 5 C18 precolumn (5 μm, 4.6 × 150 mm)	A: water with formic acid (0.1%) B: acetonitrile:water (98:2, v/v) with formic acid (0.1%) Flow rate: 0.8 mL/min	ESI-TOF-MS/MS	24	'Picual'	Japón-Luján & De Castro (2008)
	MAE Solvent: MeOH:H_2O (80:20, v/v) Extraction time: 6 min Temperature: 80 °C **SFE** Solvent: CO_2 + 6.6% EtOH Extraction time: 60 min Pressure: 150 bar Temperature: 40 °C **PLE** Solvent: H_2O and EtOH (in two different extractions) Extraction time: 20 min Pressure: 100 bar Temperature: 150 °C	C18 Eclipse Plus (1.8 μm, 4.6 × 150 mm)	A: water with acetic acid (0.5%) B: acetonitrile Flow rate: 0.8 mL/min	ESI-TOF-MS	35	'Oueslati', 'Chetoui', 'Chemlali', 'El Hor', 'Jarboui', 'Chemchali'	Taamalli *et al.* (2012a, 2012b)

	PLE Solvent: EtOH Extraction time: 20 min Pressure: 100 bar Temperature: 150 °C	Phenomenex Gemini C18 (3 μm, 2 × 150 mm)	A: water with acetic acid (0.5%) B: acetonitrile Flow rate: 0.2 mL/min	ESI-QTOF-MS/MS	45	'Hojiblanca'	Quirantes-Piné *et al.* (2013)
	Solid–liquid extraction Solvents: MeOH: H_2O (80:20, v/v) + 10 mL sodium metabisulfite (2%) (three cycles)	LiChrospher C18 column (5 μm, 4 × 250 mm with C18 precolumn)	A: water with formic acid (0.5%) B: acetonitrile: water (40:60, v/v) with formic acid (0.5%) Flow rate: 0.7 mL/min	IT-APCI-MS	115	'Bical', 'Borrenta', 'Cobrançosa', 'Coimbreira', 'Lentisca', 'Madural', 'Negrinha de Freixo', 'Redondal', 'Santulhana', 'Verdeal Transmontana'	Silva *et al.* (2006)
	Ultrasound-assisted extraction Solvents: MeOH:H_2O (80:20, v/v) (20 mL/g) Extraction time: 10 min (three cycles)	Poroshell 120 EC-C18 analytical column (2.7 μm, 4.6 × 100 mm)	A: water with acetic acid (1%) B: acetonitrile Flow rate: 0.8 mL/min	DAD-ESI-TOF-MS (λ = 350 nm)	37	'Sikitita', 'Arbequina', 'Picual'	Talhaoui *et al.* (2014)
	Solid–liquid extraction Solvents: EtOH:H_2O (60:40, v/v) with citric acid (1 g/L) (40 mL/g) Temperature: ~22 °C Extraction time: 5 h	Superspher RP-18 (4 μm, 2 × 125 mm)	A: water with acetic acid (2.5%) B: methanol Flow rate: 0.33 mL/min	DAD (λ = 350 nm)	35	'Koroneiki'	Mylonaki *et al.* (2008)
Simple phenols and derivatives	**MAE** Solvent: EtOH:H_2O (80:20, v/v) Irradiation power: 200 W Extraction time: 8 min	Zorbax Eclipse XDB C18 (5 μm, 4.6 × 150 mm) and a Kromasil 5 C18 precolumn (5 μm, 4.6 × 150 mm)	A: water with formic acid (0.1%) B: acetonitrile: water (98:2, v/v) with formic acid (0.1%) Flow rate: 0.8 mL/min	ESI-TOF-MS/MS	24	'Picual'	Japón-Luján & De Castro (2008)

(*continued*)

Table 20.2 (*Continued*)

Compounds	Extraction procedure	Stationary phase	Mobile phase and flow rate	Detector	Run time (min)	OL variety	References
	Solid–liquid extraction Solvents: MeOH:H_2O (80:20, v/v) + 10 mL sodium metabisulfite (2%) (three cycles)	LiChrospher C18 column (5 μm, 4 × 250 mm with C18 precolumn)	A: water with formic acid (0.5%) B: acetonitrile: water (40:60, v/v) with formic acid (0.5%) Flow rate: 0.7 mL/min	IT-APCI-MS	115	'Bical', 'Borrenta', 'Cobrançosa', 'Coimbreira', 'Lentisca', 'Madural', 'Negrinha de Freixo', 'Redondal', 'Santulhana', 'Verdeal Transmontana'	Silva *et al.* (2006)
	PLE Solvent: EtOH Extraction time: 20 min Pressure: 100 bar Temperature: 150 °C	Phenomenex Gemini C18 (3 μm, 2 × 150 mm)	A: water with acetic acid (0.5%) B: acetonitrile Flow rate: 0.2 mL/min	ESI-QTOF-MS/MS	45	'Hojiblanca'	Quirantes-Piné *et al.* (2013)
	Ultrasound-assisted extraction Solvents: MeOH:H_2O (80:20, v/v) (20 mL/g) Extraction time: 10 min (three cycles)	Poroshell 120 EC-C18 (2.7 μm, 4.6 × 100 mm)	A: water with acetic acid (1%) B: acetonitrile Flow rate: 0.8 mL/min	DAD-ESI-TOF-MS (λ = 325 nm)	37	'Sikitita', 'Arbequina', 'Picual'	Talhaoui *et al.* (2014)
Phenolic acids and derivatives	**PLE** Solvent: EtOH Extraction time: 20 min Pressure: 100 bar Temperature: 150 °C	Phenomenex Gemini C18 (3 μm, 2 × 150 mm)	A: water with acetic acid (0.5%) B: acetonitrile Flow rate: 0.2 mL/min	ESI-QTOF-MS/MS	45	'Hojiblanca'	Quirantes-Piné *et al.* (2013)
Secoiridoids and derivatives	**Solid–liquid extraction** Solvent: MeOH:H_2O (80:20, v/v) (~13 mL/g) Extraction time: 30 min	XTerraR RP 18 (3.5 μm, 4.6 × 150 mm) with a XTerraR RP 18 precolumn (3.5 μm, 4.6 × 10 mm)	A: water with acetic acid (0.1%) B: acetonitrile with acetic acid (0.1%) Flow rate: 1 mL/min	ESI-IT-MS/MS	80	'Koroneiki', 'Megaritiki', 'Kalamon'	Kiritsakis *et al.* (2010)

MAE Solvent: EtOH:H_2O (80:20, v/v) Irradiation power: 200 W Extraction time: 8 min	Zorbax Eclipse XDB C18 (5 μm, 4.6 × 150 mm) and a Kromasil 5 C18 precolumn (5 μm, 4.6 × 150 mm)	A: water with formic acid (0.1%) B: acetonitrile: water (98:2, v/v) with formic acid (0.1%) Flow rate: 0.8 mL/min	ESI-TOF-MS/MS	24	'Picual'	Japón-Luján & De Castro (2008)
MAE Solvent: MeOH:H_2O (80:20, v/v) Extraction time: 6 min Temperature: 80 °C	C18 Eclipse Plus (1.8 μm, 4.6 × 150 mm)	A: water with acetic acid (0.5%) B: acetonitrile Flow rate: 0.8 mL/min	ESI-TOF-MS	35	'El Hor'	Taamalli *et al.* (2012b)
Solid–liquid extraction Solvents: MeOH:H_2O (80:20, v/v) + 10 mL of sodium metabisulfite 2% (3 cycles)	LiChrospher C18 (5 μm, 4 × 250 mm with C18 precolumn)	A: water with formic acid (0.5%) B: acetonitrile: water (40:60, v/v) with formic acid (0.5%) Flow rate: 0.7 mL/min	IT-APCI-MS	115	'Bical', 'Borrenta', 'Cobrançosa', 'Coimbreira', 'Lentisca', 'Madural', 'Negrinha de Freixo', 'Redondal', 'Santulhana', 'Verdeal Transmontana'	Silva *et al.* (2006)
PLE Solvent: EtOH Extraction time: 20 min Pressure: 100 bar Temperature: 150 °C	Phenomenex Gemini C18 (3 μm, 2 × 150 mm)	A: water with acetic acid (0.5%) B: acetonitrile Flow rate: 0.2 mL/min	ESI-QTOF-MS	45	'Hojiblanca'	Quirantes-Piné *et al.* (2013)
Ultrasound-assisted extraction Solvents: MeOH:H_2O (80:20, v/v) (20 mL/g) Extraction time: 10 min (three cycles)	Poroshell 120 EC-C18 (2.7 μm, 4.6 × 100 mm)	A: water with acetic acid (1%) B: acetonitrile Flow rate: 0.8 mL/min	DAD-ESI-TOF-MS (λ = 240 nm)	37	'Sikitita', 'Arbequina', 'Picual'	Talhaoui *et al.* (2014)

(*continued*)

Table 20.2 (*Continued*)

Compounds	Extraction procedure	Stationary phase	Mobile phase and flow rate	Detector	Run time (min)	OL variety	References
Lignans	**MAE** Solvent: MeOH:H_2O (80:20, v/v) Extraction time: 6 min Temperature: 80 °C **SFE** Solvent: CO_2 + 6.6% ethanol Extraction time: 60 min Pressure: 150 bar Temperature: 40 °C **PLE** Solvent: H_2O and EtOH (in two different extractions) Extraction time: 20 min Pressure: 100 bar Temperature: 150 °C	C18 Eclipse Plus (1.8 µm, 4.6 × 150 mm)	A: water with acetic acid (0.5%) B: acetonitrile Flow rate: 0.8 mL/min	ESI-TOF-MS	35	'Oueslati', 'Chetoui', 'Chemlali', 'El Hor', 'Jarboui', 'Chemchali'	Taamalli *et al.* (2012a, 2012b)
Tocopherols (TR)	**Countercurrent Supercritical Fluid Extraction** Solvent: $scCO_2$ + 10% EtOH Pressure: 75 bar Temperature: 35°C Extraction time: 1 h	Silica analytical column (5 µm, 4 × 250 mm)	Isocratic: propan-2-ol: hexane (0.5:9.5, v/v) Flow rate: 1 mL/min	DAD (λ = 292 nm)	30	'Picual'	Tabera *et al.* (2004)

OL. Talhaoui *et al.* (2014) characterized 30 phenolic compounds from three varieties of OL with HPLC-DAD-ESI-MS, using time-of-flight (TOF) analysis (Talhaoui *et al.*, 2014). Taamalli *et al.* (2012a) identified 36 phenolic compounds using the same technique, although the additional use of tandem mass spectrometry (MS/MS) by ion trap MS allowed them to obtain fragment ions that were of structural relevance to identify target compounds in this complex matrix in the same way as in other previous studies (Silva *et al.*, 2006; Kiritsakis *et al.*, 2010). On the other hand, quadrupole time-of-flight mass spectrometry (QTOF-MS) combines high sensitivity and mass accuracy for both precursor and product ions, and provided the elemental composition of the parent and fragment ions that allowed the characterization of 48 phenolic compounds from the 'Hojiblanca' variety of olive (Quirantes-Piné *et al.*, 2013). Apart from ESI, atmospheric pressure chemical ionization (APCI) has been used as an ionization method to analyze the phenolic compounds in ten varieties of OL (Silva *et al.*, 2006). Some authors have underlined that APCI is more suitable to analyze medium-polar and lower molecular-mass compounds, whereas ESI is the best option for more polar compounds with a high molecular weight (La Torre *et al.*, 2006).

Regarding the previous extraction of bioactive compounds from OL for their subsequent LC analysis, a wide range of procedures have been described. The solvent used for the extraction has to be carefully selected, depending on the compounds of interest. Therefore, conventional extraction techniques, such as maceration or solid–liquid extraction using methanolic or ethanolic mixtures with water, proved to be effective in recovering secoiridoids, derivatives, and flavonoids from OL (Silva *et al.*, 2006; Kiritsakis *et al.*, 2010). Nevertheless, more environmentally friendly extraction techniques such as pressurized and supercritical fluid extraction (PLE and SFE, respectively), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE) have gained increasing attention over traditional techniques. Some studies concluded that SFE and PLE were more efficient in extracting less polar compounds such as apigenin, diosmetin, or luteolin, whereas MAE was more effective in extracting more polar compounds (Taamalli *et al.*, 2012b). In addition, MAE has proved to be a more efficient extraction technique than conventional extraction, due to its high efficiency and rapidity (Taamalli *et al.*, 2012b).

20.2.2 Gas chromatography (GC)

GC is the most important technique for the characterization of volatile compounds, although it can be applied to the analysis of a wide variety of compounds using a previous derivatization step. GC has been employed for the analysis of terpenes and their derivatives, fatty acids, triacylglycerols (TAGs), sterols, volatile compounds, and sugars from OL (Table 20.3). Unlike LC, the mobile phase in GC is an inert gas that carries the sample through the column. The most widely used inert gases are helium, nitrogen, and hydrogen. Hydrogen has been used to analyze fatty acids and terpenes in OL (Guinda *et al.*, 2010; Cavalheiro *et al.*, 2015). Theoretically, H_2 provides a more rapid analysis, because it has less viscosity compared with He or N_2. Capillary columns (open-tubular columns) are most widely applied to analyze compounds from OL because of their better resolution and high sensitivity compared with packed columns (Poole, 2012). The vapor pressure of compounds determines their retention time; for this reason, the oven temperature is another parameter to optimize, to maintain the column at the selected temperature. Most research programs the oven to generate a gradient of temperature to facilitate the separation (Contreras *et al.*, 2013). The gradient chosen for the analysis of terpenes, derivatives, and sugars has ranged from 50 °C to nearly 300 °C (Tabera *et al.*, 2004; Sánchez Ávila *et al.*, 2007; Guinda *et al.*, 2010), whereas for fatty acids, the final oven temperature selected was up to 230 °C. The oven temperature is determined by the carrier gas and column, and it is usually higher than the boiling point of the compound of interest. For this reason, the maximum oven temperature for analyzing triglycerides is 360 °C, due to the boiling point of glycerol at 290 °C.

GC coupled with a flame ionization detector (FID) has been effectively used to characterize nonpolar compounds from OL. Despite its versatility, when FID is used, the absence of structural information can cause errors in the characterization and quantification, if standards are not available. Therefore, coupling with MS is sometimes preferable. In this regard, GC-MS was the choice for characterizing 59 volatile compounds and 22 different sugars in OL (Gómez-González *et al.*, 2010; Konoz *et al.*, 2013).

Supercritical fluids have attracted special attention regarding extraction. Countercurrent supercritical fluid extraction (CC-SFE) has proved to be very effective in extracting sterols and triglycerides, as well as terpenes and their derivatives, from a hexane extract of OL, combining different green solvents such as supercritical CO_2 and ethanol (Tabera *et al.*, 2004). In addition to CO_2 and ethanol, chloroform, hexane, dichloromethane

Table 20.3 Some of the gas chromatography (GC) procedures for the determination of bioactive compounds in olive leaves.

Compounds	Extraction procedure	Stationary phase	GC conditions	Detection	Run time (min)	OL variety	References
Terpenes and derivatives	**Ultrasound-assisted extraction** Solvent: EtOH (30 mL/g) Preheated: 45 °C Duty cycle: 0.5 s Converter applied power: 450 W Extraction time: 20 min	Factor Four fused silica capillary column VF-5 ms (0.25 µm, 30 m × 0.25 mm)	Carrier gas: He Injector temperature: 250 °C Detector temperature: 220, 200, and 50 °C Injector: split mode (ratio 50:1)	EI-IT-MS	>55	'Hojiblanca', 'Acebuche'	Sánchez Ávila *et al.* (2007)
	Solid–liquid extraction Solvent: EtOH (20 mL/g) Temperature: 25 °C Extraction time: 1 h	Rtx-65TG Crossbond capillary column (0.1 µm, 30 m × 0.25 mm)	Carrier gas: H_2 Injector temperature: 300 °C Detector temperature: 300 °C Injector: split mode (ratio ND)	FID	ND	'Picual', 'Hojiblanca', 'Arbequina'	Guinda *et al.* (2010)
	ccSFE Solvent: $scCO_2$ + 10% EtOH Pressure: 75 bar Temperature: 35 °C Extraction time: 1 h	Silica SPB-5 capillary column (0.25 µm, 30 m × 0.25 mm)	Carrier gas: N_2 Injector temperature: 300 °C Detector temperature: 320 °C Injector: split mode (ratio 1:50)	FID	ND	'Picual'	Tabera *et al.* (2004)
Fatty acids	**Solid–liquid extraction** Solvent: hexane (5 mL/g) Extraction time: 4 h	Carbowax capillary column (0.25 µm, 15 m × 0.25 mm)	Carrier gas: N_2 Injector temperature: 230 °C Detector temperature: 250 °C Injector: ND	FID	ND	'Chemlali', 'Chemchali', 'Chetoui', 'Zarrazi'	Bahloul *et al.* (2014)

	Solid–liquid extraction Solvent: 8 mL chloroform (0.02% BHT), 16 mL MeOH, and 6.4 mL H_2O Extraction time: 30 min	ZBFFAP capillary column (0.25 μm, 0 m × 0.25 mm)	Carrier gas: H_2 Injector temperature: 240 °C Detector temperature: 240 °C Injector: split mode (ratio 1:50)	FID	46.75	'Ascolano', 'Arbosana', 'Negrinha do Freixó', 'Koroneiki', 'Grappolo'	Cavalheiro *et al.* (2015)
Triglycerides	**ccSFE** Solvent: $scCO_2$ + 10% EtOH Pressure: 75 bar Temperature: 35 °C Extraction time: 1 h	Silica SPB-5 capillary column (0.25 μm, 30 m × 0.25 mm)	Carrier gas: He Injector temperature: 360 °C Detector temperature: 365 °C Injector: split mode (ratio 1:50)	FID	ND	'Picual'	Tabera *et al.* (2004)
Sterols	**ccSFE** Solvent: $scCO_2$ + 10% EtOH Pressure: 75 bar Temperature: 35 °C Extraction time: 1 h	Silica SPB-5 capillary column (0.25 μm, 30 m × 0.25 mm)	Carrier gas: He Injector temperature: 280 °C Detector temperature: 290 °C Injector: split mode (ratio 1:50)	FID	>25	'Picual'	Tabera *et al.* (2004)
	Ultrasound-assisted extraction Solvent: dichlorometane + hexane (2:1) Power: 50 W Temperature: 20 °C Extraction time: 10 min	VF-5 ms fused silica capillary column (0.25 μm, 30 m × 0.25 mm)	Carrier gas: ND Injector temperature: 250 °C Detector temperature: 250 °C Injector: Split (ratio 50:1 for 0.5 min and 100:1 for 10 min) – splitless mode	EI-IT-MS	75	'Manzanilla', 'Picual', 'Gordal'	Orozco-Solano *et al.* (2010)
Volatile compounds	**Microwave-assisted hydrodistillation** Solvent: water Power: 700 W Extraction time: 45 min	HP5-MS capillary fused silica column (0.25 μm, 30 m × 0.25 mm)	Carrier gas: He Injector temperature: 290 °C Detector temperature: ND Injector: splitless	EI-MS	>70	ND	Konoz *et al.* (2013)

(*continued*)

Table 20.3 (*Continued*)

Compounds	Extraction procedure	Stationary phase	GC conditions	Detection	Run time (min)	OL variety	References
	Hydrodistillation (HDT) Solvent: water Extraction time: 3 h	HP5 capillary column (0.25 μm, 30 m × 0.25 mm)	Carrier gas: N_2 Injector temperature: 250 °C Detector temperature: 250 °C Injector: split mode (ratio 1:30)	FID	ND	'Neb Jemel', 'Chemchali', 'Chemlali'	Brahmi *et al.* (2012)
		DB5 capillary column (0.25 μm, 30 m × 0.25 mm)	Carrier gas: He Injector temperature: 250 °C Detector temperature: ND Injector: splitless	EI-IT-MS			
Sugars	**Ultrasound-assisted extraction** Solvent: dichloromethane:MeOH (2:1, v/v) Preheated: room temperature Duty cycle: 0.5 s Converter applied power: 450 W Extraction time: 10 min	FactorFour fused-silica capillary column VF5 ms (0.25 μm, 30 m × 0.25 mm)	Carrier gas: He Injector temperature: 280 °C Detector temperature: ND Injector: split (ratio 2:1 for 0.5 min and 100:1 for 10 min) – splitless mode	EI-CID-MS	81	'Picual', 'Manzanilla', 'Hojiblanca'	Gómez-González *et al.* (2010)

ND: Not described.

with methanol, and water have been used to extract volatile compounds, fatty acids, or sugars from OL (Gómez-González *et al.*, 2010; Brahmi *et al.*, 2012; Bahloul *et al.*, 2014). However, the analysis of some compounds requires an additional previous derivatization step to obtain volatile derivatives, which enables their separation by GC. This is the case for fatty acids, which must be converted into their corresponding fatty acid methyl esters by using a methanolic solution of potassium hydroxide prior to GC analysis (Bahloul *et al.*, 2014). A detailed example of the experimental procedure for extract preparation prior to GC analysis has been described elsewhere (Lisec *et al.*, 2006).

20.2.3 Nuclear magnetic resonance (NMR)

NMR is a spectroscopic technique used to identify molecular structures from liquid and solid matrices, without altering the sample or producing hazardous waste (Marcone *et al.*, 2013). Compared with HPLC-MS or GC-MS, NMR is much faster and can determine the composition of a sample without any pretreatment, which ensures highly reproducible results. Together with MS, NMR provides the structural information of the analyte. However, the coupling of MS with a separation technique is more extensively applied, because MS has higher sensitivity than NMR (Silva Elipe, 2003). Furthermore, this is an expensive technique, and although it has been used for the analysis of olive oil (Alonso-Salces *et al.*, 2011; Dais & Hatzakis, 2013), not many researchers have applied it to the elucidation of compounds present in OL. Karioti *et al.* (2014) isolated and analyzed two secoiridoid glycosides from an aqueous methanolic extract of OL that experienced boron deficiency (Karioti *et al.*, 2014).

20.2.4 Other techniques

CE has also been used to characterize phenolic compounds from OL. The technique consists of using an electrical field to separate the components from a complex matrix. This analytical technique coupled to TOF-MS allows the characterization of 31 phenolic compounds in leaves from the ‘Hojiblanca’ and ‘Manzanilla’ olive varieties (Arráez-Román *et al.*, 2008). Capillary electrophoresis has become an alternative or complementary technique to chromatographic separations for the analysis of phenolic compounds, because it provides a high separation efficiency, low analysis time, high-resolution power, and low consumption of samples and reagents (Hernández-Borges *et al.*, 2007). Two-dimensional electrophoresis was also used to analyze proteins from OL after a pretreatment based on phenol extraction using sodium dodecyl sulfate (Wang *et al.*, 2003).

Another simple separation technique, thin-layer chromatography (TLC), has been used to characterize five phenolic compounds and derivatives in OL by the subsequent recording of UV spectra on the layer (Heimler *et al.*, 1992). Although this separation technique has several advantages, such as its simplicity, rapidity, and low cost (Cimpoiu, 2006), its limited resolution capability and the absence of fully automated systems make it a less appealing option.

Spectroscopic techniques such as atomic absorption spectrophotometry and inductively coupled plasma optical emission spectrometry (ICP-OES) have been used to determine the mineral composition of OL (Toplu *et al.*, 2009; Cavalheiro *et al.*, 2015). These analyses require previous acidic digestion, usually employing HNO_3, $HClO_4$, or mixtures, to ensure stability and comparability with the calibration standards. However, some official methods are available to analyze minerals (OES and flame atomic absorption spectroscopy), which allow the results from different laboratories to be standardized (AOAC, 1995).

In addition, visible spectrophotometry has been used to analyze β-carotene (maximum absorbance at 480 nm) from OL extracts (Tabera *et al.*, 2004).

Figure 20.1 shows a schematic summary of the extraction and analytical techniques for characterizing functional components from olive leaves.

20.3 Future prospects

Food and pharmaceutical companies are continuously seeking new sources of bioactive compounds, and nowadays OL have received considerable attention due to their safety, low cost, and potential nutritional and therapeutic effects, which make them important candidates for the development of nutraceuticals. Due to this

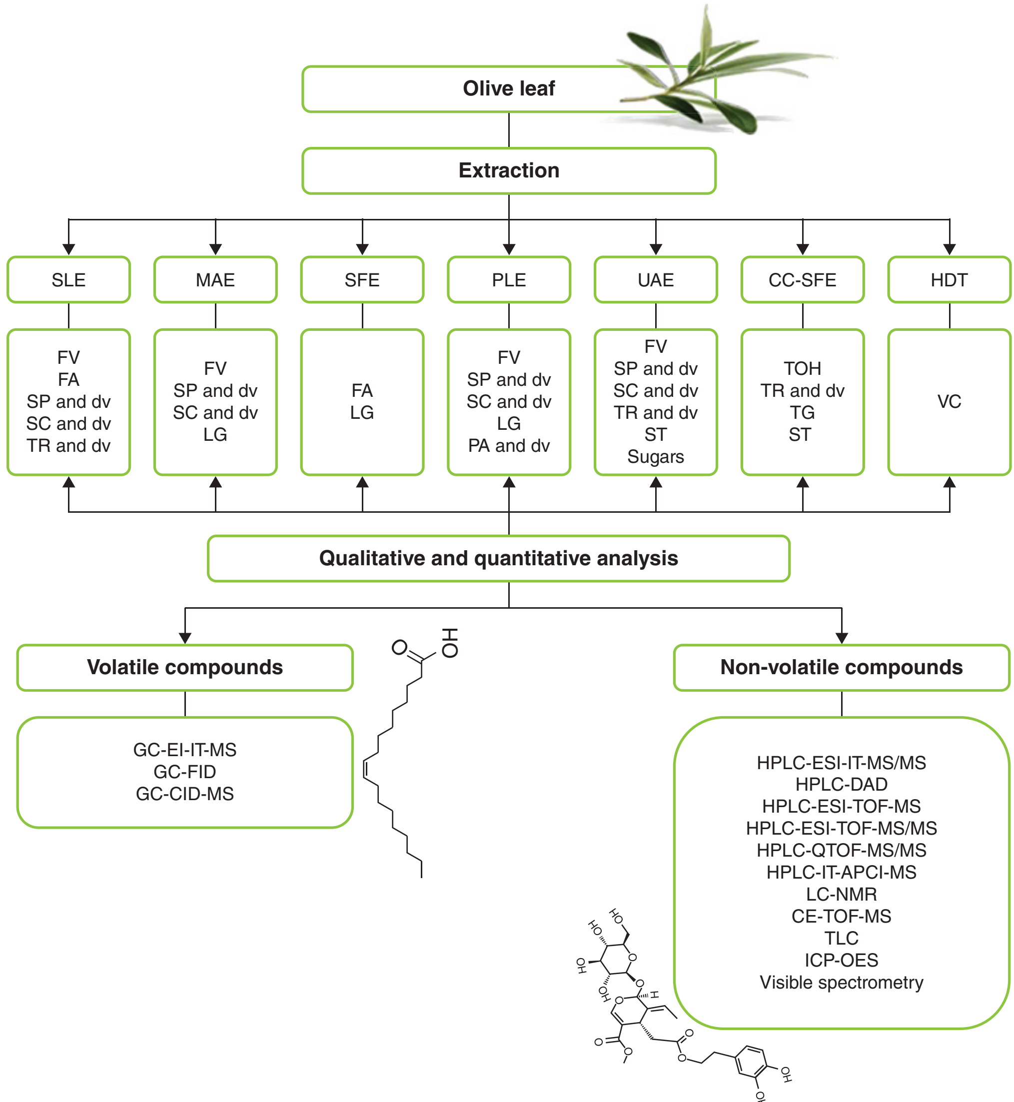

Figure 20.1 Schematic summary of the extraction and analytical techniques for characterizing functional components from olive leaves.

increasing interest in OL composition, a huge number of analytical methods based on different techniques have been reported for the analysis of different types of extracts from this matrix. Prior *et al.* (2005) described some strong reasons to standardize analytical methods, which include providing guidance for the appropriate application of assays, and the possibility to compare foods or commercial products, to control variation between products, and to provide quality standards for regulatory issues and health claims (Prior *et al.*, 2005). Therefore, and although the different health effects of OL *in vitro* and *in vivo* have been described and many available products deriving from OL exist on the market for different purposes, the lack of standardization of analyses makes it difficult to establish legislation concerning OL as nutraceuticals. Thus, regulation of the way in which the OL matrix is analyzed should be the future goal for researchers, before it can be classified as a nutraceutical by the relevant bodies.

Acknowledgments

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References

Alonso-Salces, R.M., Holland, M.V., & Guillou, C. (2011) 1H-NMR fingerprinting to evaluate the stability of olive oil. Food Control 22, 2041–2046.

AOAC (1995) Official method of analysis of AOAC International. AOAC International, Arlington, VA.

Arráez-Román, D., Sawalha, S., Segura-Carretero, A., Menendez, J., & Fernández-Gutiérrez, A. (2008) Identification of phenolic compounds in olive leaves using CE-ESI-TOF-MS. Agro Food Industry Hi-Tech 19, 18–22.

Bączek, T., Kaliszan, R., Novotná, K., & Jandera, P. (2005) Comparative characteristics of HPLC columns based on quantitative structure–retention relationships (QSRR) and hydrophobic-subtraction model. Journal of Chromatography A 1075, 109–115.

Bahloul, N., Kechaou, N., & Mihoubi, N.B. (2014) Comparative investigation of minerals, chlorophylls contents, fatty acid composition and thermal profiles of olive leaves (*Olea europaea* L.) as by-product. Grasas y Aceites, 65. doi: http://dx.doi.org/10.3989/gya.0102141

Barrajón-Catalán, E., Taamalli, A., Quirantes-Piné, R., Roldan-Segura, C., Arráez-Román, D., Segura-Carretero, A., Micol, V., & Zarrouk, M. (2015) Differential metabolomic analysis of the potential antiproliferative mechanism of olive leaf extract on the JIMT-1 breast cancer cell line. Journal of Pharmaceutical and Biomedical Analysis 105, 156–162.

Brahmi, F., Flamini, G., Issaoui, M., Dhibi, M., Dabbou, S., Mastouri, M., & Hammami, M. (2012) Chemical composition and biological activities of volatile fractions from three Tunisian cultivars of olive leaves. Medicinal Chemistry Research 21, 2863–2872.

Cavalheiro, C.V., Picoloto, R.S., Cichoski, A.J., Wagner, R., de Menezes, C.R., Zepka, L.Q., Da Cimpoiu, C. (2006) Analysis of some natural antioxidants by thin-layer chromatography and high performance thin-layer chromatography. Journal of Liquid Chromatography and Related Technologies 29, 1125–1142.

Contreras, J.A., Rockwood, A.L., Tolley, H.D., & Lee, M.L. (2013) Peak sweeping and gating using thermal gradient gas chromatography. Journal of Chromatography A 1278, 160–165.

Dais, P., & Hatzakis, E. (2013) Quality assessment and authentication of virgin olive oil by NMR spectroscopy: a critical review. Analytica Chimica Acta 765, 1–27.

El, S.N., & Karakaya, S. (2009) Olive tree (*Olea europaea*) leaves: potential beneficial effects on human health. Nutrition Reviews 67, 632–638.

Gómez-González, S., Ruiz-Jiménez, J., Priego-Capote, F., & Luque De Castro, M.D. (2010) Qualitative and quantitative sugar profiling in olive fruits, leaves, and stems by gas chromatography-tandem mass spectrometry (GC-MS/MS) after ultrasound-assisted leaching. Journal of Agricultural and Food Chemistry 58, 12292–12299.

Guinda, Á., Rada, M., Delgado, T., Gutiérrez-Adánez, P., & Castellano, J.M. (2010) Pentacyclic triterpenoids from olive fruit and leaf. Journal of Agricultural and Food Chemistry 58, 9685–9691.

Harnly, J.M., Bhagwat, S., & Lin, L. (2007) Profiling methods for the determination of phenolic compounds in foods and dietary supplements. Analytical and Bioanalytical Chemistry 389, 47–61.

Heimler, D., Pieroni, A., Tattini, M., & Cimato, A. (1992) Determination of flavonoids, flavonoid glycosides and biflavonoids in *Olea europaea* L. leaves. Chromatographia 33, 369–373.

Hernández-Borges, J., Borges-Miquel, T.M., Rodríguez-Delgado, M.Á., & Cifuentes, A. (2007) Sample treatments prior to capillary electrophoresis–mass spectrometry. Journal of Chromatography A 1153, 214–226.

Japón-Luján, R., & Luque De Castro, M.D. (2008) Liquid-liquid extraction for the enrichment of edible oils with phenols from olive leaf extracts. Journal of Agricultural and Food Chemistry 56, 2505–2511.

Karioti, A., Giocaliere, E., Guccione, C., Pieraccini, G., Gallo, E., Vannacci, A., & Bilia, A.R. (2014) Combined HPLC-DAD-MS, HPLC-MSn and NMR spectroscopy for quality control of plant extracts: the case of a commercial blend sold as dietary supplement. Journal of Pharmaceutical and Biomedical Analysis, 88, 7–15.

Kiritsakis, K., Kontominas, M.G., Kontogiorgis, C., Hadjipavlou-Litina, D., Moustakas, A., & Kiritsakis, A. (2010) Composition and antioxidant activity of olive leaf extracts from Greek olive cultivars. Journal of the American Oil Chemists' Society 87, 369–376.

Konoz, E., Abbasi, A., Moazeni, R.S., Parastar, H., & Jalali-Heravi, M. (2013) Chemometrics-assisted gas chromatographic-mass spectrometric analysis of volatile components of olive leaf oil. Journal of the Iranian Chemical Society 10, 169–179.

La Torre, G.L., Saitta, M., Vilasi, F., Pellicanò, T., & Dugo, G. (2006) Direct determination of phenolic compounds in Sicilian wines by liquid chromatography with PDA and MS detection. Food Chemistry 94, 640–650.

Lisec, J., Schauer, N., Kopka, J., Willmitzer, L., & Fernie, A.R. (2006) Gas chromatography mass spectrometry-based metabolite profiling in plants. Nature Protocols 1, 387–396.

Lockyer, S., Corona, G., Yaqoob, P., Spencer, J.P.E., & Rowland, I. (2015) Secoiridoids delivered as olive leaf extract induce acute improvements in human vascular function and reduction of an inflammatory cytokine: a randomised, double-blind, placebo-controlled, cross-over trial. British Journal of Nutrition 114, 75–83.

Marcone, M.F., Wang, S., Albabish, W., Nie, S., Somnarain, D., & Hill, A. (2013) Diverse food-based applications of nuclear magnetic resonance (NMR) technology. Food Research International 51, 729–747.

Meirinhos, J., Silva, B.M., Valentão, P., Seabra, R.M., Pereira, J.A., Dias, A., Andrade, P.B., & Ferreres, F. (2005) Analysis and quantification of flavonoidic compounds from Portuguese olive (*Olea europaea* L.) leaf cultivars. Natural Product Research 19, 189–195.

Mylonaki, S., Kiassos, E., Makris, D.P., & Kefalas, P. (2008) Optimisation of the extraction of olive (*Olea europaea*) leaf phenolics using water/ethanol-based solvent systems and response surface methodology. Analytical and Bioanalytical Chemistry 392, 977–985.

Orozco-Solano, M., Ruiz-Jiménez, J., & Luque de Castro, M.D. (2010) Ultrasound-assisted extraction and derivatization of sterols and fatty alcohols from olive leaves and drupes prior to determination by gas chromatography–tandem mass spectrometry. Journal of Chromatography A 1217, 1227–1235.

Paskovic, I., Perica, S., Pecina, M., Hancevic, K., Polic Paskovic, M., & Herak Custic, M. (2013) Leaf mineral concentration of five olive cultivars grown on calcareous soil. Journal of Central European Agriculture 14, 1471–1478.

Pereira, A.P., Ferreira, I.C.F.R., Marcelino, F., Valentão, P., Andrade, P.B., Seabra, R., Estevinho, L., Bento, A., & Pereira, J.A. (2007) Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. cv. Cobrançosa) leaves. Molecules 12, 1153–1162.

Poole, C.F. (2012) Packed columns for gas-liquid and gas-solid chromatography. In: Gas chromatography, ed. C.F. Poole. Elsevier, Oxford, 97.

Prior, R.L., Wu, X., & Schaich, K. (2005) Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry 53, 4290–4302.

Quirantes-Piné, R., Lozano-Sánchez, J., Herrero, M., Ibáñez, E., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2013) HPLC-ESI-QTOF-MS as a powerful analytical tool for characterising phenolic compounds in olive-leaf extracts. Phytochemical Analysis 24, 213–223.

Sánchez Ávila, N., Priego Capote, F., & Luque de Castro, M.D. (2007) Ultrasound-assisted extraction and silylation prior to gas chromatography-mass spectrometry for the characterization of the triterpenic fraction in olive leaves. Journal of Chromatography A 1165, 158–165.

Silva, S., Gomes, L., Leitão, F., Coelho, A.V., & Boas, L.V. (2006) Phenolic compounds and antioxidant activity of *Olea europaea* L. Fruits and leaves. Food Science and Technology International 12, 385–396.

Silva Elipe, M.V. (2003) Advantages and disadvantages of nuclear magnetic resonance spectroscopy as a hyphenated technique. Analytica Chimica Acta 497, 1–25.

Taamalli, A., Arráez-Román, D., Barrajón-Catalán, E., Ruiz-Torres, V., Pérez-Sánchez, A., Herrero, M., Ibañez, E., Micol, V., Zarrouk, M., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2012a) Use of advanced techniques for the extraction of phenolic compounds from Tunisian olive leaves: phenolic composition and cytotoxicity against human breast cancer cells. Food and Chemical Toxicology 50, 1817–1825.

Taamalli, A., Arráez-Román, D., Ibañez, E., Zarrouk, M., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2012b) Optimization of microwave-assisted extraction for the characterization of olive leaf phenolic compounds by using HPLC-ESI-TOF-MS/IT-MS2. Journal of Agricultural and Food Chemistry 60, 791–798.

Tabera, J., Guinda, Á., Ruiz-Rodríguez, A., Señoráns, F.J., Ibáñez, E., Albi, T., & Reglero, G. (2004) Countercurrent supercritical fluid extraction and fractionation of high-added-value compounds from a hexane extract of olive leaves. Journal of Agricultural and Food Chemistry 52, 4774–4779.

Talhaoui, N., Gómez-Caravaca, A.M., León, L., De la Rosa, R., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2014) Determination of phenolic compounds of 'Sikitita' olive leaves by HPLC-DAD-TOF-MS. Comparison with its parents 'Arbequina' and 'Picual' olive leaves. LWT – Food Science and Technology 58, 28–34.

Toplu, C., Uygur, V., & Yildiz, E. (2009) Leaf mineral composition of olive varieties and their relation to yield and adaptation ability. Journal of Plant Nutrition 32, 1560–1573.

Wang, W., Scali, M., Vignani, R., Spadafora, A., Sensi, E., Mazzuca, S., & Cresti, M. (2003) Protein extraction for two-dimensional electrophoresis from olive leaf, a plant tissue containing high levels of interfering compounds. Electrophoresis 24, 2369–2375.

Whelan, T.J., Gray, M.J., Slonecker, P.J., Shalliker, R.A., & Wilson, M.A. (2005) Study of the selectivity of reversed-phase columns for the separation of polycarboxylic acids and polyphenol compounds. Journal of Chromatography A 1097, 148–156.

21 Production of phenol-enriched olive oil

Kostas Kiritsakis and Dimitrios Gerasopoulos

21.1 Introduction

The Mediterranean diet, being rich in fruits, vegetables, and olive oil, has been related with decreased incidence of cardiovascular diseases and cancer, due to its high content of bioactive compounds, such as vitamins, flavonoids, and other phenols (Benavente-Garcia *et al.*, 2000; Visioli & Galli, 2002).

During the last few years, a great deal of research effort has been devoted toward the enrichment of olive oil with antioxidants from natural sources, such as plants and herbs, creating products characterized as "gourmet" (Damechki *et al.*, 2001). This can be justified by the ability of these compounds to protect not only the human organism from diseases related to the harmful effects of free radicals in the body, but also the product itself from deterioration due to oxidation. Enriched olive oils have a prolonged shelf life and increased resistance to undesirable alterations during cooking or storage (Salta *et al.*, 2007), while they preserve their initial nutritional and organoleptic qualities (Antoun & Tsimidou, 1997).

21.2 Olive oil phenolic compounds and their functional properties

Olive oil is acknowledged as the healthiest edible oil source, particularly due to its phenolic compounds and their beneficial functional effects to the human organism, which include antioxidant, antiatherogenic, antithrombotic, and other properties (Salvador *et al*., 2003; Corominas-Faja *et al*., 2014; Servili *et al*., 2014). Olive oil phenolic compounds are also effective against cancer (Simopoulos, 2001; Kris-Etherton *et al*., 2002; Tapiero *et al*., 2002) and present anti-inflammatory (Trichopoulou & Lagiou, 1997) and antimicrobial effects (Umar Lule & Xia, 2005). Valls *et al.* (2015) found that an olive oil enriched with its own phenolic compounds had a positive effect on endothelial function in hypertensive patients. Finally, these phenols, besides their bioactivity, also contribute to olive oil's special taste and aroma (Bendini *et al.*, 2007).

Besides polyphenols, virgin olive oil (VOO) consists of a variety of important compounds, such as α-tocopherol, β-carotene, chlorophyll, phytosterols, terpenic acids, squalene, flavonoids, and simple phenols (Visioli & Galli, 2002), some of which also exhibit functional properties, such as powerful antioxidant activity (Bradley & Min, 1992; Baldioli *et al*., 1996). This increased antioxidant ability, along with its fatty acid composition (characterized by high monounsaturated fatty acids content) (Aparicio *et al.*, 1999), give olive oil high resistance to oxidative deterioration.

Olive oil phenols are considered to be an excellent defense system against reactive oxygen species (ROS). More specifically, they prevent oxidative stress–related diseases that are connected to ROS, such as cancer, coronary heart disease, and even Alzheimer's disease (Trichopoulou *et al.*, 2003). In addition, olive oil phenols are considered an important part of its quality evaluation, as they are responsible for its flavor, aroma, and oxidation protection (Andrewes *et al.*, 2003). The positive linear relationship between the total content of phenolic compounds and oil stability has long been established (Gutfinger, 1981).

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The phenolic compounds in olive oil (phenolic alcohols, phenolic acids, secoiridoid derivatives, lignans, and flavonoids), which are associated with bitter and pungent taste, oxidative stability, and shelf life (Angerosa *et al.*, 2000), can be divided into three categories. These are oleuropein; ligstroside aglycones and their derivatives; and simple phenols, phenethyl alcohol derivatives, cinnamic and benzoic acids, and other phenolic compounds recently identified as lignans (Brenes *et al.*, 2000). All these phenols/polyphenols exhibit different properties that contribute to olive oil's effect on the human organism. For example, oleocanthal, the dialdehydic form of decarboxymethyl ligstroside aglycone (p-HPEA-EDA), which is responsible for the pungency associated with some extra virgin olive oils (Andrewes, 2003; Karkoula *et al.*, 2012), is considered a natural nonsteroidal anti-inflammatory drug. Oleocanthal possesses inhibitory ability on cyclooxygenases (COX-1 and -2), similar to ibuprofen (Beauchamp *et al.*, 2005). Recent research on oleocanthal showed that it acts therapeutically on inflammatory degenerative joint diseases as well (Iacono *et al.*, 2010). In addition, oleocanthal is stable in the gastric juice and acts against *Helicobacter pylori*, a bacterium considered to be responsible for peptic ulcers and some types of gastric cancer (Romero *et al.*, 2007).

Oleuropein is a glycosylated phenolic compound that contributes to the bitterness of olives. Its degradation products – 3,4-dihydroxyphenylethanol (3,4-DHPEA) or hydroxytyrosol; the dialdehydic form of elenolic acid conjugated with hydroxytyrosol (3,4-DHPEA-EDA), also known as oleacein; as well as oleuropein aglycone (3,4-DHPEA-EA) – are some of the most important olive oil phenols that present great antioxidant activity (Baldioli *et al.*, 1996). Oleuropein is proven to have vasodilating, antispasmodic, antihypertensive, and anti-arrhythmic properties (Petroni *et al.*, 1995). Oleacein has similar activities to oleocanthal, but also displays anti–breast cancer properties (Menendez *et al.*, 2009), as well as antioxidant activities (Paiva-Martins *et al.*, 2009). Anticancer and anti–cardiovascular disease properties were also connected to caffeic acid, *p*-coumaric acid, pinoresinol, and verbascoside (Figure 21.1).

Finally, the importance of phenolic compounds in preventing oxidation during storage has also been reported (Kiritsakis & Dugan, 1984; Kiritsakis *et al*., 2002; Bendini *et al*., 2009). Recently, Suarez *et al.* (2011) studied the stability of a phenol-enriched olive oil during storage and found out that phenolic enrichment of the oils extended their shelf life, delaying the appearance of peroxides and improving their oxidative stability.

21.3 Effect of the extraction process on olive oil functional compounds

The functional components that give olive oil its unique characteristics and its nutritional and biological properties are affected by a number of parameters. These parameters can be grouped into three categories: those acting during oil formation in the fruit (variety, ripeness and health of the fruit, age of the tree, and environmental and cultivation conditions) (Tura *et al.*, 2007); those acting during the mechanical oil extraction, where these components are transferred from the olive fruit to the oily phase (temperature, amount of water added, and extraction procedure) (Ranalli *et al.*, 2001); and oil storage conditions (Kiritsakis *et al.*, 2002).

Mechanical procedures include the phases of crushing, malaxation, and extraction. Each part of the process, together with the fruit characteristics, specifies the formation of volatile compounds and the release of phenolic antioxidants, which greatly influence the quality of VOO (De Stefano *et al.*, 1999). The total phenolic content is significantly affected by the extraction technique employed (Amirante *et al.*, 2001) and the conditions applied (Artajo *et al*., 2006; Gómez-Rico *et al*., 2009; Clodoveo, 2012).

The phenolic compounds are generally more soluble in the water than in the oily phase. As a result, only a very small fraction of the phenols present in the olive fruit is released into the oily phase during the extraction process. Thus, during processing, a large amount of the antioxidants is lost not only in the wastewater but also in the solid remains (pomace) (Rodis *et al.*, 2002). The most important loss of the different phenolic groups present in olive paste occurs in the solid phase (wet pomace), and the low lipophilic behavior of the phenolic structures leads to low concentrations in VOO.

One of the most commonly used methods of olive processing is the continuous centrifugation system, which has replaced the classic mill, also known as "three-phase" or "two-phase" systems, depending on the method used to separate olive oil from the by-products. These methods employ a metal crusher to break the

(–)-Oleocanthal (inhibits COX-1, COX-2 and scavenges ROS)

Oleuropein (vasodilator, coronary vasodilator, antispasmodic, antihypertensive, antiarrhythmic, binds with Aβ and scavenges ROS)

Hydroxytyrosol (inhibits platelet aggregation and scavenges ROS)

1-(3′-methoxy-4′-hydroxy-phenyl)-6,7-dihydroxy-isochroman (inhibits platelet aggregation and scavenges ROS)

1-phenyl-6,7-dihydroxy-isochroman (inhibits platelet aggregation and scavenges TOS)

Caffeic acid (exhilarates CNS and scavenges ROS)

p-coumaric acid (antihyperlipidaemia and scavenges ROS)

Pinoresinol (inhibits cAMP phosphodiesterase and scavenges ROS)

Verbascoside (anti-inflammatory, inhibits 5-lipoxidase and scavenges ROS)

Ligstroside

Demethyloleuropein

Figure 21.1 Structures and pharmacological effects of olive oil phenols. *Source*: Yang *et al.* (2007). Reproduced with permission of Elsevier.

olive fruit and release the oil, a malaxer to aggregate the oil drops, and a horizontal centrifuge, the decanter, for the separation of different phases (Kiritsakis, 1998). The continuous method has reduced the storage time of the fruit before processing and therefore the fermentation processes, leading to higher quality oils, as they lacked the *atrojado* sensory defect (Salvador *et al.*, 2003).

21.3.1 Crushing

Olive paste is prepared by crushing the olive fruit. This way, the fruit's tissues break and the oil drops are released. It is a critical step that affects the quality of the final VOO produced. During the crushing stage, enzymes that are responsible for the transformation of polar phenolic and volatile compounds are triggered. The crushing conditions exert a major effect on the concentration of these components, mostly the hydroxytyrosol derivatives, which leads to alterations in the organoleptic characteristics and oxidative stability of olive oil. Different olive crushers are used nowadays, such as hammer mills, toothed crushers, or blade cutters. It has been shown that the type of mill employed affects the volatile composition and other minor components (Inarejos-García *et al.*, 2011).

The hammer crusher is considered to be the strongest of the crushing mills. Compared to the traditional stone mills, it leads to higher phenolic extraction from the tissues (Caponio *et al.*, 2003). The result is more bitter oils with higher antioxidant capacity. A hammer crusher employs fixed or mobile grids with holes of different diameters, so the intensity of crushing can be controlled, depending on the maturity of the fruit, the cultivar, and the desired oil characteristics. Grid hole size and rotation speed can affect the composition and the quality of the produced VOO. As the diameter gets smaller, the extraction of the phenolic compounds produces better results (Inarejos-García *et al.*, 2011). Moreover, when blade or stone crushers are used, a significantly higher amount of volatiles is produced in olive oil, especially Z-3-hexen-1-ol, hexanal, and E-2-hexenal (Inarejos-García *et al.*, 2011).

21.3.2 Malaxation

After the crushing process, oil droplets are distributed within the olive mash and can be lost with the by-products. The next step of the extraction process is malaxation, the mixing of the crushed fruit, which helps coalescence, where small oil droplets coalesce into large droplets and form the lipid phase, which is more easily mechanically separated by subsequent centrifugation. Thus, slow malaxation enables the production of large oil droplets and leads to a continuous oil phase (Angerosa *et al.*, 2001).

The conditions applied during malaxation define the breaking up of the oil–water emulsions formed during crushing. As a result, malaxation is probably the most crucial step in the olive oil extraction procedure that determines not only the quantity but also the quality of the produced olive oil, altering both the phenolic and the volatile compounds of the final product (Di Giovacchino *et al.*, 2002; Gómez-Rico *et al.*, 2009). While adequate malaxation time is essential for the merge of oil droplets by ensuring the breakage of the emulsions and the maximization of the yield, it must be determined carefully to avoid causing a reduction in the major phenolics and the overall quality of the oil. Studies have demonstrated that malaxation time also has an important effect on resistance to oxidation of the olive oil produced. A longer malaxation procedure favors oxidative degradation of the phenolic compounds (chemical or enzymatic) and increases the presence of oil volatiles (Lercker *et al.*, 1999; Servili *et al.*, 2003).

It has been proven that higher oil yields are obtained by malaxing at higher temperatures as the viscosity of the mix is decreased (Amirante *et al.*, 2002). While temperature increase seems on one hand to downgrade the volatile content of the final product (Di Giovachino *et al.*, 2002), on the other hand it favors the phenolic content as the applied malaxation temperature rises from 20 to 40 °C (Gómez-Rico *et al.*, 2009). However, oils obtained using temperatures over 40 °C were rejected because of heated or burnt "off flavors" (Boselli *et al.*, 2009). This enhancement of the phenolic content is attributed to the increase in the partition coefficients of the phenolic compounds between the water and the oily phases in the olive paste (Rodis *et al.*, 2002). Furthermore, the increase in total phenolics at high malaxation temperatures was accompanied by decreases in bitter and pungent sensory characteristics (Boselli *et al.*, 2009). Nevertheless, contrasting results indicating an inverse relationship between malaxation temperature and phenolic content have also been reported (Servili *et al.*, 2003).

Table 21.1 Effect of malaxation temperature on the total phenolic content and oxidative stability.

Cultivar	Temperature		
	25 °C	**35 °C**	**45 °C**
	TPC (mg/kg)		
'Frantoio'/'Leccino'	84.7	133	143
'Coratina'	172	201	241
	Induction period (h)		
'Frantoio'/'Leccino'	21.4	28.9	29.4
'Coratina'	43.7	47.8	53.9

Source: Boselli *et al.* (2009). Reproduced with permission of Elsevier.

At the stages of crushing and malaxation, several enzymes that are involved in the generation and transformation of polar phenolic and volatile compounds are triggered. This has an immediate effect on the concentration of these compounds and, as a consequence, on the taste, aroma, and stability of the produced oil, depending on processing conditions (Di Giovacchino *et al.*, 2002; Gómez-Rico *et al.*, 2009). The effect of malaxation temperature on the total phenolic content and oxidative stability of olive oils from two different cultivars is shown in Table 21.1. The oxidative stability is expressed as hours of induction period, calculated with the Rancimat method.

21.3.3 Decantation

At the final stage of the procedure, centrifugal separation of the different phases takes place. The three-phase decanter is the first type that was released in the market and used for olive oil extraction. It is called three-phase because this type of centrifugal decanter allows the separation of three flows of matter (i.e., olive oil, pomace, and the wastewater), and it needs lukewarm water added to dilute olive paste (Kiritsakis, 1998) in order to facilitate oil acquisition. Consequently, the use of this system demands a serious amount of water for its function and generates a considerable volume of wastewater (80–100 L/100 kg of olives) (Nergiz & Unal, 1991; Di Giovacchino *et al*., 1994). Moreover, the phenols and o-diphenols are highly soluble in water, thus a significant amount is drifted with the wastewater (Di Giovacchino, 2001). The addition of water to the olive paste also causes a significant reduction of the glycosidic and flavonoid compounds through glycosidic modification or degradation (Boskou, 2000). As a result, the three-phase decanters produce olive oil with significantly reduced phenolic content.

At the beginning of the 1990s, a new type of decanter was released to the market, the two-phase centrifugal decanter, that didn't require the addition of lukewarm water and was able to separate the oily phase from the olive paste without producing vegetable wastewater. This new type of decanters produces olive oils that exhibit a higher total phenolic content and are more resistant to oxidation (Gimeno *et al.*, 2002). This has led to an improvement of oil quality, as the natural phenols and o-diphenols contents were positively affected by the elimination of added water (Ranalli & Angerosa, 1996). At the same time, by minimizing the amount of wastewater, the production costs decreased (Caponio *et al.*, 2003).

21.4 Enhancement of olive oil's antioxidant content

Phenolic compounds play a significant part in the human diet because they exhibit important health benefits. The main sources of dietetic phenols are plants. Olive oil cannot be considered a main source of phenols, because its phenol concentration is between 20 and 800 mg/kg, which is not an adequate daily intake, considering limitations in caloric supply (Artajo *et al.*, 2006). Additionally, the percentage of the olive oil's phenolic content absorbed by humans is about 60% (Vissers *et al*., 2004; Jacobs *et al*., 2009).

For many years, synthetic antioxidants have been used as food additives. Such substances are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ), which is the most powerful synthetic antioxidant. However, research has indicated that these compounds may present a health risk for consumers, as they have been connected to cancer and carcinogenesis (Prior, 2004). For that reason, the use of TBHQ as a food additive is restricted in some countries, and BHA has been removed from the generally recognized as safe (GRAS) list of compounds. Thus, a majority of the industries that produce food additives, cosmetics, and pharmaceuticals have recognized the necessity of using healthier, safer, and cheaper alternative compounds. In order to achieve phenolic enhancement that is free from health risk, natural antioxidants that could replace the synthetic ones have been studied (Yanishlieva & Marinova, 2001). Tocopherols, ascorbic acid, rosemary extracts, lycopene, and some flavonoids and olive polyphenols from either olive mill wastewaters or olive leaves are now available in the form of food additives, as replacements for synthetic products (Artajo *et al.*, 2006).

21.4.1 Sources and methods of olive oil enrichment in natural antioxidants

In order to increase the phenolic compounds of olive oil, a number of methods have been studied. The majority of them occupies common by-products of the olive oil's extraction process, as olive cake, olive mill wastewater, or olive leaves, because they carry high concentrations of the desirable substances. The phenolic compounds that exist in these by-products are retrieved by extraction and, after appropriate processing, returned to the produced olive oil. However, alternative sources, such as herbs and vegetables, have also been studied.

21.4.1.1 Natural sources of antioxidants

In recent years, a plethora of natural sources of antioxidants and methods of enrichment have been reported. The research conducted includes spices and vegetables (e.g., oregano and spinach) in the form of powder (Lee *et al.*, 2002; Houhoula *et al.*, 2003); spice extracts (oregano, rosemary, mint, sage, hyssop, thyme, oat, and tea leaves) (Tian & White, 1994; Gordon & Kourimska, 1995; Yanishlieva *et al.*, 1997; Abdalla & Roozen, 1999; Che Man & Tan, 1999; Lolos *et al.*, 1999; Zandi & Gordon, 1999); leafy vegetables such as cabbage, coriander, hongone (sessile joyweed), and spinach (Shyamala *et al.*, 2005); as well as direct coextraction of vegetables in oil (Benakmoum *et al.*, 2008). Artajo *et al.* (2006) added pure phenolic compounds to refined olive oil as a lipid matrix, in order to check their effect on antioxidant stability and the bitterness index.

Nevertheless, a common problem exists when enriching olive oil with phenolic extracts. This involves the difficulty in dispersion, because this kind of emulsions is highly unstable, as the natural trend is the separation of the oil and water phases (Drelich *et al.*, 2010). In order to overcome this problem, the use of emulsifiers has been proposed to stabilize the phenolic compounds added. Suarez *et al.* (2011) identified lecithin as the best emulsifier to prepare enriched olive oils. It was observed that it allowed the hydroethanolic particles in the oil to be stabilized and limited the increase in the bitterness that occurred as a consequence of the phenolic enrichment.

21.4.1.2 Olive cake

A rich source of the desired compounds is the main by-product of the olive oil extraction process, olive cake. It is possible to extract the main phenolic compounds from olive cake, in order to enrich olive oil, using new extraction procedures that allow reduction of the extraction time and solvent consumption while increasing the efficiency of extraction (Suarez *et al.*, 2009).

21.4.1.3 Olive mill wastewater

Another method under investigation is recycling the olive mill wastewater from the decanter, because it shows an important concentration in phenolic compounds. These phenolic compounds engage in different

mechanisms against free radical attack. According to Fki *et al.* (2005), lower peroxide values and higher stability were measured in oils with an added olive mill wastewater extract. Wastewater can be used immediately after its collection from the decanter, as soon as it is produced, instead of ordinary water, to dilute the olive paste that enters the decanter. According to studies, the increase in the total phenolic content of the oil is about 30%, with an additional reduction in the volume of vegetable wastewater of about 35–40% (Di Giovacchino *et al.*, 2001). Recycling olive-vegetation water, apart from providing additives for the production of enriched olive oil, also resolves pollution problems, as it is very rich in phenols with high hydrophilicity and antimicrobial activity (Servili *et al.*, 2011).

Servili *et al.* (2011) utilized olive-vegetation water, adding a crude phenolic concentrate (CPC) during the olive oil extraction process at an industrial scale, trying to improve the bioactive phenol content in the final product. The CPC was produced from enzymatically treated olive-vegetation water by a three-phase membrane system. The prior enzymatic treatment of the olive-vegetation water was performed by depolymerizing enzymes with pectinase and hemicellulosic activities. Microfiltration, ultrafiltration, and reverse osmosis were used in order to recover the phenolic compounds. The CPC produced was added at the malaxation step, and the procedure was carried on normally. Table 21.2 shows the phenolic and volatile composition of CPC.

21.4.1.4 Olive leaves

Recent research has shown that phenolic extracts obtained from all parts of the olive plant (fruit, leaves, and pomace, and even olive wood extracts) show remarkable antioxidant activity (Farag *et al*., 2003; Brahmi *et al*., 2013; Rahmanian *et al*., 2015; Salido *et al*., 2015; Talhaoui *et al*., 2015). Olive leaves, together with olive pomace and olive mill wastewater, are the major by-products of the olive oil extraction process. Usually about 10% of the total weight of the olives that arrive at the olive oil industries is olive leaves. They are considered a cheap raw material that can be used as a useful source of food additives (Briante *et al.*, 2002),

Table 21.2 Phenolic and volatile composition of CPC.

Compound	Concentration
Phenols	**(g/L)**
Hydroxytyrosol	0.03 ± 0.003
Tyrosol	0.01 ± 0.001
Oleacein	16.9 ± 1.7
Oleocanthal	n.d.
Verbascoside	2.4 ± 0.2
Total phenols	19.3 ± 1.7
Volatile compounds	**(μg/L)**
Aldehydes	
Hexanal	1010 ± 75.5
(E)-2-Pentenal	395 ± 25.6
(E)-2-Hexenal	3280 ± 246
Alcohols	
1-Penten-3-ol	6930 ± 381
1-Pentanol	970 ± 63.1
(E)-2-Penten-1-ol	5120 ± 333
1-Hexanol	9550 ± 406
(E)-3-Hexen-1-ol	910.0 ± 65.1
(Z)-3-Hexen-1-ol	5735.0 ± 315
(Z)-2-Hexen-1-ol	1432.0 ± 78.8
Esters	
Hexyl acetate	n.d.
(Z)-3-Hexenyl acetate	n.d.

Source: Servili *et al.* (2011). Reproduced with permission of Elsevier.

Table 21.3 Antioxidant ability of the olive leaf extracts from three different cultivars added to olive oil, as expressed by the Oxidative Stability Index (OSI).

	OSI value (h)	
Cultivar	**Methanol extract**	**Methanol/water (60/40) extract**
'Megaritiki'	27.24 ± 1.51	27.50 ± 1.44
'Kalamon'	28.12 ± 1.63	27.91 ± 1.28
'Koroneiki'	27.83 ± 1.32	28.42 ± 1.33
Control	22.00 ± 1.05	

Source: Kiritsakis *et al.* (2010). Reproduced with permission of Springer.

since several studies have observed that they provide a rich source of natural antioxidants (Bouaziz & Sayadi, 2005). Bouaziz *et al.* (2008) managed to obtain a high amount of oleuropein from an olive leaf extract, with a concentration that reached 14% on a dry mass basis. Several flavonoids were also present in adequate concentrations (Savournin *et al.*, 2001).

The antioxidant capacity of olive leaves has been attributed to the presence of phenols, which act as free radical scavengers; the principal compounds found in olive leaves were oleuropein, related secoiridoids, and other derivatives (Kiritsakis *et al.*, 2010; Kontogianni & Gerothanassis, 2012). Table 21.3 shows the improvement of antioxidant ability of a commercial olive oil when enriched with olive leaf extracts from three different cultivars, as expressed by the Oxidative Stability Index (OSI).

The olive leaf extract lowers blood pressure in animals and increases blood flow in the coronary arteries, to relieve arrhythmia and prevent intestinal muscle spasms (Garcia *et al.*, 2000). In old times, olive leaves were used as a folk remedy for combating diseases, such as malaria (Lee *et al.*, 2009). These characteristics of olive leaves are attributed to the bioavailability of their phenolic compounds and derivatives (Meirinhos *et al*., 2005; Herrero *et al*., 2011; Kontogianni & Gerothanassis, 2012).

Olive leaves have been used to enhance oils' oxidative stability in the form of juice (Farag *et al.*, 2007), methanol extract (Salta *et al.*, 2007), and ethanol extract (Japon-Lujan *et al.*, 2006). Recently, olive leaves were coextracted with olive oil with very good results (Nenadis *et al.*, 2010). Alternatively, several quantities of olive leaves were added to over-mature olives before the olive oil extraction procedure, resulting in a product with improved organoleptic characteristics (Di Giovacchino *et al.*, 1996).

Enriching olive oil with phenolic compounds extracted from olive leaves has been reported to impart similar stability to refined olive oil as that found in virgin olive oil (Bouaziz *et al.*, 2010). However, there are a few aspects that need to be taken into consideration when olive leaves or olive leaf extracts are added to olive oil, such as possible color alterations. Moreover, besides an expected change in the organoleptic characteristics of a phenol-enriched olive oil, chlorophyll content might be high, causing photooxidation (Damechki *et al.*, 2001). Chlorophylls can act as photosensitizers, due to their ability to transfer energy from light to triplet oxygen, giving singlet oxygen that reacts with unsaturated fatty acids and forms hydroperoxides. Nevertheless, chlorophylls act as antioxidants in the dark, protecting the oil from oxidation (Kiritsakis, 1998).

21.4.1.5 Enzymes

In order to improve the yield and quality of olive oil, the use of enzyme formulations, mostly in liquid form, during the extraction process has also been studied (Ranalli & De Mattia, 1997; Garcia *et al*., 2001). Ranalli *et al.* (2004) found that adding enzymes during olive oil extraction increases the olive oil yield as well as the concentration of constituents that dissolve in the oily phase. Several key compounds, such as phenols, tocopherols, lipochromes, and flavor compounds, exhibited an increase in olive oils obtained with enzymatic application in the olive paste before the malaxation stage. In addition, the concentration of lignans 1-acetoxypinoresinol and pinoresinol slightly increased (Chiacchierini *et al.*, 2007). The total effect of the enzymes on the phenolic content in oils depends on the olive cultivar used (Ranalli & Serraiocco, 1995). It has been determined that three types of enzymes, pectinases, cellulases, and hemicellulases, were found to be essential for extraction of the oil from olives (Galante *et al.*, 1998).

Table 21.4 Effect of the addition of Olivex + Glucanex during malaxation on the phenolic compounds.

	Concentration mg/kg	
Compound	**Control**	**Enzymatically treated**
Hydroxytyrosol	0.6 ± 0.1	1.0 ± 0.1
Tyrosol	1.6 ± 0.1	2.0 ± 0.1
Vanillic acid	0.9 ± 0.1	1.1 ± 0.1
Vanillin	0.2 ± 0.1	0.3 ± 0.1
p-Coumaric acid	0.2 ± 0.1	0.2 ± 0.1
Oleacein	142.4 ± 12.0	246.9 ± 4.9
Oleocanthal	15.7 ± 1.4	18.5 ± 0.5
1-Acetoxypinoresinol	96.0 ± 3.1	99.5 ± 1.3
Pinoresinol	65.3 ± 10.6	67.1 ± 1.5
Oleuropein aglycones	8.6 ± 0.8	12.6 ± 0.5
Luteolin	6.2 ± 0.2	6.6 ± 0.2
Apigenin	1.5 ± 0.1	1.5 ± 0.1
Orthodiphenols	188.7 ± 12.6	319.2 ± 5.0
Non-orthodiphenols	181.4 ± 11.1	190.2 ± 2.1

Source: Garcia *et al.* (2001). Reproduced with permission of Elsevier.

One example of an enzyme complex that is tested is rapidase, consisting of pectolytic, cellulolytic, and hemicellulolytic enzyme species, which degrade the fruit colloids (pectins, hemicelluloses, proteins, etc.), emulsifying the minute oil droplets. Rapidase showed higher quality in concentration of phenols, volatile compounds, tocopherols, carotenes, and chlorophylls, and a lower oxidative deterioration than in oils without enzymatic treatment (Ranalli *et al.*, 2004). According to studies in which commercial enzyme Olivex+Novoferm 12 is added, the complexation of hydrophilic phenols with polysaccharides is reduced and the concentration of free phenols in the pastes increases, along with their release in the oils and the wastewater (Vierhuis *et al.*, 2001). When Bioliva, another natural enzyme extract, was used, minor components like phenols, tocopherols, volatiles, carotenes, xanthophylls, and chlorophylls increased their concentration into the oily phase along with flavor and shelf life (Ranalli *et al.*, 2004). Furthermore, Cytolase 0 is a frequently used combination of enzymes, consisting of pectinases (from *Aspergillus*), cellulases, and hemicellulases (from *Trichoderma*), plus some minor enzymes, which all exist in olive fruit, but are lost during the oil extraction process. Addition of Cytolase 0 replaces and even enhances them (Ranalli *et al.*, 1999). Novoferm 12 is a pectolytic enzyme preparation of *Aspergillus niger* origin, Glucanex is an enzyme formulation rich in glucosidases, while Olivex is an enzyme preparation derived from *Aspergillus aculeatus*, which seems to give good oil extraction and better stability when stored. Table 21.4 shows the effect in the phenolic compounds when adding an Olivex+Glucanex mixture in 'Arbequina' pastes during malaxation (Garcia *et al.*, 2001).

One of the enzymatic methods also used to improve the desired characteristics of olive oil and its by-products includes the use of macerating enzymes during olive oil extraction, which can lead to increased extraction (up to 2 kg oil per 100 kg olives under cold processing conditions), better centrifugal fractionation of the oily must, higher levels of antioxidants and vitamin E in oils, slow induction of rancidity, overall improvement in plant efficiency, and low oil content in the wastewater (Galante *et al.*, 1998). In addition, olive wastewater exhibited a higher concentration of phenolics when the pastes were treated with enzymes (Garcõa *et al.*, 2001).

It is important to note that the enzymes studied are naturally present inside the olive fruit, but deactivated during the extraction process. As a result, the replacement of these enzymatic species is appropriate, taking into consideration the role they play in determining the final product quality. Finally, the enzymatic complexes, due to their water solubility, after producing their positive effects on composition of oil analytical fractions, get washed out with the olive mill wastewater (Ranalli & De Mattia, 1997), implying that oil composition is not modified.

The role of the various enzymes present in the enzyme preparations is not yet determined. According to one theory, the enzymes degrade the olive cell wall and change the rheological behavior of the paste

(Coimbra *et al.*, 1994). Another theory is that by degrading the olive cell wall, they help the oil droplets gradually merge into larger droplets until they form the oil phase, which is more easily separated (Ranalli & De Mattia, 1997).

21.4.1.6 Alternative methods of enrichment

Alternative ways to improve the quality of the final products have also been tested. Yousfi *et al.* (2009) treated olives with modified atmosphere and ethylene and found that modified atmospheres induced off-flavor development in the oil extracted, producing a significant reduction in the overall grading of their sensory quality, which was intensified by the ethylene addition.

According to research conducted by Tekaya *et al.* (2013), fertilization can change the levels of antioxidant compounds. Appropriate fertilizers can be used to obtain oils of the highest quality, as the levels of the antioxidants present may be influenced by nutrient availability. Furthermore, innovative solvents and methods of extraction have also been attempted, in order to maximize the amount of antioxidants recovered from the raw material. Recently, ethyl lactate, an environmentally friendly solvent, has been examined as an extraction solvent with promising results (Ishida & Chapman, 2009). Aparicio and Alcalde (2009) thoroughly investigated solutions of ethyl-lactate in water, including studying phase behavior, thermodynamic properties, spectroscopic studies, and molecular dynamics simulations. Kiritsakis *et al.* (2010) used solvents of increasing polarity (petroleum ether, dichloromethane, methanol, and methanol/water; 60:40, v/v) to extract phenols from olive leaves. As mentioned before, Servili *et al.* (2011) employed microfiltration, ultrafiltration, and reverse osmosis to recover the phenolic compounds from olive-vegetation water. Finally, Sahin and Sampli (2013) applied ultrasound technology to optimize phenol extraction from an olive leaf extract.

The use of emulsifiers in order to stabilize the phenolic compounds in olive oil has also been studied. According to Suarez *et al.* (2011), lecithin increases the oxidative stability of the phenol-enriched oils, as lecithin exhibits amphiphilic behavior, forming reverse micelles that contain the hydro-ethanolic solution of the extract, and therefore stabilizes the added phenolic compounds in olive oil. The method of encapsulation in order to enhance solubility in lipid matrices has been investigated. Spigno *et al.* (2013) applied encapsulation to increase the antioxidant efficiency of a hazelnut paste enriched with a phenolic grape marc (pomace) extract. The extract dispersibility increased, improving the antioxidant activity of the final product. Kaderides *et al.* (2015) also enhanced the antioxidant stability of hazelnut paste by adding an encapsulated phenolic extract from pomegranate peels. Finally, Mourtzinos *et al.* (2007) studied the use of an olive leaf extract encapsulated in β-cyclodextrin. The encapsulation increased the aqueous solubility of the polyphenolic residue from olive leaf by more than 150%, suggesting that encapsulated olive leaf extract can be used as a food additive with the advantage of higher aqueous solubility. Moreover, oleuropein, which was the main constituent of olive leaf extract, is protected inside the cyclodextrin cavity from decomposition, thus increasing the stability of the extract.

21.5 Conclusion

The option of olive oil enrichment with phenols in order to achieve a higher phenolic content is of great importance, as it is necessary to increase the daily intake of these beneficial compounds without increasing caloric intake at the same time. However, when enhancing olive oil to increase its phenolic compounds, there are three parameters to take under consideration. First, the phenol extract has to be dispersed and stabilized in the oil matrix; and, second, a way has to be found to make sure that these phenolic compounds are stable during the olive oil's shelf life. Finally, the real effect of the additives has to be tested and evaluated for their possible interaction with the other components existing naturally in the product.

References

Abdalla, E.A., & Roozen, J.P. (1999) Effect of plant extracts on the oxidative stability of sunflower oil and emulsion. Food Chemistry 64, 323–329.

Amirante, R., Cini, E., Montel, G.L., & Pasqualome, A. (2001) Influence of mixing and extraction parameters on virgin olive oil quality. Grasas y Aceites 52, 198–201.

Amirante, P., Dugo, G., & Gomez, T. (2002) Influence of technological innovation in improving the quality of extra virgin olive oil. Olivae 93, 34–42.

Andrewes, P., Busch, J.L.H., De Joode, T., Groenewegen, A., & Alexandre, H. (2003) Sensory properties of virgin olive oil polyphenols: indentification of deacetoxy-ligstroside aglycon as a key contributor to pungency. Journal of Agriculture and Food Chemistry 51, 1415–1420.

Angerosa, F., Mostallino, R., Basti, C., & Vito, R. (2000) Virgin olive oil odor notes: their relationship with volatile compounds from the lipoxigenase pathway and secoiridoid compounds. Food Chemistry 68, 283–287.

Angerosa, F., Mostallino, R., Basti, C., & Vito, R. (2001) Influence of malaxation temperature and time on the quality of virgin olive oils. Food Chemistry 72, 19–28.

Antoun, N., & Tsimidou, M. (1997) Gourmet olive oils: stability and consumer acceptability studies. Food Research International 30, 131–136.

Aparicio, R., Roda, L., Albi, M.A., & Gutierrez, F. (1999) Effect of various compounds on virgin olive oil stability measured by Rancimat. Journal of Agriculture and Food Chemistry 47, 4150–4155.

Aparicio, S., & Alcalde, R. (2009) Insights into the ethyl lactate+water mixed solvent. Journal of Physical Chemistry B 113, 14257–14269.

Artajo, L.S., Romero, M.P., Morello, J.R., & Motilva, M.J. (2006) Enrichment of refined olive oil with phenolic compounds: evaluation of their antioxidant activity and their effect on the bitter index. Journal of Agricultural and Food Chemistry 54, 6079–6088.

Baldioli, M., Servili, M., Perretti, G., & Montedoro, G. (1996) Antioxidant activity of tocopherols and phenolic compounds in virgin olive oil. Journal of the American Oils Chemists' Society 73, 1589–1593.

Beauchamp, G.K., Keast, R.S.J., Morel, D., Lin, J., Pika, J., Han, Q., Lee, C., Smith, A.B., & Breslin, P.A.S. (2005) Phytochemistry: ibuprofen like activity in extra-virgin olive oil. Nature 437, 45–46.

Benakmoum, A., Abbeddou, S., Ammouche, A., Kefalas, P., & Gerasopoulos D. (2008) Valorisation of low quality edible oil with tomato peel waste. Food Chemistry 110, 684–690.

Benavente-Garcia, O., Castillo, J., Lorente, J., Ortuno A., & Del Rio J.A. (2000) Antioxidant activity of phenolics extracted from *Olea europaea* L. leaves. Food Chemistry 68, 457–462.

Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gómez-Caravaca, A.M., Segura-Carretero, A., Fernández-Gutiérrez, A., & Lercker, G. (2007) Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods: an overview of the last decade. Molecules 12, 1679–1719.

Bendini, A., Cerretani, L., Salvador, M.D., Fregapane, G., & Lercker, G. (2009) Stability of the sensory quality of virgin olive oil during storage: an overview. Journal of Food Science 21, 389–406.

Boselli, E., Di Lecce, G., Strabbioli, R., Pieralisi, G., & Frega, N.G. (2009) Are virgin olive oils obtained below 27C better than those produced at higher temperatures? LWT – Food Science and Technology 4, 748–757.

Boskou, D. (2000) Mediterranean diets. In: World review nutrition & diet, ed. A.P. Simopoulos & F. Visioli. Karger, Basel, Switzerland.

Bouaziz, M., Fki, I., Ayadi, M., Jemai, H., & Sayadi, S. (2010) Stability of refined olive oil and olive-pomace oil added by phenolic compounds from olive leaves. European Journal of Lipid Science and Technology 112, 894–905.

Bouaziz, M., Fki, I., Jemai, H., Ayadi, M., & Sayadi, S. (2008) Effect of storage on refined and husk olive oils composition: stabilization by addition of natural antioxidants from Chemlali olive leaves. Food Chemistry 108, 253–262.

Bouaziz, M., & Sayadi, S. (2005) Isolation and evaluation of antioxidants from leaves of a Tunisian cultivar olive tree. European Journal of Lipid Science and Technology 107, 118–125.

Bradley, D.G., & Min, D.B. (1992) Singlet oxygen oxidation of foods. Critical Reviews in Food Science and Nutrition 31, 211–236.

Brahmi, F., Mechri, B., Dhibi, M., & Hammami, M. (2013) Variations in phenolic compounds and antiradical scavenging activity of *Olea europaea* leaves and fruits extracts collected in two different seasons. Industrial Crops & Products 49, 256–264.

Brenes, M., Hidalgo, F.J., Garcia, A., Rios, J.J., Garcia, P., Zamora, R., & Garrido, A. (2000) Pinoresinol and 1-acetoxypinoresinol, two new phenolic compounds identified in olive oil. Journal of the American Oils Chemists' Society 7, 715–720.

Briante, R., La Cara, F., Febbraio, F., Patumi, M., & Nucci, R. (2002) Bioactive derivatives from oleuropein by a biotransformation on *Olea europaea* leaf extracts. Journal of Biotechnology 93, 109–119.

Caponio, F., Gomes, T., Summo, C., & Pasqualone, A. (2003) Influence of the type of olive-crusher used on the quality of extra virgin olive oils. European Journal of Lipid Science & Technology 105, 201–206.

Che Man, Y.B., & Tan, C.P. (1999) Effects of natural and synthetic antioxidants on changes in refined bleached and deodorized palm olein during deep fat frying of potato chips. Journal of the American Oil Chemists' Society 76, 331–339.

Chiacchierini, E., Mele, G., Restuccia, D., & Vinci, G. (2007) Impact evaluation of innovative and sustainable extraction technologies on olive oil quality. Trends in Food Science & Technology 18, 299–305.

Clodoveo, M.L. (2012) Malaxation: Influence on virgin olive oil quality. Past, present and future-An overview. Trends in Food Science & Technology 25, 13–23.

Coimbra, M.A., Waldron, K.W., & Selvendran, R.R. (1994) Isolation and characterization of cell wall polymers from olive pulp (*Olea europea* L.). Carbohydrate Research 252, 245–262.

Corominas-Faja, B., Santangelo, E., Cuyàs, E., Micol, V., Joven, J., Ariza, X., Segura-Carretero, A., García, J. & Menendez, J.A. (2014) Computer aided discovery of biological activity spectra for anti-aging and anti-cancer olive oil oleuropeins. AGING 6, 731–741.

Damechki, M., Sotiropoulou, S., & Tsimidou, M. (2001) Antioxidant and pro-oxidant factors in oregano and rosemary gourmet olive oils. Grasas y Aceites 52, 207–213.

De Stefano, G., Piacquadio, P., Servili, M., Di Giovacchino, L., & Sciancalepore, V. (1999) Effect of extraction systems on the phenolic composition of virgin olive oils. Fett-Lipid 101, 328–332.

Di Giovacchino, L., Angerosa, F., & Di Giacinto, L. (1996) Effect of mixing leaves with olives on organoleptic quality of oil obtained by centrifugation. Journal of the American Oil Chemists' Society 73, 371–374.

Di Giovacchino, L., Costantini, N., Serraiocco, A., Surricchio, G., & Basti, C. (2001) Natural antioxidants and volatile compounds of virgin olive oils obtained by two or three-phases centrifugal decanters. European Journal of Lipid Science and Technology 103, 279–285.

Di Giovacchino, L., Sestili, S., & Di Vincenzo, D. (2002) Influence of olive processing on virgin olive oil quality. European Journal of Lipid Science and Technology 104, 587–601.

Di Giovacchino, L., Solinas, M., & Miccoli, M. (1994) Effect of extraction systems on the quality of virgin olive oil. Journal of the American Oils Chemists' Society 71, 1189–1194.

Drelich, A., Gomez, F., Clausse, D., & Pezron, I. (2010) Evolution of water-in-oil emulsions stabilized with solid particles: influence of added emulsifier. Colloids and Surfaces A: Physicochemical and Engineering Aspects 365, 171–177.

Farag, R.S., El-Baroty, G.S., & Basuny, A.M. (2003) The influence of phenolic extracts obtained from the olive plant (cvs. Picual and Kronakii), on the stability of sunflower oil. International Journal of Food Science & Technology 38, 81–87.

Farag, R.S., Mahmoud, E.A., & Basuny, A.M. (2007) Use crude olive leaf juice as a natural antioxidant for the stability of sunflower oil during heating. International Journal of Food Science & Technology 42, 107–115.

Fki, I., Allouche, N., & Sayadi, S. (2005) The use of polyphenolic extract, purified hydroxytyrosol and 3,4-dihydroxyphenyl acetic acid from olive mill wastewater for the stabilization of refined oils: a potential alternative to synthetic antioxidants. Food Chemistry 93, 197–204.

Galante, Y.M., De Conti, A., & Monteverdi, R. (1998) Application of trichoderma enzymes in food and feed industries. In: Trichoderma & gliocladium enzymes, biological control and commercial applications, ed. G.F. Harman & C.P. Kubicek. Taylor & Francis, London.

Garcia, A., Brenes, M., Moyano, M.J., Alba, J., Garcia, P., & Garrido, A. (2001) Improvement of phenolic compound content in virgin olive oils by using enzymes during malaxation. Journal of Food Engineering 48, 189–194.

Garcia, O.B., Castillo, J., Lorente, J., Ortuno, A., & Delrio, J. (2000) Antioxidant activity of phenolics extracted from *Olea europaea* L. leaves. Food Chemistry 68, 457–462.

Gimeno, E., Castellote, A.I., Lamuela-Raventos, R.M., De La Torre, M.C., & Lopez-Sabater, M.C. (2002) The effects of harvest and extraction methods on the antioxidant content (phenolics, a-tocopherol and b-carotene) in virgin olive oil. Food Chemistry 78, 207–211.

Gómez-Rico, A., Inarejos-García, A., Salvador, M.D., & Fregapane, G. (2009) Effect of malaxation conditions on phenol and volatile profiles in olive paste and the corresponding virgin olive oils (*Olea europaea* L. cv. Cornicabra). Journal of Agricultural & Food Chemistry 57, 3587–3595.

Gordon, M.H., & Kourimska, L. (1995) The effect of antioxidants on changes in oils during heating and deep frying. Journal of the Science of Food & Agriculture 68, 347–353.

Gutfinger, T. (1981) Polyphenols in olive oils. Journal of the American Oils Chemists' Society 58, 966–968.

Herrero, M., Temirzoda, T.N., Segura-Carretero, A., & Quirantes, R. (2011) New possibilities for the valorization of olive oil by-products. Journal of Chromatography A 1218, 7511–7520.

Houhoula, D.P., Oreopoulou, V., & Tzia, C. (2003) Antioxidant efficiency of oregano during frying and storage of potato chips. Journal of the Science of Food & Agriculture 83, 1499–1503.

Iacono, A., Gomez, R., Sperry, J., Conde, J., Bianco, G., Meli, R., Reino, J.J.G., Smith, A.B., & Gualillo, O. (2010) Effect of oleocanthal and its derivatives on inflammatory response induced by lipopolysaccharide in a murine chondrocyte cell line. Arthritis Rheumatism 62, 1675–1682.
Inarejos-García, A.M., Salvador, M.D., & Fregapane, G. (2011) Effect of crushing on olive paste and virgin olive oil minor components. European Food Research & Technology 232, 441–451.
Ishida, B.K., & Chapman, M.H. (2009) Carotenoid extraction from plants using a novel, environmentally friendly solvent. Journal of Agricultural & Food Chemistry. 57, 1051–1059.
Jacobs, D.R., Gross, M.D., & Tapsell, L.C. (2009) Food synergy: an operational concept for understanding nutrition. American Journal of Clinical Nutrition 89, 1543–1548.
Japon-Lujan, R., Luque-Rodriguez, J.M., & Luque De Castro, M.D. (2006) Dynamic ultrasound-assisted extraction of oleuropein and related biophenols from olive leaves. Journal of Chromatography A 1108, 76–82.
Kaderides, K., Goula, A.M., & Adamopoulos, K.G. (2015) A process for turning pomegranate peels into a valuable food ingredient using ultrasound-assisted extraction and encapsulation. Innovative Food Science and Emerging Technologies 31, 204–215.
Kiritsakis, A. (Ed.) (1998) Olive oil: from the tree to the table, 2nd ed. Food & Nutrition Press, Trumbull, CT.
Kiritsakis, A., & Dugan, L. (1984) Effect of selected storage conditions and packaging materials on olive oil quality. Journal of the American Oils Chemists' Society 61, 1868–1870.
Kiritsakis, A., Kanavouras, A., & Kiritsakis, K. (2002) Review: chemical analysis-quality control and packaging issues of olive oil. European Journal of Lipids Science & Technology 104, 628–638.
Kiritsakis, K., Kontominas, M.G., Kontogiorgis, C., Hadjipavlou-Litina, D., Moustakas, A., & Kiritsakis, A. (2010) Composition and antioxidant activity of olive leaf extracts from Greek olive cultivars. Journal of the American Oils Chemists' Society 87, 369–376.
Kontogianni, V.G., & Gerothanassis, I.P. (2012) Phenolic compounds and antioxidant activity of olive leaf extracts. Natural Product Research 26, 186–189.
Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E., & Etherton, T.D. (2002) Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. American Journal of Medicine 113, 71–88.
Lee, J., Lee, S., Lee, H., Park, K., & Choe, E. (2002) Spinach (*Spinacia oleracea*) powder as a natural food-grade antioxidant in deep-fat-fried products. Journal of Agricultural & Food Chemistry 50, 5664–5669.
Lee, O.H., Lee, B.Y., Lee, J., Lee, H.B., Son, J.Y., Park, C.S., Shetty, K., & Kim, Y.C. (2009) Assessment of phenolics-enriched extract and fractions of olive leaves and their antioxidant activities. Bioresource Technology 100, 6107–6113.
Lercker, G., Frega, N., Bocci, F., & Mozzori, M. (1999) Volatile constituents and oxidative stability of virgin olive oils: influence of the kneading of the olive paste. Grasas y Aceites 50, 26–29.
Lolos, M., Oreopoulou, V., & Tzia, C. (1999) Oxidative stability of potato chips: effect of frying oil type, temperature and antioxidants. Journal of the Science of Food & Agriculture 79, 1524–1528.
Meirinhos, J., Silva, B.M., Valentão, P., & Seabra, R.M. (2005) Analysis and quantification of flavonoidic compounds from Portuguese olive (*Olea europaea* L.) leaf cultivars. Natural Product Research 19, 189–195.
Menendez, J.A., Vazquez-Martin, A., Oliveras-Ferraros, C., Garcia-Villalba, R., Carrasco-Pancorbo, A., Fernandez-Gutierrez, A., & Segura-Carretero, A. (2009) Extra-virgin olive oil polyphenols inhibit HER2 (erbb-2)-induced malignant transformation in human breast epithelial cells: relationship between the chemical structures of extra-virgin olive oil secoiridoids and lignans and their inhibitory activities on the tyrosine kinase activity of HER2. International Journal of Oncology 34, 43–51.
Mourtzinos, I., Salta, F., Yannakopoulou, K., Chiou, A., & Karathanos, V.T. (2007) Encapsulation of olive leaf extract in β-cyclodextrin. Journal of Agriculture and Food Chemistry 55, 8088–8094.
Nenadis, N., Moutafidou, A., Gerasopoulos, D., & Tsimidou, M.Z. (2010) Quality characteristics of olive leaf-olive oil preparations. European Journal of Lipid Science & Technology 112, 1337–1344.
Nergiz, C., & Unal, K. (1991) Effect of method of extraction on the total polyphenol, 1,2-diphenol content and stability of virgin olive oil. Journal of the Science of Food and Agriculture 56, 79–84.
Paiva-Martins, F., Fernandes, J., Rocha, S., Nascimento, H., Vitorino, R., Amado, F., Borges, F., Belo, L., & Silva, A.S. (2009) Effects of olive oil polyphenols on erythrocyte oxidative damage. Molecular Nutrition & Food Research 53, 609–616.
Prior, R.L. (2004) Absorption and metabolism of anthocyanins: potential health effects. In: Phytochemicals: mechanisms of action, ed. M. Meskin, W.R. Bidlack, A.J. Davies, D.S. Lewis, & R.K. Randolph. CRC Press, Boca Raton, FL.
Rahmanian, N., Jafari, S.M., & Wani, T.A. (2015) Bioactive profile, dehydration, extraction and application of the bioactive components of olive leaves. Trends in Food Science & Technology 42, 150–172.
Ranalli, A., & Angerosa, F. (1996) Integral centrifuges for olive oil extraction: the qualitative characteristics of products. Journal of the American Oils Chemists' Society 73, 417–421.

Ranalli, A., & De Mattia, G. (1997) Characterisation of olive oil produced with a new enzyme processing aid. Journal of the American Oils Chemists' Society 74, 1105–1113.

Ranalli, A., & Serraiocco, A. (1995) Effect induced by a pectolytic adjuvant in olive oil extraction by the present technological systems: pluriannual research results. La Rivista Italiana delle Sostanze Grasse 72, 355–364.

Ranalli, A., Contento, S., Schiavone, C., & Simone, N. (2001) Malaxing temperature affects volatile and phenol composition as well as other analytical features of virgin olive oil. European Journal of Lipid Science and Technology 103, 228–238.

Ranalli, A., Lucera, L., Contento, S., Simone, N., & Del Re, P. (2004) Bioactive constituents, flavors and aromas of virgin oils obtained by processing olives with a natural enzyme extract. European Journal of Lipid Science & Technology 106, 187–197.

Ranalli, A., Sgaramella, A., & Surricchio, G. (1999) The new "Cytolase 0" enzyme processing aid improves quality and yields of virgin olive oil. Food Chemistry 66(4), 443–454.

Rodis, P.S., Karathanos, V.T., & Mantzavinou, A. (2002) Partitioning of olive oil antioxidants between oil and water phases. Journal of Agricultural & Food Chemistry 50, 596–601.

Romero, C., Medina, E., Vargas, J., Brenes, M., & De Castro, A. (2007) In vitro activity of olive oil polyphenols against *Helicobacter pylori*. Journal of Agriculture and Food Chemistry 55, 680–686.

Sahin, S., & Samli, R. (2013) Optimization of olive leaf extract obtained by ultrasound-assisted extraction with response surface methodology. Ultrasonics Sonochemistry 20, 595–602.

Salido, S., Pérez-Bonilla, M., Adams, R.P., & Altarejos, J. (2015) Phenolic components and antioxidant activity of wood extracts from 10 main Spanish olive cultivars. Journal of Agricultural & Food Chemistry 63, 6493–6500.

Salta, F.N, Mylona, A., Chiou, A., Boskou, G., & Andrikopoulos, N.K. (2007) Oxidative stability of edible vegetable oils enriched in polyphenols with olive leaf extract. Food Science & Technology International 13, 413–421.

Salvador, M.D., Aranda, F., Gomez-Alonso, S., & Fregapane, G. (2003) Influence of extraction system, production year and area on Cornicabra virgin olive oil: a study of five crop seasons. Food Chemistry 80, 359–366.

Savournin, C., Baghdikian, B., Elias, R., Dargouth-Kesraoui, F., Boukef, K. & Balansard, G. (2001) Rapid high-performance liquid chromatography analysis for the quantitative determination of oleuropein in *Olea europaea* leaves. Journal of Agriculture and Food Chemistry. 49, 618–621.

Servili, M., Esposto, S., Veneziani, G., Urbani, S., Taticchi, A., Di Maio, I., Selvaggini, R., Sordini, B., & Montedoro, G. (2011) Improvement of bioactive phenol content in virgin olive oil with an olive-vegetation water concentrate produced by membrane treatment. Food Chemistry 124, 1308–1315.

Servili, M., Selvaggini, R., Taticchi, A., Esposto, S., & Montedoro, G.F. (2003) Air exposure time of olive pastes during the extraction process and phenolic and volatile composition of virgin olive oil. Journal of the American Oils Chemists' Society 80, 685–695.

Servili, M., Sordini, B., Esposto, S., Urbani, S., Veneziani, G., Di Maio, I., Selvaggini, R., & Taticchi. A. (2014) Biological activities of phenolic compounds of extra virgin olive oil. Antioxidants 3, 1–23.

Shyamala, B.N., Gupta, S., Jyothi Lakshmi, A., & Prakash, J. (2005) Leafy vegetable extracts-antioxidant activity and effect on storage stability of heated oil. Innovative Food Science & Emerging Technologies 6, 239–245.

Simopoulos, A.P. (2001) The Mediteranean diets: what is so important about the diet of Greece? The scientific evidence. Journal of Nutrition 131, 3065–3073.

Spigno, G., Donsu, F., Amendola, D., Sessa, M., Ferrari, G., & De Faveri, D.M. (2013) Nanoencapsulation systems to improve solubility and antioxidant efficiency of a grape marc extract into hazelnut paste. Journal of Food Engineering 114, 207–214.

Suarez, M., Romero, M.P., Ramo, T., & Motilva, M.J. (2011) Stability of a phenol-enriched olive oil during storage. European Journal of Lipid Science and Technology 113, 894–903.

Suarez, M., Romero, M.P., Ramo, T., Macia, A., & Motilva, M.J. (2009) Methods for preparing phenolic extracts from olive cake for potential application as food antioxidants. Journal of Agriculture and Food Chemistry 57, 1463–1472.

Talhaoui, N., Taamalli, A., Gómez-Caravaca, A.M., Fernández-Gutiérrez, A., & Segura-Carretero, A. (2015) Phenolic compounds in olive leaves: analytical determination, biotic and abiotic influence and health benefits. Food Research International 77, 92–108.

Tapiero, H., Tew, K.D., Nguyen, B.G., & Mathe, G. (2002) Polyphenols: do they play a role in the prevention of human pathologies? Biomedicine & Pharmacotherapy 56, 200–207.

Tekaya, M., Mechri, B., Bchir, A., Attia, F., Cheheb, H., Daassa, M., & Hammami, M. (2013) Enhancement of antioxidants in olive oil by foliar fertilization of olive trees. Journal of the American Oils Chemists' Society 90, 1377–1386.

Tian, L.L., & White, P.J. (1994) Antipolymerization activity of oat extract in soy bean and cottonseed oils under frying conditions. Journal of the American Oils Chemists' Society 71, 1087–1094.

Trichopoulou, A., Costacou, T., Bamia, C., & Trichopoulos, D. (2003) Adherence to a Mediterranean diet and survival in a Greek population. New England Journal of Medicine 348, 2599–2608.

Trichopoulou, A., & Lagiou, P. (1997) Healthy traditional Mediterranean diet: an expression of culture, history and lifestyle. Nutrition Reviews 55, 383–389.

Tura, D, Gigliotti, C., Pedo, S., Failla, O., Bassi, D., & Serraiocco, A. (2007) Influence of cultivar and site of cultivation on levels of lipophilic and hydrophilic antioxidants in virgin olive oils (*Olea europea* L.) and correlations with oxidative stability. Scientia Horticulturae 112, 108–119.

Umar Lule, S., & Xia, W. (2005) Food phenolics, pros and cons: a review. Food Reviews International 21, 367–388.

Valls, R.M., Farràs, M., Suárez, M., Fernández-Castillejo, S., Fitó, M., Konstantinidou, V., Fuentes, F., López-Miranda, J., Giralt, M., Covas, M.I., Motilva. M.J., & Solà, R. (2015) Effects of functional olive oil enriched with its own phenolic compounds on endothelial function in hypertensive patients. A randomised controlled trial. Food Chemistry 167, 30–35.

Vierhuis, E., Servili, M., Baldioli, M., Schols, H.A., Voragen, A.G.I., & Montedoro, G.F. (2001) Effect of enzyme treatment during mechanical extraction of olive oil on phenolic compounds and polysaccharides. Journal of Agricultural & Food Chemistry 49, 1218–1223.

Visioli, F., & Galli, C. (2002) Biological properties of olive oil phytochemicals. Critical Reviews in Food Science and Nutrition 42, 209–221.

Vissers, M.N., Zock, P.L., & Katan, M.B. (2004) Bioavailability and antioxidant effects of olive oil phenols in humans: a review. European Journal of Clinical Nutrition 58, 955–965.

Yang, D.P., Kong, D.X., & Zhang, H.Y. (2007) Multiple pharmacological effects of olive oil phenols. Food Chemistry 104, 1269–1271.

Yanishlieva, N.V., & Marinova, E.M. (2001) Stabilisation of edible oils with natural antioxidants. European Journal of Lipid Science & Technology 103, 752–767.

Yanishlieva, N.V., Marinova E.M., Marekov, I.N., & Gordon, M.H. (1997) Effect of ethanol extract from summer savory (*Saturejae hortensis* L) on the stability of sunflower oil at frying temperature. Journal of the Science of Food & Agriculture 74, 524–530.

Yousfi, K., Cayuela, J.A., & Garcia, J.M. (2009) Effect of temperature, modified atmosphere and ethylene during olive storage on quality and bitterness level of the oil. Journal of the American Oils Chemists' Society 86, 291–296.

Zandi, P., & Gordon, M.H. (1999) Antioxidant activity of extracts from old tea leaves. Food Chemistry 64, 285–288.

22 Olives and olive oil: a Mediterranean source of polyphenols

Anna Tresserra-Rimbau and Rosa M. Lamuela-Raventós

22.1 Introduction

Olive oil and olives are extensively consumed in the Mediterranean countries. Apart from being rich in monounsaturated fatty acids, these food products have a unique phenolic profile with interesting physiological properties. Extra virgin olive oil (EVOO) and olives contain tyrosols, flavones, phenolic acids, and lignans, the most prevalent polyphenols being oleuropein and its derivatives (e.g., 3,4-DHPEA-EDA, 3,4-DHPEA-EA, and oleuropein-aglycone). Thus, the polar fraction is a complex mixture of phenolic acids, simple phenols and their derivatives, lignans, and flavones. Due to the biological importance of the oil and its unique character, analytical methods based on chromatographic techniques have been developed to analyze this complex phenolic mixture, using mass spectrometry and liquid chromatography and possibly coupled to a nuclear magnetic resonance (NMR) machine (Petrakis, 2006; Bendini *et al*., 2007). Experimental, clinical, and epidemiological studies have supported the beneficial health effects of these compounds. Olive and olive oil polyphenols act as antioxidants and radical scavengers with antitumor and anti-inflammatory properties. In particular, their beneficial effects on plasma lipid levels and oxidative damage resulted in a positive health claim being accepted by the European Food Safety Authority (EFSA).

Although olive oil has a low smoke point compared to seed oils, virgin olive oil (VOO) is more protected against oxidation and degradation. VOO is considered the best oil for cooking and frying, due to its lipid profile and phenolic composition. Moreover, the antioxidant activity of vegetables has been shown to increase after they are cooked with VOO. However, more epidemiological and intervention clinical trials are still necessary to evaluate the effects of cooking with VOO.

22.2 Phenolic profile of olives and olive oils

Natural polyphenols occur in plants as secondary metabolites. Polyphenols are responsible for most of the sensory properties of plant-based foods, such as bitterness, astringency, and oxidative stability (Pandey & Rizvi, 2009; Li *et al*., 2014). Thousands of different polyphenols have been identified in plants, usually in conjugated forms, with one or more sugar residues linked to hydroxyl groups, although linkage with other compounds (amines, carboxylic and organic acids, other polyphenols, or lipids) is also possible (Del Rio *et al.*, 2013). According to the number of phenol rings that they contain and the structural elements that bind these rings, polyphenols are usually divided into several classes: flavonoids, phenolic acids, stilbenes, and lignans (Figure 22.1). Some polyphenols do not fit in any of these classes and are grouped as “other polyphenols” (Spencer *et al*., 2008; Pandey & Rizvi, 2009), which is the case of tyrosols in olives and olive oils.

The composition of olive oil is 98% fatty acids, mainly oleic acid (C18:1), and 2% other minor components, such as squalene, pigments, tocopherols, waxes, and the polar fraction. It is in this polar fraction where polyphenols are located. The phenolics of olive oil contain mainly tyrosols such as hydroxytyrosol and

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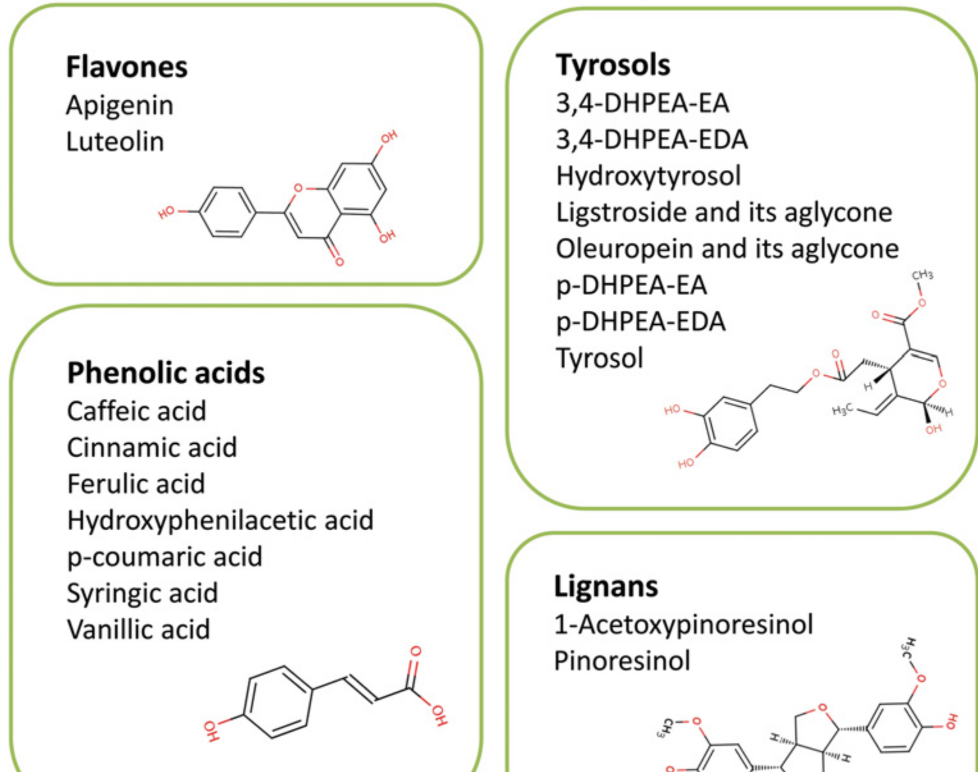

Figure 22.1 Polyphenol groups from olive oil and their main individual compounds.

its derivatives (ligstroside, oleuropein, 3,4-DHPEA-EA, and 3,4-DHPEA-EDA), known as secoiridoids, as well as lignans, flavonoids, and phenolic acids. The secoiridoid structure is composed of a phenethyl alcohol (hydroxytyrosol or tyrosol) and elenolic acid in its glucosidic or aglyconic form. The secoiridoid oleuropein, a coumarin-like compound abundant in the Oleaceae family, is an ester of hydroxytyrosol (3,4-DHPEA) and elenolic acid (EA) glucoside, whereas ligstroside is an ester of tyrosol (*p*-HPEA) and elenolic acid (EA) glucoside. The phenethyl alcohols can also be linked to the dialdehydic form of elenolic acid (EDA), leading to 3,4-DHPEA-EDA and *p*-HPEA-EDA (Bendini *et al.*, 2007). Fresh VOO mostly contains complex secoiridoid aglycones, while stored oils contain higher amounts of free phenols such as tyrosol and hydroxytyrosol due to the enzymatic liberation of their respective aglycones. This contributes to the loss of bitterness of fresh olive oil over time (Koidis & Boskou, 2014).

Olive oils also contain low amounts of phenolic acids, including benzoic and cinnamic acid derivatives, which have been associated with color and sensory properties, as well as with health-related effects (Bendini *et al.*, 2007). Caffeic, cinnamic, ferulic, *p*- and *o*-coumaric, syringic, potocatechuic, and vanillic acids are some examples of phenolic acids that have been identified in VOO. Olives are richer in phenolic acids than olive oil, with black olives containing higher levels of flavones, flavonols, and anthocyanins, which are responsible for their color. The only flavonoids that have been described in green olives and olive oil are apigenin and luteolin, both of them belonging to the flavone class.

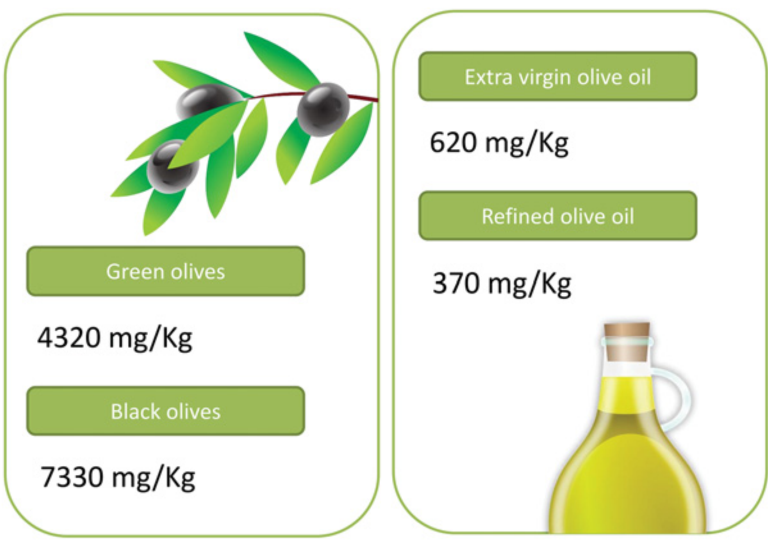

Figure 22.2 A comparison of polyphenolic content of olives and olive oils.

The lignan fraction of VOO has been used to characterize the olive cultivar. 1-acetoxypinoresinol has been identified and quantified in olive oil, whereas larisciresinol and secoisolariciresinol have been quantified in olives after hydrolysis. Pinoresinol has been quantified in both olive oil and olives (Brenes *et al.*, 2002). The total phenolic content varies, depending on the olive cultivar, agronomic conditions, the methodology used to obtain the oil, and storage conditions. The total polyphenol level of EVOO may be between 100 and 800 mg/kg. When the oil is refined, the concentration decreases to less than 10 mg/kg. Olives are richer in polyphenols, especially the black cultivars, with concentrations that range from 4000 to more than 7000 mg/kg, as shown in Figure 22.2 (Neveu *et al.*, 2010).

Table 22.1 shows the concentration of flavonoids, lignans, phenolic acids, and other polyphenols in black and green olives and extra virgin, virgin, and refined olive oil. Data were obtained from the Phenol-Explorer database (www.phenol-explorer.eu), the first comprehensive database on polyphenol content in foods, developed by the INRA (Institut National de la Recherche Agronomique). Concentrations are derived from the systematic collection of scientific publications and are based on different chromatographic methods. In the case of phenolic acids and lignans from olives, chromatography is applied after hydrolysis, which is needed to release phenolic compounds otherwise inaccessible for analysis. Data show that black olives have the

Table 22.1 Concentration of different polyphenol classes measured in olives and olive oil (mg/kg).

Olives and olive oil samples	Polyphenol class			
	Flavonoids	**Lignans**	**Phenolic acids**	**Other polyphenols**
Black olives, raw	1600 ± 250	00.7 ± 00.1*	3080 ± 200*	2660 ± 340
Green olives, raw	05.6 ± 03.1	00.3 ± 00.1*	2210 ± 130*	2110 ± 260
Extra virgin olive oil	15.3 ± 05.7	10.8 ± 01.7	03.1 ± 00.2	595 ± 80
Virgin olive oil	02.3 ± 00.2	28.1 ± 13.6	05.3 ± 00.4	541 ± 44
Refined olive oil	01.5 ± 00.6	31.6 ± 11.7	n.d.	336 ± 39

Note: "Refined" oils are deodorized, neutralized, and degummed. Concentrations expressed as mg/kg (mean ± standard deviation).
n.d.: Not detected.
*Chromatography after hydrolysis.
Source: Data from www.phenol-explorer.eu.

highest polyphenol content, especially phenolic acids and flavonoids, compared to green olives and olive oils. In contrast, refined olive oils have the lowest values of all polyphenol classes except for lignans.

22.3 Analytical approaches to characterize the phenolic profile of olives and olive oils

Accurate quantification of polyphenols in olives and olive oils is hampered by their highly diverse chemical structures and wide range of concentrations. Another issue is the difficulty of finding suitable pure standards, particularly for secoiridoids and lignans. However, several analytical methods to separate, determine, and quantify polyphenols have been developed in recent years. A key step in the characterization of the phenolic profile of olive oil is sample preparation. Liquid–liquid extraction, usually with methanol and water, or solid-phase extraction (SPE) is the method generally used (Pellegrini *et al.*, 2001). Reverse-phase high-performance liquid chromatography (RP-HPLC) is used to separate and analyze the isolated polar fraction of olive oil, with C18 columns, a gradient elution of the mobile phase of acetonitrile and water, and reading at 280 nm with an ultraviolet (UV) or diode array detector (DAD), which is the wavelength at which most polyphenols absorb. Alternative methods of detection are fluorescence, colorimetric electrode array, and amperometric detectors (Bendini *et al.*, 2007).

The on-line coupling of LC with one-dimensional ^{1}H and ^{13}C NMR (LC-NMR) not only allows quantification of phenolics but also provides structural information. However, the most widely used method to identify, characterize, and quantify polyphenols in olive oil is mass spectrometry coupled to liquid chromatography (LC-MS). Atmospheric pressure chemical ionization (APCI) and electrospray ionization (EI) are commonly used, applying both positive and negative ionization. In general, the negative ion mode has greater sensitivity for polyphenols, but results from the positive ion mode are complementary (Ryan *et al.*, 1999; Gutiérrez-Rosales *et al.*, 2003; Carrasco-Pancorbo *et al.*, 2005; Alarcón Flores *et al.*, 2012; Vallverdú-Queralt *et al.*, 2014; Gosetti *et al.*, 2015). Ionization efficiency of the analytes also depends on the composition of the mobile phase and pH. The selection of the analyzer is based on the sensitivity and selectivity required; ion trap and triple quadrupole (QqQ) provide structural elucidation with additional selectivity and sensitivity due to the possibility of performing tandem MS (MS/MS) or multiple-stage MS (MS^{n}), and time-of-flight (TOF) allows excellent mass accuracy (Bristow & Webb, 2003).

GC is not often chosen because of the extra derivatization step required and because some phenolic compounds do not survive the high temperature of the column. Capillary electrophoresis coupled to different detectors (UV, flame ionization and electrochemical detectors, and MS) is a suitable alternative to chromatographic methods, especially for routine analysis to control and monitor industrial processes. This method is relatively fast, requires small sample size and electrolyte consumption, and has good separation efficiency (Bonoli *et al.*, 2003; Frazier & Papadopoulou, 2003; Carrasco-Pancorbo *et al.*, 2004).

Finally, the Folin-Ciocalteu method is a simple but nonspecific spectrophotometric assay that can be useful when an estimated value is required. This method is based on the reaction of free hydroxyl groups (or any reducing agent) with a mixture of phosphomolybdate and phosphotungstate that gives a blue complex in basic conditions, the absorbance of which is proportional to the concentration (Singleton *et al.*, 1999; Prior *et al.*, 2005). The characterization of the phenolic profiles in olives is similar to that in olive oil, but it requires an extra sample preparation step. Generally, olives are ground and sometimes dried before being washed with a nonpolar solvent (usually hexane) to remove lipophilic compounds. Polyphenols are then extracted with methanol or ethanol. The obtained extracts are filtered and concentrated before being analyzed with colorimetric or chromatographic methods (Marsilio *et al.*, 1999; Morelló *et al.*, 2004, 2005). When GC is chosen, an additional derivatization step with trimethylsilyl (TMS) or a combination of N, N–bis(trimethyl–silyl)trifluoro-acetamide (BSTFA), and trimethylchlorosilane (TMCS) is needed (Marsilio *et al.*, 1999; Petrakis, 2006).

22.3.1 Sensory properties of VOO linked to polyphenols

The characteristic flavor of VOO is due to a unique combination of volatile and phenolic compounds, which define its aroma and taste, respectively (Angerosa *et al.*, 1999). Bitterness and pungency, positive attributes in

olive oil, depend on the quality and amount of polyphenols in the product. Some phenols enhance the tasting perception of bitterness, while others elicit the pungency, astringency, and metallic attributes. Oleuropein aglycone and other secoiridoid derivatives of hydroxytyrosol are the main contributors to bitterness of olive oil (García *et al.*, 2001; Vitaglione *et al.*, 2013). On the other hand, *p*-HPEA-EDA (ligstroside-aglycone dialdehyde), the secoiridoid that derives from tyrosol, is related to throat irritation (Beauchamp *et al.*, 2005).

22.4 Stability of polyphenols: cooking effects

An advantage of using VOO for cooking, compared to other oils, is its thermal stability, which is due to its triacylglycerol content, its low level of polyunsaturated fatty acids, and its antioxidants, consisting mainly of carotenes, polyphenols, and tocopherols (Velasco & Dobarganes, 2002; Servili *et al*., 2004; Olivero-David *et al*., 2014). During frying, oil temperature usually varies from 160 to 240 °C, with an optimal value of around 180 °C. Temperature and the presence of oxygen are factors that can initiate oxidation, which produces a chemical degeneration of the oil, and an accumulation of free radicals and other oxidized substances with a potential deleterious effect on health. The resistance of an oil to oxidation depends on its fatty acid and antioxidant profile. Olive oil is rich in oleic acid, a monounsaturated fatty acid that is less oxidizable than polyunsaturated fatty acids, as well as being rich in antioxidants. The amount of antioxidants present in VOO depends on several factors, namely the cultivar and ripening stage of the olives, culture conditions, the processing method used to obtain the oil, and, finally, the storage conditions (Brenes *et al*., 2002; Gimeno *et al*., 2002; Velasco & Dobarganes 2002; Bonoli *et al*., 2004; Chatzilazarou *et al*., 2006; Ben Youssef *et al*., 2010; Franco *et al*., 2014). VOO obtained from green olives contains more antioxidants and is therefore more stable during processing, frying, or storage. Polyphenols, which are sensitive to heat and temperature, are the main compounds responsible for oil stability during frying (Esposto *et al*., 2015), and VOO rich in phenolic content has been shown to be quite stable during cooking (Olivero-David *et al*., 2014; Esposto *et al*., 2015).

Traditionally, Mediterranean countries have used VOO to cook vegetables (Spanish *sofrito*, Catalan *sanfaina*, Italian *pisto*, etc.). It has recently been demonstrated that during the cooking process, phenols are transferred from the oil to the vegetables, whose antioxidant capacity is thereby enhanced (Ramírez-Anaya *et al*., 2015). We also observed similar results in tomato sauce, which was sampled every 15 minutes during preparation, from time zero until 1 hour, using two different proportions of VOO (5 or 10%) (Figure 22.3) (Vallverdú-Queralt *et al*., 2014).

As Figure 22.4 shows, the sauce prepared with the higher proportion of VOO was richer in tyrosol and hydroxytyrosol, indicating that when more olive oil was added, a higher amount of phenolics was transferred

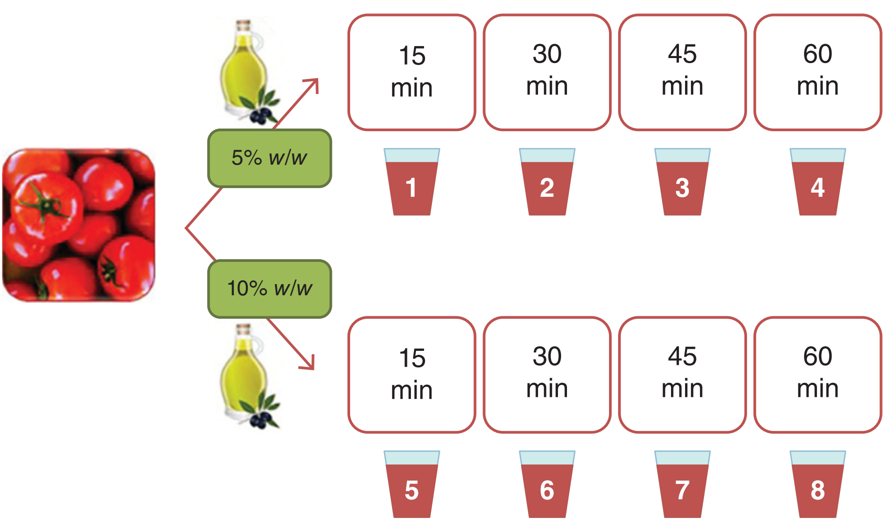

Figure 22.3 Cooking time and olive oil addition on tomato sauce.

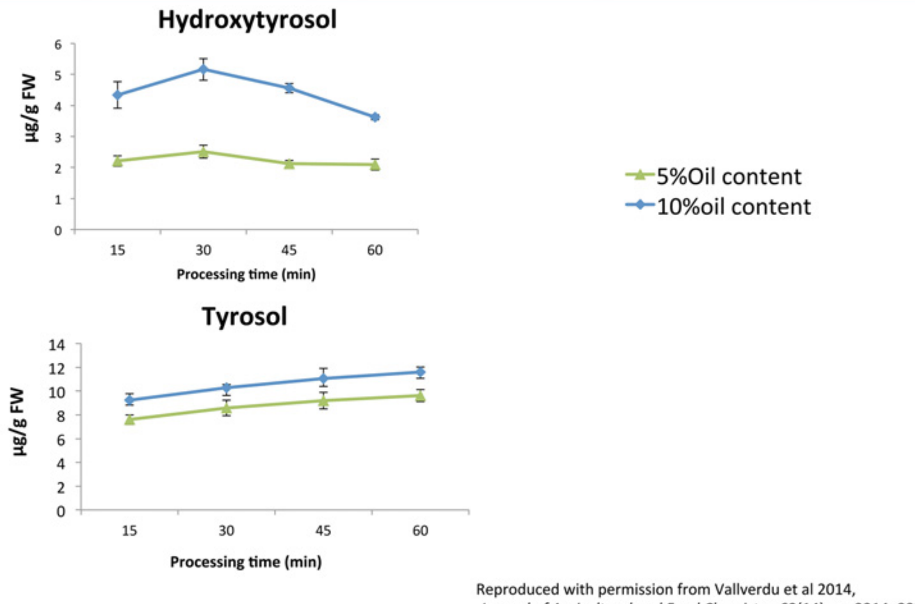

Figure 22.4 Changes in tomato sauce phenolics from VOO addition over time, using two different concentrations of VOO (5 and 10%).

to the tomato sauce. However, a decrease in the concentration of some tomato flavonoids and cinnamic acids was also observed during cooking, while caffeic acid increased. Consequently, increasing the amount of VOO enriched the tomato sauce with oil-derived polyphenol compounds, and a longer cooking time increased the amount of caffeic acid and tyrosol, while reducing the content of other polyphenols.

22.4.1 Nutritional effects of cooking

The use of VOO for cooking is controversial because of its low smoke point (165–190 °C) compared to vegetable oils. However, as mentioned in this chapter, the high level of antioxidants in VOO and its fatty acid profile offset this. While the effect of added oil on carotenoid absorption and bioavailability has been extensively studied in tomatoes (Gärtner *et al*., 1997; Fielding *et al*., 2005; Arranz *et al*., 2015; Vallverdú-Queralt *et al*., 2015), carrots, and spinach (Rich *et al.*, 2003a, 2003b), few studies have focused on phenolic bioavailability in vegetables cooked in VOO. When olive oil is added during tomato sauce processing, besides enriching the sauce with oil-derived polyphenols, the bioavailability of tomato polyphenols is enhanced (Martínez-Huélamo *et al.*, 2015). This effect is also correlated with a decrease in anti-inflammatory parameters.

Deep fat frying, a widely used cooking method, causes hydrolysis of triacylgycerols, oxidation of fatty acids, and deactivation of antioxidant compounds. In addition, polymerization (Choe & Min, 2007), which increases the foaming, viscosity, density, and content of free fatty acids, polar materials, and polymeric compounds in the frying oils, would occur (Velasco *et al.*, 2004). The oxidation products may be absorbed by the fried food and ingested (Velasco *et al*., 2004; Casal *et al*., 2010), with deleterious effects on health. However, when the oil is rich in phenolic compounds, these antioxidants protect the oil from degradation. In a randomized crossover intervention study, obese participants received four breakfasts consisting of milk and muffins made with different oils (VOO, sunflower oil, or a mixed seed oil supplemented with

either dimethylpolysiloxane or natural polyphenols from olive mill wastewater) (Perez-Herrera *et al.*, 2012, 2013). Phenolic compounds from VOO seemed to exert a protective effect against postprandial oxidative stress, anti-inflammatory response, and antiatherogenic properties compared to sunflower oil. Therefore, polyphenol-rich VOO would appear to be the most suitable oil for cooking and frying, since its lipid profile and antioxidants protect it from degradation. An additional benefit is that the phenolic compounds in the oil are transferred to the food, thus increasing its antioxidant capacity. Although intervention studies have shown the beneficial effects of using antioxidant-rich VOO, epidemiological data to evaluate the effect of frying with different oils are still lacking. Furthermore, in order to corroborate the role of VOO polyphenols in cooking, more intervention studies are required to explore the effect of cooking different foods, for different durations, using VOO with varying phenolic contents.

22.5 Health effects of olive and olive oil polyphenols

Consumption of olive oil polyphenols contributes to the protection of blood lipids from oxidative damage. This health claim was approved by the EFSA in 2011. Since the use of health claims in the European Union was harmonized in 2006, only a few foods and ingredients have met the stringent requirements accorded by the EFSA. Double-blind, randomized, placebo-controlled trials in humans that demonstrate cause–effect relationships are crucial for approval. Moreover, the effective amount of food (or food constituent) has to be easily consumed in the context of a balanced diet. That is the case of polyphenols in EVOO, since 5 mg of hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) can be consumed daily (in approximately two tablespoons of oil) in order to obtain the desired benefits (EFSA Panel on Dietetic Products, 2011). Nevertheless, the health benefits of olive oil polyphenols go far beyond those related to the health claim.

The benefits of consuming olive oil have been extensively reported and are summarized in Figure 22.5 (Covas *et al.*, 2009; Lucas *et al.*, 2011; Cárdeno *et al.*, 2013; Martín-Peláez *et al.*, 2013; Vitaglione *et al.*, 2013; Barbaro *et al.*, 2014). Traditionally, the health effects of olive oil were attributed to a high content of oleic acid, but it is currently accepted that olive oil is more than a healthy fat; its phenolic fraction also plays an important role in its health-promoting attributes due to anticarcinogenic, antiatherogenic, anti-inflammatory, and antimicrobial activities.

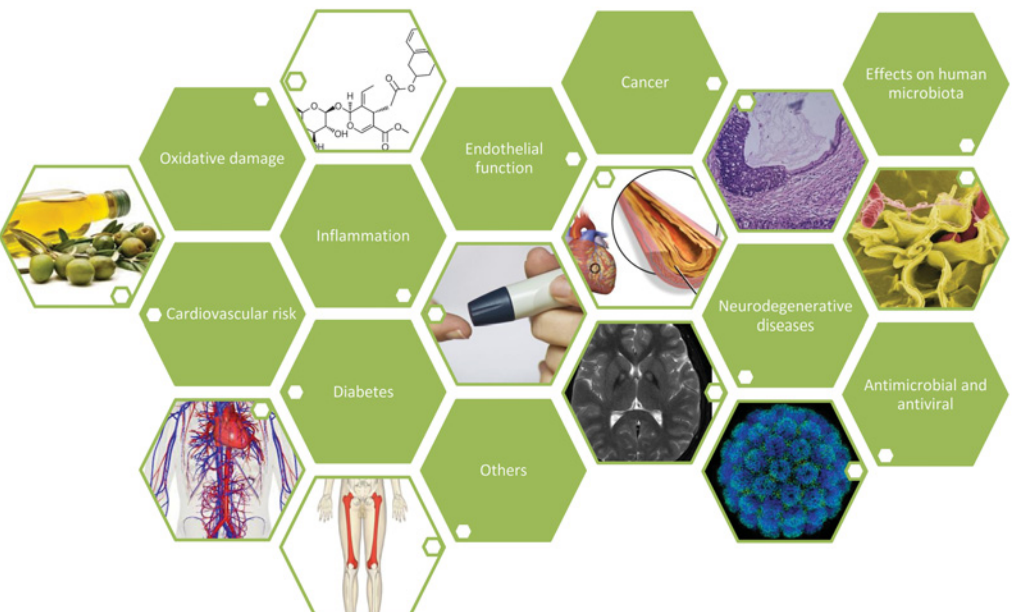

Figure 22.5 Beneficial effects of olive oil polyphenols in different diseases.

22.5.1 Bioavailability of olive oil polyphenols

Dietary intake of polyphenols from olive oil was estimated to be around 22 mg in an elderly Spanish population (the PREDIMED [PREvención con DIetaMEDiterránea] cohort), provided by 4–5 tablespoons. The PREDIMED study was a large, multicenter, randomized, controlled, clinical trial aimed at assessing the effects of two Mediterranean diets, supplemented with either EVOO or nuts, versus a low-fat diet in a Spanish population, aged 55–80 years and at high cardiovascular risk (Martínez-González *et al*., 2012; Estruch *et al*., 2013). Together with olives, VOO provided 11% of the total polyphenol intake in this population, being the fourth polyphenol contributor in the diet (Tresserra-Rimbau *et al.*, 2013). Most of these polyphenols were elenolic esters and oleuropein- and ligstroside-aglycons, with a small amount of free hydroxytyrosol and tyrosol. The question is: to what extent do these polyphenols reach the target tissues where they can exert beneficial effects?

The majority of research on the bioavailability of olive oil phenolic compounds has focused on hydroxytyrosol, tyrosol, and oleuropein. Visioli *et al.* (2000b) found that approximately 98% of hydroxytyrosol and tyrosol from olive oil was absorbed in a dose–response manner. Another study conducted in healthy humans with an ileostomy and subjects with a colon showed that absorption of ligstroside-aglycone, hydroxytyrosol, tyrosol, and oleuropein-aglycone was at least 55–66% of the ingested dose. This study also demonstrated that the small intestine was the major site of absorption, since ileostomy and normal subjects showed similar results (Vissers *et al.*, 2002). The mechanism by which absorption occurs depends on the different polarities and sizes of the various phenolic compounds (Vissers *et al.*, 2002). For instance, the absorption of the polar tyrosol and hydroxytyrosol has been postulated to occur via passive diffusion, whereas oleuropein-glycoside, also polar but larger, may be absorbed via a glucose transporter after diffusion through the lipid bilayer of the epithelial cell membrane (Manna *et al.*, 2000).

Non-absorbed phenolic compounds may also exert antioxidant activity in the gastrointestinal tract, since isolated polyphenols can scavenge free radicals formed by the fecal matrix and the epithelial cells of the intestine (Manna *et al.*, 1997; Owen *et al.*, 2000). However, the beneficial effects attributed to olive oil can only be explained if its bioactive compounds are not only absorbed but also available in target tissues. In the colon, phenolics are broken down by macrobiotics and converted to other phenolics, which has a notable effect on their bioavailability. Phenolic compounds from olive oil are subject to phase I (hydrogenation, hydroxylation, and methylation) and phase II (mainly glucuronidation and sulfation) biotransformation. Thus, glucuronoid conjugates of the polyphenol classes in olive oil have been detected in urine and plasma, whereas the presence of sulfated metabolites has scarcely been reported (Gómez-Romero *et al.*, 2012). The reactions of methylation, glucuronidation, and sulfation take place through the respective action of catechol-*O*-methyl transferases (COMT), uridine-5′-diphosphate glucurunosyl transferases (UDPGT), and sulfotransferases (SULT) (Manach *et al.*, 2004). The metabolism of lignans has only been studied more recently. After incubation of free pinoresinol using differentiated Caco-2/TC7 cell monolayers, pinoresinol glucuronide and sulfate conjugates were formed (Soler *et al.*, 2010). Flavonoids are typically methylated and glucuronidated. The flavonoids methyl-monogluguronides of apigenin and luteolin have been identified in human urine (García-Villalba *et al.*, 2010).

More than 60 metabolites were identified in human urine samples of ten healthy volunteers after the intake of 50 mL of EVOO, ten of which were proposed as biomarkers. In kinetic studies, the concentration of most of these compounds reached a maximum in the first two hours (García-Villalba *et al.*, 2010). Once polyphenols are circulating through the bloodstream, they are able to reach tissues and organs.

Based on studies on the amount and form in which olive oil polyphenols are excreted, we can conclude that at least 5% of ingested olive oil polyphenols are recovered in the urine as glucuronide conjugates of hydroxytyrosol and tyrosol. The remaining polyphenols are metabolized into other compounds, such as *O*-methylated hydroxytyrosol or monosulfate conjugates. However, some of these results have been obtained only in animal studies (Gómez-Romero *et al.*, 2012), so further research is needed to elucidate the mechanisms of absorption, metabolism, and excretion of other key phenolic compounds from olive oil in humans.

22.5.2 Protection against oxidative damage and inflammation

Reactive oxygen species (ROS) are chemically reactive molecules produced by the normal metabolism of organisms. Under environmental stress, the level of ROS increases and can damage lipids, DNA, and

proteins. Besides oxidative damage, ROS activate pro- and anti-inflammatory cytokines (Valko *et al.*, 2007). Oxidation and inflammation are interrelated processes, and both are involved in the etiology of chronic diseases such as hypertension, insulin resistance, metabolic syndrome, cardiovascular diseases (CVDs), cancer, and age-related pathologies.

Olive oil polyphenols have been shown to scavenge ROS under natural and chemically simulated oxidative stress conditions. Oleuropein, for instance, has an antioxidant potential similar to that exerted by ascorbic acid and α-tocopherol (vitamins C and E, respectively) (Visioli *et al.*, 1998). Oleuropein can also scavenge oxidants such as hypochlorous acid (HClO) and nitric oxide (NO) (Visioli *et al.*, 1998, 2002). Moreover, in a human intervention study, Covas *et al.* (2006b) found that total plasma antioxidant activity increased after ingestion of olive oil polyphenols. Levels of F2-isoprostane in urine samples also decreased when participants consumed polyphenol-rich olive oils in two different clinical studies (Visioli *et al.*, 2000a; Ruano *et al.*, 2005).

Elevated concentrations of pro-inflammatory agents in serum, such as thromboxane B2 (TXB2) and leukotriene B4 (LTB4), are well-known risk factors for CVDs. Some investigators have described that TXB2 and LTB4 concentrations decrease in a dose–response manner with the phenolic concentration of olive oil (Léger *et al.*, 2005; Visioli *et al.*, 2005; Covas *et al.*, 2006a; Bogani *et al.*, 2007). Other pro-inflammatory agents, such as interleukin-6 (IL6) and C-reactive protein (CRP), also decreased after consumption of olive oil in a placebo crossover randomized trial with coronary heart patients (Fitó *et al.*, 2008). In a subsample of the PREDIMED study, serum markers of atheroma plaque stability were measured after 12 months of intervention. Inflammatory biomarkers of atherosclerosis and plaque vulnerability such as CRP, IL6, sICAM, and P-selectin were significantly reduced in the Mediterranean diet group supplemented with olive oil compared to the low-fat diet group (Casas *et al.*, 2014). Moreover, a higher consumption of VOO was significantly associated with a significant reduction of plasma tumor necrosis factor receptor-60 (TNFR60) concentration (Urpi-Sarda *et al.*, 2012).

22.5.3 Cardiovascular diseases, LDL, HDL, and endothelial function

Elevated levels of total cholesterol and low-density lipoprotein (LDL) cholesterol are well-known risk factors for atherosclerosis, which is the primary cause of CVD, whereas elevated levels of high-density lipoprotein (HDL) cholesterol have protective, anti-inflammatory properties. Moreover, LDL oxidation causes damage to the vascular wall, stimulating macrophage uptake and formation of foam cells, which in turn results in the formation of plaque within the arterial wall (Patrick & Uzick, 2001; Fitó *et al.*, 2005).

Polyphenols from olive oil decrease the incidence of CVDs by acting on their main risk factors. Thus, apart from decreasing oxidized LDL cholesterol (Covas *et al.*, 2006a; Bogani *et al.*, 2007; Castañer *et al.*, 2011, 2012; Moreno-Luna *et al.*, 2012), they can also increase HDL particles (Covas *et al.*, 2006a; Castañer *et al.*, 2012; Hernáez *et al.*, 2014) and HDL anti-inflammatory activity (Loued *et al.*, 2013). The metabolic activity of olive oil polyphenols can be related to their capacity to bind LDL and decrease the oxidation of these particles. Several authors have found hydroxytyrosol, tyrosol, and their metabolites in human lipoprotein fractions after intake of VOO but not refined olive oil (Bonanome *et al.*, 2000; Gimeno *et al.*, 2002, 2007; de la Torre-Carbot *et al.*, 2007, 2010). This is important since oxidized LDL (oxLDL) plays a key role in the development of atherosclerosis.

Endothelial function was improved in clinical intervention trials with hypercholesterolemic individuals, patients with early atherosclerosis, and healthy volunteers (Bogani *et al.*, 2007; Moreno-Luna *et al.*, 2012; Widmer *et al.*, 2013). The mechanisms through which atherosclerosis was attenuated included lipid lowering, inhibition of LDL oxidation, suppression of inflammatory factors (Pacheco *et al.*, 2007), and prevention of macrophage activation. According to Castañer *et al.* (2012), polyphenols from olive oil reduced LDL oxidation, not only by scavenging free radicals but also by modulating gene expression. Concretely, polyphenol-rich olive oil reduced CD40L gene expression, downstream products, and related genes involved in atherogenic and inflammatory processes in humans. In addition to the effects on plasma lipids and endothelial function, some authors reported a beneficial effect of polyphenols on blood pressure (Castañer *et al.*, 2012; Moreno-Luna *et al.*, 2012). Lesions on the vascular epithelium stimulate endothelial adhesion molecule expression, platelet activity, and aggregation. Circulating monocytes are attracted by these molecules and

adhere to the endothelium, becoming foam cells and forming fatty streaks. It has been demonstrated that olive oil polyphenols can inhibit platelet aggregation and decrease homocysteine (Dell'Agli *et al.*, 2007; Manna *et al.*, 2009).

The final results of the PREDIMED study concluded that Mediterranean diets reduced the incidence of major cardiovascular events (stroke, myocardial infarction, and cardiovascular death) by approximately 30%. The multivariable-adjusted hazard ratios (HRs) were 0.70 (95% confidence interval [CI], 0.54 to 0.92) and 0.72 (95% CI, 0.54 to 0.96) for the group assigned to a Mediterranean diet with EVOO and the group assigned to a Mediterranean diet with nuts, respectively, versus the control group (Estruch *et al.*, 2013). Within the PREDIMED trial, an observational prospective cohort study found that olive oil consumption, especially the EVOO, was associated with reduced cardiovascular risk. Comparing the highest versus the lowest tertiles, the multivariable-adjusted HRs were 0.65 (95% CI, 0.47 to 0.89) for total olive oil and 0.61 (95% CI, 0.44 to 0.85) for EVOO. Total olive oil consumption was also significantly associated with 48% reduced risk of cardiovascular mortality. For each 10 g/d increase in EVOO consumption, CVD and mortality risk decreased by 10 and 7%, respectively. However, in this study, no significant associations were found between olive oil consumption and cancer and all-cause mortality (Guasch-Ferré *et al.*, 2014).

In a post-hoc analysis of the same trial, EVOO in the context of a Mediterranean dietary pattern significantly reduced the risk of atrial fibrillation (HR 0.62; 95% CI, 0.45–0.85) compared with the low-fat diet group, whereas no effect was found for the Mediterranean diet with nuts, suggesting the important role of olive oil in the prevention of arrhythmias (Martínez-González *et al.*, 2014). These findings are supported by other prospective cohort studies. In the Three-City Study in France, high olive oil consumption and high plasma oleic acid as an indirect biological marker of olive oil intake were associated with lower incidence of stroke in older subjects (Samieri *et al.*, 2011). Within the EPICOR (long-tErm follow-up of antithrombotic management Patterns In acute CORonary syndrome patients) study, a strong inverse association was found between increasing consumption of olive oil and coronary heart disease risk (Bendinelli *et al.*, 2011). Similarly, it was found that olive oil was associated with a reduced risk of overall mortality, CVD mortality, and incident coronary heart disease events in a large Mediterranean cohort within the EPIC-Spain (The European Prospective Investigation into Cancer and Nutrition) study (Buckland *et al.*, 2012a, 2012b).

22.5.4 Protection against cancer

Several epidemiological studies have demonstrated that a Mediterranean diet decreased the risk of some types of cancer, especially those of the digestive tract (Grosso *et al*., 2013; Gotsis *et al*., 2015). This could partially be attributed to the high consumption of olive oil in Mediterranean countries. In fact, a meta-analysis of 19 observational studies concluded that a higher intake of olive oil was associated with lower risks of having any type of cancer, breast cancer, and a cancer of the digestive system (Psaltopoulou *et al.*, 2011). Moreover, a review of Italian case–control studies showed that olive oil was inversely related to colorectal, breast, and other cancers, mainly those of the upper digestive and respiratory tract (La Vecchia & Bosetti, 2006). Similarly, in a Belgian case–control study, authors found an inverse linear association between olive oil intake and bladder cancer risk (Brinkman *et al.*, 2011). In support of these findings, a study with mice concluded that olive leaf extract and oleuropein prevented chronic ultraviolet B (UVB)-induced skin damage, carcinogenesis, and tumor growth (Kimura & Sumiyoshi, 2009). Several *in vitro* studies have reported protective effects of olive oil polyphenols against DNA damage, a precursor for human carcinogenesis, as well as antiproliferative and proapoptotic effects in different cancer cell lines, namely leukemia tumor cells, colorectal carcinoma cells, and breast cancer cells (Casaburi *et al.*, 2013). These results agreed with those from a randomized crossover trial conducted in healthy postmenopausal women consuming olive oils with different concentrations of polyphenols. This study showed a reduction of DNA damage by consumption of polyphenol-rich olive oil (Salvini *et al.*, 2006).

Several mechanisms have been proposed to explain the anticancer properties of olive oil, including the reduction of environmental and food carcinogen bioavailability, protection against oxidative stress, the inhibition of enzymes related to tumor promotion and metastasis, and a direct effect on nucleic acids and nucleoproteins, among others (Cárdeno *et al*., 2013; Martín-Peláez *et al*., 2013; Barbaro *et al*., 2014).

22.5.5 Neuroprotective effect

Some evidence has also emerged about olive oil and neurodegenerative diseases. Polyphenols from olive oil have shown neuroprotective effects *in vivo* and *in vitro*. These compounds are able to protect against cerebral ischemia, spinal cord injury, Huntington's disease, and Alzheimer's disease. Nevertheless, at present, many of the mechanisms behind these protective actions have not been fully elucidated (Khalatbary, 2013).

Studies in animals revealed that oleuropein and hydroxytyrosol play important roles in preventing or attenuating Parkinson's and Alzheimer's diseases. For instance, oleuropein counteracts amyloid plaque generation and deposition, and inhibits Tau aggregation, which are characteristics of Alzheimer's disease (González-Correa *et al*., 2008; Daccache *et al*., 2011; Carito *et al*., 2014; Sarbishegi *et al*., 2014). Other authors also examined the effects of EVOO on learning and memory in SAMP8 mice, an age-related learning/memory impairment model associated with increased amyloid-β protein and brain oxidative damage. Their findings suggested that EVOO has beneficial effects on learning and memory deficits related to the overproduction of amyloid-β protein by reversing oxidative damage in the brain (Farr *et al*., 2012). Results from intervention studies about olive oil and neuroprotection are very scarce, since most of the available studies were performed in experimental animals. However, a long-term intervention with a Mediterranean diet supplemented with EVOO resulted in a better cognitive function in comparison with a low-fat diet in a subsample of the PREDIMED trial (Martínez-Lapiscina *et al*., 2013). Hydroxytyrosol is even capable of crossing the blood–brain barrier. However, data in this field are still very limited, and further investigation is needed.

According to an *in vitro* study with human retinal pigment epithelial cells (ARPE-19), age-related macular degeneration, which in most cases leads to blindness, can also be prevented with hydroxytyrosol (Zhu *et al*., 2010).

22.5.6 Other effects

In vitro results with olive oil polyphenols such as oleuropein, hydroxytyrosol, and tyrosol have demonstrated potent antimicrobial activity against several strains of bacteria responsible for intestinal and respiratory infections (Barbaro *et al*., 2014). For instance, decarboxymethyl ligstroside inhibits the growth of *Helicobacter pylori* (Romero *et al*., 2007). Polyphenols from olive oil can also improve the human macrobiotics, since most of them reach the lower parts of the gastrointestinal tract, where they can be metabolized. This stimulates the growth of beneficial bacteria such as *Lactobacillus* (Martín-Peláez *et al*., 2013).

In a clinical trial with healthy subjects, EVOO in the frame of a Mediterranean-style diet improved postprandial glucose and LDL cholesterol levels. Corroborating these results, a higher olive oil intake has recently been associated with a modestly lower risk of type 2 diabetes in women from a US cohort (Guasch-Ferré *et al*., 2015). Other *in vitro* and *in vivo* studies have linked olive oil and its bioactive compounds to hepatoprotection, a decrease in chronic inflammatory disorders, nutrigenomic effects, and bone health (Puel *et al*., 2008; Martín-Peláez *et al*., 2013; Barbaro *et al*., 2014). However, data in these fields are still scarce, and more, better designed studies, especially in humans, are required.

22.6 Conclusion

The effects of virgin olive oil on human health have been clearly demonstrated in epidemiological and intervention clinical trials. VOO, due to its lipid profile and rich phenolic composition, has unique sensory and health properties. The complexity of the phenolic profile of olives and VOO requires mass spectrometry or nuclear magnetic resonance spectroscopy for its determination. Initially, the properties of olives and VOO were attributed to their high antioxidant levels, but it has become clear that multiple mechanisms are involved, including an impact on gut macrobiotics that may have protective effects against CVD and cancer neurological diseases.

Controversy still surrounds the use of VOO for cooking, since it has a lower smoke point than other oils. Nevertheless, it is now known that during the cooking process, polyphenols from VOO are transferred to vegetables, thereby increasing their antioxidant capacity. It would thus appear that VOO is the best oil to use for cooking in terms of health benefits, but more epidemiological and interventional clinical trials are required to evaluate the effect of cooking with VOO.

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References

Alarcón Flores, M.I., Romero-González, R., Garrido Frenich, A., & Martínez Vidal, J.L. (2012) Analysis of phenolic compounds in olive oil by solid-phase extraction and ultra high performance liquid chromatography-tandem mass spectrometry. Food Chemistry 134, 2465–2472.

Angerosa, F., Basti, C., & Vito, R. (1999) Virgin olive oil volatile compounds from lipoxygenase pathway and characterization of some Italian cultivars. Journal of Agricultural and Food Chemistry 47, 836–839.

Arranz, S., Martínez-Huélamo, M., Vallverdu-Queralt, A., Valderas-Martinez, P., Illán, M., Sacanella, E., Escribano, E., Estruch, R., & Lamuela-Raventos, R. M. (2015) Influence of olive oil on carotenoid absorption from tomato juice and effects on postprandial lipemia. Food Chemistry 168, 203–210.

Barbaro, B., Toietta, G., Maggio, R., Arciello, M., Tarocchi, M., Galli, A., & Balsano, C. (2014) Effects of the olive-derived polyphenol oleuropein on human health. International Journal of Molecular Sciences 15, 18508–18524.

Beauchamp, G. K., Keast, R.S.J., Morel, D., Lin, J., Pika, J., Han, Q., Lee, C.-H., Smith, A.B., & Breslin, P.A.S. (2005) Phytochemistry: ibuprofen-like activity in extra-virgin olive oil. Nature 437, 45–46.

Bendinelli, B., Masala, G., Saieva, C., Salvini, S., Calonico, C., Sacerdote, C., Agnoli, C., Grioni, S., Frasca, G., Mattiello, A., Chiodini, P., Tumino, R., Vineis, P., Palli, D., & Panico, S. (2011) Fruit, vegetables, and olive oil and risk of coronary heart disease in Italian women: the EPICOR Study. American Journal of Clinical Nutrition 93, 275–283.

Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gómez-Caravaca, A.M., Segura-Carretero, A., Fernández-Gutiérrez, A., & Lercker, G. (2007) Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. Molecules (Basel, Switzerland) 12, 1679–1719.

Ben Youssef, N., Zarrouk, W., Carrasco-Pancorbo, A., Ouni, Y., Segura-Carretero, A., Fernández-Gutiérrez, A., Daoud, D., & Zarrouk, M. (2010) Effect of olive ripeness on chemical properties and phenolic composition of Chétoui virgin olive oil. Journal of the Science of Food and Agriculture 90, 199–204.

Bogani, P., Galli, C., Villa, M., & Visioli, F. (2007) Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. Atherosclerosis 190, 181–186.

Bonanome, A., Pagnan, A., Caruso, D., Toia, A., Xamin, A., Fedeli, E., Berra, B., Zamburlini, A., Ursini, F., & Galli, G. (2000) Evidence of postprandial absorption of olive oil phenols in humans. Nutrition, Metabolism, and Cardiovascular Diseases 10, 111–120.

Bonoli, M., Bendini, A., Cerretani, L., Lercker, G., & Toschi, T.G. (2004) Qualitative and semiquantitative analysis of phenolic compounds in extra virgin olive oils as a function of the ripening degree of olive fruits by different analytical techniques. Journal of Agricultural and Food Chemistry 52, 7026–7032.

Bonoli, M., Montanucci, M., Gallina Toschi, T., & Lercker, G. (2003) Fast separation and determination of tyrosol, hydroxytyrosol and other phenolic compounds in extra-virgin olive oil by capillary zone electrophoresis with ultraviolet-diode array detection. Journal of Chromatography A 1011, 163–172.

Brenes, M., García, A., Dobarganes, M. C., Velasco, J., & Romero, C. (2002) Influence of thermal treatments simulating cooking processes on the polyphenol content in virgin olive oil. Journal of Agricultural and Food Chemistry 50, 5962–5967.

Brinkman, M.T., Buntinx, F., Kellen, E., Van Dongen, M.C.J.M., Dagnelie, P.C., Muls, E., & Zeegers, M.P. (2011) Consumption of animal products, olive oil and dietary fat and results from the Belgian case-control study on bladder cancer risk. European Journal of Cancer (Oxford, England: 1990) 47, 436–442.

Bristow, A.W.T., & Webb, K.S. (2003) Intercomparison study on accurate mass measurement of small molecules in mass spectrometry. Journal of the American Society for Mass Spectrometry 14, 1086–1098.

Buckland, G., Mayén, A.L., Agudo, A., Travier, N., Navarro, C., Huerta, J.M., Chirlaque, M. D., Barricarte, A., Ardanaz, E., Moreno-Iribas, C., Marín, P., Quirós, J.R., Redondo, M.L., Amiano, P., Dorronsoro, M., Arriola, R., Molina-Montes, E., Sánchez, M.J., & Gonzalez, C.A. (2012a) Olive oil intake and mortality within the Spanish population (EPIC-Spain). American Journal of Clinical Nutrition 96, 142–149.

Buckland, G., Travier, N., Barricarte, A., Ardanaz, E., Moreno-Iribas, C., Sánchez, M.J., Molina-Montes, E., Chirlaque, M.D., Huerta, J.M., Navarro, C., Redondo, M.L., Amiano, P., Dorronsoro, M., Larrañaga, N., &

Gonzalez, C.A. (2012b) Olive oil intake and CHD in the European Prospective Investigation into Cancer and Nutrition Spanish cohort. British Journal of Nutrition 108, 2075–2082.
Cárdeno, A., Sánchez-Hidalgo, M., & Alarcón-de-la-Lastra, C. (2013) An up-date of olive oil phenols in inflammation and cancer: molecular mechanisms and clinical implications. Current Medicinal Chemistry 20, 4758–4776.
Carito, V., Venditti, A., Bianco, A., Ceccanti, M., Serrilli, A. M., Chaldakov, G., Tarani, L., De Nicolò, S., & Fiore, M. (2014) Effects of olive leaf polyphenols on male mouse brain NGF, BDNF and their receptors TrkA, TrkB and p75. Natural Product Research 28, 1970–1984.
Carrasco-Pancorbo, A., Cerretani, L., Bendini, A., Segura-Carretero, A., Gallina-Toschi, T., & Fernandez-Gutiérrez, A. (2005) Analytical determination of polyphenols in olive oils. Journal of Separation Science 28, 837–858.
Carrasco Pancorbo, A., Cruces-Blanco, C., Segura Carretero, A., & Fernández Gutiérrez, A. (2004). Sensitive determination of phenolic acids in extra-virgin olive oil by capillary zone electrophoresis. Journal of Agricultural and Food Chemistry 52, 6687–6693.
Casaburi, I., Puoci, F., Chimento, A., Sirianni, R., Ruggiero, C., Avena, P., & Pezzi, V. (2013) Potential of olive oil phenols as chemopreventive and therapeutic agents against cancer: a review of in vitro studies. Molecular Nutrition & Food Research 57, 71–83.
Casal, S., Malheiro, R., Sendas, A., Oliveira, B. P. P., & Pereira, J. A. (2010) Olive oil stability under deep-frying conditions. Food and Chemical Toxicology 48, 2972–2979.
Casas, R., Sacanella, E., Urpí-Sardà, M., Chiva-Blanch, G., Ros, E., Martínez-González, M.-A., Covas, M.-I., Salas-Salvadó, J., Fiol, M., Arós, F., & Estruch, R. (2014) The effects of the mediterranean diet on biomarkers of vascular wall inflammation and plaque vulnerability in subjects with high risk for cardiovascular disease: a randomized trial. PLoS One 9, e100084.
Castañer, O., Covas, M.-I., Khymenets, O., Nyyssonen, K., Konstantinidou, V., Zunft, H.-F., de la Torre, R., Muñoz-Aguayo, D., Vila, J., & Fitó, M. (2012) Protection of LDL from oxidation by olive oil polyphenols is associated with a downregulation of CD40-ligand expression and its downstream products in vivo in humans. American Journal of Clinical Nutrition 95, 1238–1244.
Castañer, O., Fitó, M., López-Sabater, M.C., Poulsen, H.E., Nyyssönen, K., Schröder, H., Salonen, J.T., De la Torre-Carbot, K., Zunft, H.-F., De la Torre, R., Bäumler, H., Gaddi, A.V, Saez, G.T., Tomás, M., & Covas, M.-I. (2011) The effect of olive oil polyphenols on antibodies against oxidized LDL. A randomized clinical trial. Clinical Nutrition (Edinburgh, Scotland) 30, 490–493.
Chatzilazarou, A., Gortzi, O., Lalas, S., Zoidis, E., & Tsaknis, J. (2006) Physicochemical changes of olive oil and selected vegetable oils during frying. Journal of Food Lipids 13, 27–35.
Choe, E., & Min, D.B. (2007) Chemistry of deep-fat frying oils. Journal of Food Science 72, R77–R86.
Covas, M.-I., de la Torre, K., Farré-Albaladejo, M., Kaikkonen, J., Fitó, M., López-Sabater, C., Pujadas-Bastardes, M.A., Joglar, J., Weinbrenner, T., Lamuela-Raventós, R.M., & de la Torre, R. (2006a) Postprandial LDL phenolic content and LDL oxidation are modulated by olive oil phenolic compounds in humans. Free Radical Biology & Medicine 40, 608–616.
Covas, M.-I., Konstantinidou, V., & Fitó, M. (2009) Olive oil and cardiovascular health. Journal of Cardiovascular Pharmacology 54, 477–482.
Covas, M.-I., Nyyssönen, K., Poulsen, H. E., Kaikkonen, J., Zunft, H.-J. F., Kiesewetter, H., Gaddi, A., de la Torre, R., Mursu, J., Bäumler, H., Nascetti, S., Salonen, J. T., Fitó, M., Virtanen, J., & Marrugat, J. (2006b) The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. Annals of Internal Medicine 145, 333–341.
Daccache, A., Lion, C., Sibille, N., Gerard, M., Slomianny, C., Lippens, G., & Cotelle, P. (2011) Oleuropein and derivatives from olives as Tau aggregation inhibitors. Neurochemistry International 58, 700–707.
de la Torre-Carbot, K., Chávez-Servín, J.L., Jaúregui, O., Castellote, A.I., Lamuela-Raventós, R.M., Fitó, M., Covas, M.-I., Muñoz-Aguayo, D., & López-Sabater, M.C. (2007) Presence of virgin olive oil phenolic metabolites in human low density lipoprotein fraction: determination by high-performance liquid chromatography-electrospray ionization tandem mass spectrometry. Analytica Chimica Acta 583, 402–410.
de la Torre-Carbot, K., Chávez-Servín, J.L., Jaúregui, O., Castellote, A.I., Lamuela-Raventós, R.M., Nurmi, T., Poulsen, H.E., Gaddi, A.V, Kaikkonen, J., Zunft, H.-F., Kiesewetter, H., Fitó, M., Covas, M.-I., & López-Sabater, M.C. (2010) Elevated circulating LDL phenol levels in men who consumed virgin rather than refined olive oil are associated with less oxidation of plasma LDL. Journal of Nutrition 140, 501–508.
Dell'Agli, M., Maschi, O., Galli, G.V., Fagnani, R., Dal Cero, E., Caruso, D., & Bosisio, E. (2007) Inhibition of platelet aggregation by olive oil phenols via cAMP-phosphodiesterase. British Journal of Nutrition 99, 945–951.
Del Rio, D., Rodriguez-Mateos, A., Spencer, J.P.E., Tognolini, M., Borges, G., & Crozier, A. (2013) Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. Antioxidants & Redox Signaling 18, 1818–1892.

EFSA Panel on Dietetic Products, N. and A. (NDA). (2011) Scientific Opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL cholesterol concentrations (ID 1639), mainte. EFSA Journal 9, 1–25.

Esposto, S., Taticchi, A., Di Maio, I., Urbani, S., Veneziani, G., Selvaggini, R., Sordini, B., & Servili, M. (2015) Effect of an olive phenolic extract on the quality of vegetable oils during frying. Food Chemistry 176, 184–192.

Estruch, R., Ros, E., Salas-Salvadó, J., Covas, M.-I., Corella, D., Arós, F., Gómez-Gracia, E., Ruiz-Gutiérrez, V., Fiol, M., Lapetra, J., Lamuela-Raventos, R.M., Serra-Majem, L., Pintó, X., Basora, J., Muñoz, M.A., Sorlí, J.V., Martínez, J.A., & Martínez-González, M.A. (2013) Primary prevention of cardiovascular disease with a Mediterranean diet. New England Journal of Medicine 368, 1279–1290. 0028-4793

Farr, S.A., Price, T.O., Dominguez, L.J., Motisi, A., Saiano, F., Niehoff, M.L., Morley, J.E., Banks, W.A., Ercal, N., & Barbagallo, M. (2012) Extra virgin olive oil improves learning and memory in SAMP8 mice. Journal of Alzheimer's Disease 28, 81–92.

Fielding, J.M., Rowley, K.G., Cooper, P., & O'Dea, K. (2005) Increases in plasma lycopene concentration after consumption of tomatoes cooked with olive oil. Asia Pacific Journal of Clinical Nutrition 14, 131–136.

Fitó, M., Cladellas, M., de la Torre, R., Martí, J., Alcántara, M., Pujadas-Bastardes, M., Marrugat, J., Bruguera, J., López-Sabater, M.C., Vila, J., & Covas, M.I. (2005) Antioxidant effect of virgin olive oil in patients with stable coronary heart disease: a randomized, crossover, controlled, clinical trial. Atherosclerosis 181, 149–158.

Fitó, M., Cladellas, M., de la Torre, R., Martí, J., Muñoz, D., Schröder, H., Alcántara, M., Pujadas-Bastardes, M., Marrugat, J., López-Sabater, M.C., Bruguera, J., & Covas, M.I. (2008) Anti-inflammatory effect of virgin olive oil in stable coronary disease patients: a randomized, crossover, controlled trial. European Journal of Clinical Nutrition 62, 570–574.

Franco, M.N., Galeano-Díaz, T., López, O., Fernández-Bolaños, J.G., Sánchez, J., De Miguel, C., Gil, M.V., & Martín-Vertedor, D. (2014) Phenolic compounds and antioxidant capacity of virgin olive oil. Food Chemistry 163, 289–298.

Frazier, R.A., & Papadopoulou, A. (2003) Recent advances in the application of capillary electrophoresis for food analysis. Electrophoresis 24, 4095–4105.

García, J.M., Yousfi, K., Mateos, R., Olmo, M., & Cert, A. (2001) Reduction of oil bitterness by heating of olive (*Olea europaea*) fruits. Journal of Agricultural and Food Chemistry 49, 4231–4235.

García-Villalba, R., Carrasco-Pancorbo, A., Nevedomskaya, E., Mayboroda, O. A., Deelder, A. M., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2010) Exploratory analysis of human urine by LC-ESI-TOF MS after high intake of olive oil: understanding the metabolism of polyphenols. Analytical and Bioanalytical Chemistry 398, 463–475.

Gärtner, C., Stahl, W., & Sies, H. (1997) Lycopene is more bioavailable from tomato paste than from fresh tomatoes. American Journal of Clinical Nutrition 66, 116–122.

Gimeno, E., Castellote, A.I., Lamuela-Raventós, R.M., De la Torre, M.C., & López-Sabater, M.C. (2002) The effects of harvest and extraction methods on the antioxidant content (phenolics, α-tocopherol, and β-carotene) in virgin olive oil. Food Chemistry 78, 207–211.

Gimeno, E., de la Torre-Carbot, K., Lamuela-Raventós, R.M., Castellote, A.I., Fitó, M., de la Torre, R., Covas, M.I., & López-Sabater, M.C. (2007) Changes in the phenolic content of low density lipoprotein after olive oil consumption in men: a randomized crossover controlled trial. British Journal of Nutrition 98, 1243–1250.

Gimeno, E., Fitó, M., Lamuela-Raventós, R.M., Castellote, A.I., Covas, M., Farré, M., de La Torre-Boronat, M.C., & López-Sabater, M.C. (2002) Effect of ingestion of virgin olive oil on human low-density lipoprotein composition. European Journal of Clinical Nutrition 56, 114–120.

Gómez-Romero, M., García-Villalba, R., Carrasco-Pancorbo, A., & Fernández-Gutiérrez, A. (2012) Metabolism and bioavailability of olive oil polyphenols. In: Olive oil: constituents, quality, health properties and bioconversions, ed. D. Boskou. InTech, Rijeka, Croatia, 333–356.

González-Correa, J.A., Navas, M.D., Lopez-Villodres, J.A., Trujillo, M., Espartero, J.L., & De La Cruz, J.P. (2008) Neuroprotective effect of hydroxytyrosol and hydroxytyrosol acetate in rat brain slices subjected to hypoxia-reoxygenation. Neuroscience Letters 446, 143–146.

Gosetti, F., Bolfi, B., Manfredi, M., Calabrese, G., & Marengo, E. (2015) Determination of eight polyphenols and pantothenic acid in extra-virgin olive oil samples by a simple, fast, high-throughput and sensitive ultra high performance liquid chromatography with tandem mass spectrometry method. Journal of Separation Science [Epub ahead of print].

Gotsis, E., Anagnostis, P., Mariolis, A., Vlachou, A., Katsiki, N., & Karagiannis, A. (2015) Health benefits of the Mediterranean Diet: an update of research over the last 5 years. Angiology 66, 304–318.

Grosso, G., Buscemi, S., Galvano, F., Mistretta, A., Marventano, S., La Vela, V., Drago, F., Gangi, S., Basile, F., & Biondi, A. (2013) Mediterranean diet and cancer: epidemiological evidence and mechanism of selected aspects. BMC Surgery 13 Suppl 2, S14, 1–9.

Guasch-Ferré, M., Hruby, A., Salas-Salvadó, J., Martínez-González, M.A., Sun, Q., Willett, W.C., & Hu, F.B. (2015) Olive oil consumption and risk of type 2 diabetes in US women. American Journal of Clinical Nutrition 102, 479–486.

Guasch-Ferré, M., Hu, F.B., Martínez-González, M.A., Fitó, M., Bulló, M., Estruch, R., Ros, E., Corella, D., Recondo, J., Gómez-Gracia, E., Fiol, M., Lapetra, J., Serra-Majem, L., Muñoz, M.A., Pintó, X., Lamuela-Raventós, R.M., Basora, J., Salas-Salvadó, J. (2014) Olive oil intake and risk of cardiovascular disease and mortality in the PREDIMED Study. BMC Medicine 12, 1–12.

Gutiérrez-Rosales, F., Ríos, J.J., & Gómez-Rey, M.L. (2003) Main polyphenols in the bitter taste of virgin olive oil: structural confirmation by on-line high-performance liquid chromatography electrospray ionization mass spectrometry. Journal of Agricultural and Food Chemistry 51, 6021–6025.

Hernáez, Á., Fernández-Castillejo, S., Farràs, M., Catalán, Ú., Subirana, I., Montes, R., Solà, R., Muñoz-Aguayo, D., Gelabert-Gorgues, A., Díaz-Gil, Ó., Nyyssönen, K., Zunft, H.-J.F., de la Torre, R., Martín-Peláez, S., Pedret, A., Remaley, A.T., Covas, M.-I., & Fitó, M. (2014) Olive oil polyphenols enhance high-density lipoprotein function in humans: a randomized controlled trial. Arteriosclerosis, Thrombosis, and Vascular Biology 34, 2115–2119.

Khalatbary, A.R. (2013) Olive oil phenols and neuroprotection. Nutritional Neuroscience 16, 243–249.

Kimura, Y., & Sumiyoshi, M. (2009) Olive leaf extract and its main component oleuropein prevent chronic ultraviolet B radiation-induced skin damage and carcinogenesis in hairless mice. Journal of Nutrition 139, 2079–2086.

Koidis, A., & Boskou, D. (2014) Virgin olive oil: losses of antioxidant polar phenolic compounds due to storage, packaging, and culinary uses. In Processing and impact on active components in food, ed. V.R. Preddy. Elsevier Science, Amsterdam, 267–274

La Vecchia, C., & Bosetti, C. (2006) Diet and cancer risk in Mediterranean countries: open issues. Public Health Nutrition 9, 1077–1082.

Léger, C.L., Carbonneau, M.A., Michel, F., Mas, E., Monnier, L., Cristol, J.P., & Descomps, B. (2005) A thromboxane effect of a hydroxytyrosol-rich olive oil wastewater extract in patients with uncomplicated type I diabetes. European Journal of Clinical Nutrition 59, 727–730.

Li, A.-N., Li, S., Zhang, Y.-J., Xu, X.-R., Chen, Y.-M., & Li, H.-B. (2014) Resources and biological activities of natural polyphenols. Nutrients, 6, 6020–6047.

Loued, S., Berrougui, H., Componova, P., Ikhlef, S., Helal, O., & Khalil, A. (2013) Extra-virgin olive oil consumption reduces the age-related decrease in HDL and paraoxonase 1 anti-inflammatory activities. British Journal of Nutrition 110, 1272–1284.

Lucas, L., Russell, A., & Keast, R. (2011) Molecular mechanisms of inflammation. Anti-inflammatory benefits of virgin olive oil and the phenolic compound oleocanthal. Current Pharmaceutical Design, 17, 754–768.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004) Polyphenols: food sources and bioavailability. American Journal of Clinical Nutrition 79, 727–747.

Manna, C., Galletti, P., Cucciolla, V., Moltedo, O., Leone, A., & Zappia, V. (1997) The protective effect of the olive oil polyphenol (3,4-dihydroxyphenyl)-ethanol counteracts reactive oxygen metabolite-induced cytotoxicity in Caco-2 cells. Journal of Nutrition 127, 286–292.

Manna, C., Galletti, P., Maisto, G., Cucciolla, V., D'Angelo, S., & Zappia, V. (2000) Transport mechanism and metabolism of olive oil hydroxytyrosol in Caco-2 cells. FEBS Letters 470, 341–344.

Manna, C., Napoli, D., Cacciapuoti, G., Porcelli, M., & Zappia, V. (2009) Olive oil phenolic compounds inhibit homocysteine-induced endothelial cell adhesion regardless of their different antioxidant activity. Journal of Agricultural and Food Chemistry 57, 3478–3482.

Marsilio, V., Lanza, B., & Lombardi, D.S. (1999) Quality improvement of table olives by use of an oleuropein degrading strain of Lactobacillus plantarum. In Acta Horticulturae, 474, 601–604.

Martínez-González, M.Á., Corella, D., Salas-Salvadó, J., Ros, E., Covas, M.I., Fiol, M., Wärnberg, J., Arós, F., Ruíz-Gutiérrez, V., Lamuela-Raventós, R.M., Lapetra, J., Muñoz, M.Á., Martínez, J.A., Sáez, G., Serra-Majem, L., Pintó, X., Mitjavila, M.T., & Estruch, R. (2012) Cohort profile: design and methods of the PREDIMED study. International Journal of Epidemiology 41, 377–385.

Martínez-González, M.Á., Toledo, E., Arós, F., Fiol, M., Corella, D., Salas-Salvadó, J., Ros, E., Covas, M.I., Fernández-Crehuet, J., Lapetra, J., Muñoz, M.A., Fitó, M., Serra-Majem, L., Pintó, X., Lamuela-Raventós, R.M., Sorlí, J. V, Babio, N., & Alonso, A. (2014) Extravirgin olive oil consumption reduces risk of atrial fibrillation: the PREDIMED (Prevención con Dieta Mediterránea) trial. Circulation 130, 18–26.

Martínez-Huélamo, M., Tulipani, S., Estruch, R., Escribano, E., Illán, M., Corella, D., & Lamuela-Raventós, R.M. (2015) The tomato sauce making process affects the bioaccessibility and bioavailability of tomato phenolics: a pharmacokinetic study. Food Chemistry 173, 864–872.

Martínez-Lapiscina, E.H., Clavero, P., Toledo, E., San Julián, B., Sanchez-Tainta, A., Corella, D., Lamuela-Raventós, R.M., Martínez, J.A., & Martínez-Gonzalez, M.Á. (2013) Virgin olive oil supplementation and

long-term cognition: the PREDIMED-NAVARRA randomized, trial. Journal of Nutrition Health & Aging 17, 544–552.

Martín-Peláez, S., Covas, M.I., Fitó, M., Kušar, A., & Pravst, I. (2013) Health effects of olive oil polyphenols: recent advances and possibilities for the use of health claims. Molecular Nutrition & Food Research 57, 760–771.

Morelló, J.-R., Motilva, M.-J., Tovar, M.-J., & Romero, M.-P. (2004) Changes in commercial virgin olive oil (cv Arbequina) during storage, with special emphasis on the phenolic fraction. Food Chemistry 85, 357–364.

Morelló, J.-R., Romero, M.-P., Ramo, T., & Motilva, M.-J. (2005) Evaluation of l-phenylalanine ammonia-lyase activity and phenolic profile in olive drupe (*Olea europaea* L.) from fruit setting period to harvesting time. Plant Science 168, 65–72.

Moreno-Luna, R., Muñoz-Hernandez, R., Miranda, M.L., Costa, A.F., Jimenez-Jimenez, L., Vallejo-Vaz, A.J., Muriana, F.J.G., Villar, J., & Stiefel, P. (2012) Olive oil polyphenols decrease blood pressure and improve endothelial function in young women with mild hypertension. American Journal of Hypertension 25, 1299–1304.

Neveu, V., Perez-Jiménez, J., Vos, F., Crespy, V., du Chaffaut, L., Mennen, L., Knox, C., Eisner, R., Cruz, J., Wishart, D., & Scalbert, A. (2010) Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. Database: The Journal of Biological Databases and Curation 2010, bap024.

Olivero-David, R., Mena, C., Pérez-Jimenez, M.A., Sastre, B., Bastida, S., Márquez-Ruiz, G., & Sánchez-Muniz, F.J. (2014) Influence of Picual olive ripening on virgin olive oil alteration and stability during potato frying. Journal of Agricultural and Food Chemistry 62, 11637–11646.

Owen, R.W., Spiegelhalder, B., & Bartsch, H. (2000) Generation of reactive oxygen species by the faecal matrix. Gut 46, 225–232.

Pacheco, Y.M., Bemúdez, B., López, S., Abia, R., Villar, J., & Muriana, F.J.G. (2007) Minor compounds of olive oil have postprandial anti-inflammatory effects. British Journal of Nutrition 98, 260–263.

Pandey, K.B., & Rizvi, S.I. (2009) Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity 2, 270–278.

Patrick, L., & Uzick, M. (2001) Cardiovascular disease: C-reactive protein and the inflammatory disease paradigm: HMG-CoA reductase inhibitors, alpha-tocopherol, red yeast rice, and olive oil polyphenols. A review of the literature. Alternative Medicine Review 6, 248–271.

Pellegrini, N., Visioli, F., Buratti, S., & Brighenti, F. (2001) Direct analysis of total antioxidant activity of olive oil and studies on the influence of heating. Journal of Agricultural and Food Chemistry 49, 2532–2538.

Perez-Herrera, A., Delgado-Lista, J., Torres-Sanchez, L.A., Rangel-Zuñiga, O.A., Camargo, A., Moreno-Navarrete, J.M., Garcia-Olid, B., Quintana-Navarro, G.M., Alcala-Diaz, J.F., Muñoz-Lopez, C., Lopez-Segura, F., Fernandez-Real, J.M., Luque de Castro, M.D., Lopez-Miranda, J., & Perez-Jimenez, F. (2012) The postprandial inflammatory response after ingestion of heated oils in obese persons is reduced by the presence of phenol compounds. Molecular Nutrition & Food Research 56, 510–514.

Perez-Herrera, A., Rangel-Zuñiga, O.A., Delgado-Lista, J., Marin, C., Perez-Martinez, P., Tasset, I., Tunez, I., Quintana-Navarro, G.M., Lopez-Segura, F., Luque de Castro, M.D., Lopez-Miranda, J., Camargo, A., & Perez-Jimenez, F. (2013) The antioxidants in oils heated at frying temperature, whether natural or added, could protect against postprandial oxidative stress in obese people. Food Chemistry 138, 2250–2259.

Petrakis, C. (2006). Olive oil extraction. In: Olive oil: chemistry and technology, 2nd ed., ed. D. Boskou. AOCS Press, Champaign, IL, 193–223.

Prior, R.L., Wu, X., & Schaich, K. (2005) Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry 53, 4290–4302.

Psaltopoulou, T., Kosti, R.I., Haidopoulos, D., Dimopoulos, M., & Panagiotakos, D.B. (2011) Olive oil intake is inversely related to cancer prevalence: a systematic review and a meta-analysis of 13,800 patients and 23,340 controls in 19 observational studies. Lipids in Health and Disease 10, 1–16.

Puel, C., Mardon, J., Agalias, A., Davicco, M.-J., Lebecque, P., Mazur, A., Horcajada, M.-N., Skaltsounis, A.-L., & Coxam, V. (2008) Major phenolic compounds in olive oil modulate bone loss in an ovariectomy/inflammation experimental model. Journal of Agricultural and Food Chemistry 56, 9417–9422.

Ramírez-Anaya, J.D.P., Samaniego-Sánchez, C., Castañeda-Saucedo, M.C., Villalón-Mir, M., & de la Serrana, H.L.-G. (2015) Phenols and the antioxidant capacity of Mediterranean vegetables prepared with extra virgin olive oil using different domestic cooking techniques. Food Chemistry 188, 430–438.

Rich, G.T., Faulks, R.M., Wickham, M.S.J., & Fillery-Travis, A. (2003a) Solubilization of carotenoids from carrot juice and spinach in lipid phases: II. Modeling the duodenal environment. Lipids 38, 947–956.

Rich, G.T., Faulks, R.M., Wickham, M.S.J., Fillery-Travis, A., Bailey, A.L., Faulks, R.M., Parker, M.L., Wickham, M.S.J., & Fillery-Travis, A. (2003b) Solubilization of carotenoids from carrot juice and spinach in lipid phases: I. Modeling the gastric lumen. Lipids 38, 947–956.

Romero, C., Medina, E., Vargas, J., Brenes, M., & De Castro, A. (2007) In vitro activity of olive oil polyphenols against *Helicobacter pylori*. Journal of Agricultural and Food Chemistry 55, 680–686.

Ruano, J., Lopez-Miranda, J., Fuentes, F., Moreno, J. A., Bellido, C., Perez-Martinez, P., Lozano, A., Gómez, P., Jiménez, Y., & Pérez Jiménez, F. (2005) Phenolic content of virgin olive oil improves ischemic reactive hyperemia in hypercholesterolemic patients. Journal of the American College of Cardiology 46, 1864–1868.

Ryan, D., Robards, K., Prenzler, P., Jardine, D., Herlt, T., & Antolovich, M. (1999) Liquid chromatography with electrospray ionisation mass spectrometric detection of phenolic compounds from Olea europaea. Journal of Chromatography A 855, 529–537.

Salvini, S., Sera, F., Caruso, D., Giovannelli, L., Visioli, F., Saieva, C., Masala, G., Ceroti, M., Giovacchini, V., Pitozzi, V., Galli, C., Romani, A., Mulinacci, N., Bortolomeazzi, R., Dolara, P., & Palli, D. (2006) Daily consumption of a high-phenol extra-virgin olive oil reduces oxidative DNA damage in postmenopausal women. British Journal of Nutrition 95, 742–751.

Samieri, C., Féart, C., Proust-Lima, C., Peuchant, E., Tzourio, C., Stapf, C., Berr, C., & Barberger-Gateau, P. (2011) Olive oil consumption, plasma oleic acid, and stroke incidence: the Three-City Study. Neurology 77, 418–425.

Sarbishegi, M., Mehraein, F., & Soleimani, M. (2014) Antioxidant role of oleuropein on midbrain and dopaminergic neurons of substantia nigra in aged rats. Iranian Biomedical Journal 18, 16–22.

Servili, M., Selvaggini, R., Esposto, S., Taticchi, A., Montedoro, G., & Morozzi, G. (2004) Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. Journal of Chromatography A 1054, 113–127.

Singleton, V., Orthofer, R., & Lamuela-Raventos, R.M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Oxidants and Antioxidants 299, 152–178.

Soler, A., Romero, M.P., Macià, A., Saha, S., Furniss, C.S.M., Kroon, P.A., & Motilva, M.J. (2010) Digestion stability and evaluation of the metabolism and transport of olive oil phenols in the human small-intestinal epithelial Caco-2/TC7 cell line. Food Chemistry 119, 703–714.

Spencer, J.P.E., Abd El Mohsen, M.M., Minihane, A.-M., & Mathers, J.C. (2008) Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. British Journal of Nutrition 99, 12–22.

Tresserra-Rimbau, A., Medina-Remón, A., Pérez-Jiménez, J., Martínez-González, M.A., Covas, M.I., Corella, D., Salas-Salvadó, J., Gómez-Gracia, E., Lapetra, J., Arós, F., Fiol, M., Ros, E., Serra-Majem, L., Pintó, X., Muñoz, M.A., Saez, G.T., Ruiz-Gutiérrez, V., & Lamuela-Raventós, R.M. (2013) Dietary intake and major food sources of polyphenols in a Spanish population at high cardiovascular risk: the PREDIMED study. Nutrition, Metabolism, and Cardiovascular Diseases 23, 953–959.

Urpi-Sarda, M., Casas, R., Chiva-Blanch, G., Romero-Mamani, E.S., Valderas-Martínez, P., Arranz, S., Andres-Lacueva, C., Llorach, R., Medina-Remón, A., Lamuela-Raventos, R.M., & Estruch, R. (2012) Virgin olive oil and nuts as key foods of the Mediterranean diet effects on inflammatory biomarkers related to atherosclerosis. Polyphenols and Health 65, 577–583.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M., & Telser, J. (2007) Free radicals and antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry & Cell Biology 39, 44–84.

Vallverdú-Queralt, A., Regueiro, J., de Alvarenga, J.F.R., Torrado, X., & Lamuela-Raventos, R.M. (2014) Home cooking and phenolics: Effect of thermal treatment and addition of extra virgin olive oil on the phenolic profile of tomato sauces. Journal of Agricultural and Food Chemistry 62, 3314–3320.

Vallverdú-Queralt, A., Regueiro, J., de Alvarenga, J.F.R., Torrado, X., & Lamuela-Raventos, R.M. (2015) Carotenoid profile of tomato sauces: effect of cooking time and content of extra virgin olive oil. International Journal of Molecular Sciences 16, 9588–9599.

Velasco, J., & Dobarganes, C. (2002) Oxidative stability of virgin olive oil. European Journal of Lipid Science and Technology 104, 661–676.

Velasco, J., Marmesat, S., Bordeaux, O., Márquez-Ruiz, G., & Dobarganes, C. (2004) Formation and evolution of monoepoxy fatty acids in thermoxidized olive and sunflower oils and quantitation in used frying oils from restaurants and fried-food outlets. Journal of Agricultural and Food Chemistry 52, 4438–4443.

Visioli, F., Bellomo, G., & Galli, C. (1998) Free radical-scavenging properties of olive oil polyphenols. Biochemical and Biophysical Research Communications 247, 60–64.

Visioli, F., Caruso, D., Galli, C., Viappiani, S., Galli, G., & Sala, A. (2000a) Olive oils rich in natural catecholic phenols decrease isoprostane excretion in humans. Biochemical and Biophysical Research Communications 278, 797–799.

Visioli, F., Caruso, D., Grande, S., Bosisio, R., Villa, M., Galli, G., Sirtori, C., & Galli, C. (2005) Virgin Olive Oil Study (VOLOS): vasoprotective potential of extra virgin olive oil in mildly dyslipidemic patients. European Journal of Nutrition 44, 121–127.

Visioli, F., Galli, C., Bornet, F., Mattei, A., Patelli, R., Galli, G., & Caruso, D. (2000b) Olive oil phenolics are dose-dependently absorbed in humans. FEBS Letters 468, 159–160.
Visioli, F., Poli, A., & Gall, C. (2002) Antioxidant and other biological activities of phenols from olives and olive oil. Medicinal Research Reviews 22, 65–75.
Vissers, M.N., Zock, P.L., Roodenburg, A.J.C., Leenen, R., & Katan, M.B. (2002). Olive oil phenols are absorbed in humans. Journal of Nutrition 132, 409–417.
Vitaglione, P., Savarese, M., Paduano, A., Scalfi, L., Fogliano, V., & Sacchi, R. (2013) Healthy virgin olive oil: a matter of bitterness. Critical Reviews in Food Science and Nutrition 55, 1808–1818.
Widmer, R.J., Freund, M.A., Flammer, A.J., Sexton, J., Lennon, R., Romani, A., Mulinacci, N., Vinceri, F.F., Lerman, L.O., & Lerman, A. (2013) Beneficial effects of polyphenol-rich olive oil in patients with early atherosclerosis. European Journal of Nutrition 52, 1223–1231.
Zhu, L., Liu, Z., Feng, Z., Hao, J., Shen, W., Li, X., Sun, L., Sharman, E., Wang, Y., Wertz, K., Weber, P., Shi, X., & Liu, J. (2010) Hydroxytyrosol protects against oxidative damage by simultaneous activation of mitochondrial biogenesis and phase II detoxifying enzyme systems in retinal pigment epithelial cells. Journal of Nutritional Biochemistry 21, 1089–1098.

23 Bioactive components from olive oil as putative epigenetic modulators

Tea Bilusic

23.1 Introduction

The development of new scientific disciplines, such as epigenetics, offers new perspectives in investigation of healthy potential of olive oil's bioactive components. Experimentally, epigenetics explores factors that modulate imprinting and gene silencing in mammals without change to the DNA sequence. Epigenetic changes are heritable, cell-type specific, and reversible. This chapter describes main epigenetic mechanisms in human cells as well as the environmental factors, such as food, that affect epigenetic mechanisms; and it summarizes the current knowledge on the effect of olive oil's bioactive components on epigenetic mechanisms.

23.2 Epigenetics as a new scientific challenge

> We are all guardians of our genome. The way that people live and their lifestyle no longer just affects them, but may have a knock-on effect for their children and grandchildren.
>
> Professor Marcus E. Pembrey (University College, London)

Recent scientific studies have proven the complexity of dynamic interactions between the human body and environmental factors, including diet as one of the most important contributors. Therefore, there is the constant challenge for developing powerful techniques that allow better detailed understanding of complex relations between the entire human genome and the environment as well as the changes in human genome that are characteristic of pathological states. In this relation, Professor Marcus E. Pembrey of University College, London, made the statement that opens this chapter. Thus, in that sense, the genomic revolution, which started with the Human Genome Project (1990–2003), has introduced a new dimension into biological and biomedical research. An immense amount of structural information about individual genes has been obtained. Therefore, by completing the sequencing of the human genome, it was indispensable to integrate the obtained data into a global model suitable for better understanding of the mechanisms of diseases and to predict novel therapeutic strategies. Due to continuous advances in genetics, comparative genomics, biochemistry, and bioinformatics, the integration of numerous data was made possible (Collins *et al.*, 2003). DNA microarray technology has revolutionized scientific research in the field of biology and biomedicine, allowing study of the expression patterns across an entire genome. Such progress has overturned the idea that just one gene performs only one job (e.g., coding for proteins). It was shown that a very small percentage of the genome (less than 2%) acts according to the classical definition of the gene as a protein-coding sequence, while most of the non-protein-coding DNA ("junk DNA") plays an important regulatory function (Meloni, 2014). Recent descriptions of the genome as a "vast reactive system" embedded in a complex regulatory network indicates the beginning of the so-called "postgenomic era" (Keller, 2011). More than ever, the complexity of the living organism, and its continuous and dynamic interaction with environmental

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factors, represent a constant challenge to scientists, especially because of the rapid rise of chronic diseases worldwide that represent a big social and public health problem. Hence, predicting the contribution of genes to complex disorders and determining the interactions between genes and the environment during any disease process are huge tasks (Peltonen & McKusick, 2001). This task is extremely difficult because of the complexity of the organism itself as well as the broad range of environmental signals that can involve the cellular environment around the DNA, the entire organism, and, in the case of human beings, their social and cultural dynamics (Meloni, 2014).

Epigenetics is a very fast-growing field in the postgenomic era. The term "epi-," derived from the Greek word meaning "over, above, outer," implies that epigenetic mechanisms act on genes via altering the gene expression and regulation without modifying the DNA sequence. To understand the main difference between genetics and epigenetics, it is necessary to point out that genetics deals with the transmission and processing of information about DNA, whereas epigenetics deals with its interpretation and integration with information from other sources (Jablonka & Lamb, 2002). Haig (2004) mentioned two independent origins of the term "epigenetics" in the twentieth century. The first one is referred to Conrad H. Waddington (1942), the British developmental biologist, paleontologist, geneticist, and philosopher, who used the term "epigenetics" to specify interactions between genes and the environment; and the second one is referred to the American geneticist David Nanney (1958), who used the same term to describe systems of cellular heredity that were not based on the DNA sequence. Waddington's concept of epigenetics includes three aspects: *canalization* (capacity of the organism of certain populations to produce the same phenotype regardless of the extent of genetic and environmental variations), *genetic assimilation* (relation between the organism and the environment results in an acquired phenotype that becomes part of the developmental process of the organism), and *epigenetic landscape* (certain area in chromatin characterized with cytosine methylation and histone modifications) (Kovalchuk & Kovalchuk, 2012). The definition of epigenetics, formulated at the Cold Spring Harbor Epigenetics Meeting in 2008, is the following: "An epigenetic trait is a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence" (Berger *et al.*, 2009).

Recent definitions of epigenetic mechanisms within the current scientific state of the knowledge have restarted a debate among scientists about evolution and heredity. Some scientists believe that biological thinking about heredity and evolution is undergoing a revolutionary change, and their concept of the epigenetic model is very similar to the Lamarckian model of inheritance (Monk, 1995; Jablonka & Lamb, 2002). In fact, the idea of an epigenetics-like concept appeared 200 years ago, when the French naturalist, Jean-Baptiste Lamarck (1744–1829), proposed the "theory of inheritance of acquired traits," known as Lamarckism. This concept was rejected by the English biologist Sir William Lawrence (1783–1867) and by the German biologist August Weismann (1834–1914). In 1859, English naturalist Charles Darwin (1809–1882) published the work *The Origin of Species*, in which he suggested that species in the population evolve through a process of natural selection. Darwin accepted that environmental factors play a critical role in shaping species' evolution. Darwin's theory of evolution and natural selection is still the reason for many scientific discussions. British biologist and anthropologist Alfred Russel Wallace (1823–1913) and August Weismann have proposed the theory known as "Neo-Darwinism," which suggested that evolution occurs without mechanisms of inheritance of acquired characteristics. Until that time, technological and scientific expansion has occurred, and the idea of developmental process associated with genetic and epigenetic regulation is generally accepted. The epigenetic revolution evolved in the early 2000s, when scientists began reporting the influence of environmental factors on chemical modifications to DNA and histones that resulted in gene inhibition or activation (Dias & Ressler, 2014). In recent years, epigenetics has become the focus of modern medicine, located as the connection among genetics, developmental biology, and ecology, which are all together the essence of the framework of evolutionary biology, as shown in Figure 23.1.

The capacity of the plasticity in cell and organism phenotypes is also one of the amazing facts demonstrated by epigenetic studies. Genetically identical cells or organisms can completely differ structurally and functionally (e.g., monozygotic twins) (Jablonka & Lamb, 2002). Similarly, the difference between queen bee and worker bee is epigenetic, not genetic, because it is influenced by the type of the honey fed to larvae, not by the genotype. Due to recent advances in technology of DNA sequencing and in the development of computational and mathematical models, better understanding of the organism's development was achieved. In addition, the prediction of cell differentiation processes and reprogramming events was allowed. These advances have helped in identifying and understanding epigenetic mechanisms within the mammalian genome (Kim *et al.*, 2009).

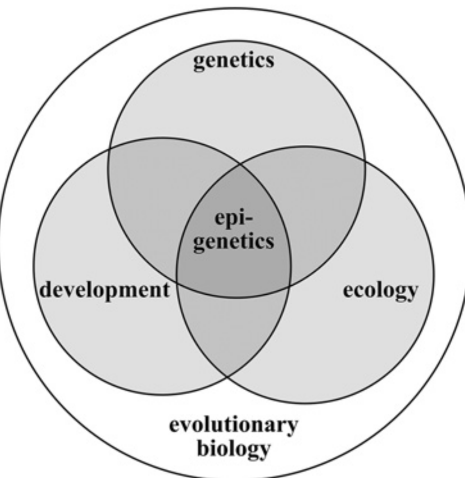

Figure 23.1 Epigenetics, ecology, and developmental biology as a part of evolutionary biology. Epigenetics represents a bridge between genetics, ecology, and developmental biology. *Source*: Jablonka and Lamb (2002). Reproduced with permission of John Wiley & Sons.

23.3 Types of epigenetic modifications

Genetics and epigenetics study heritable changes. Genetics is the study of heritable changes in gene activity or function due to direct alterations to a DNA sequence, such as point mutations, deletions, insertions, and translocation (Moore *et al.*, 2013). On the other hand, epigenetics is the study of gene activity or function that is not related to the direct alterations in DNA sequence. Epigenetics modifications include: DNA methylation, histone modifications (acetylation, methylation, ubiquitination, phosphorylation, and biotinylation), microRNA changes, genomic imprinting, and nucleosome remodeling pattern regulation (Choi & Friso, 2009). DNA methylation and posttranslational histone modifications are considered as two major epigenetic mechanisms, which are heritable during the cell division process.

23.3.1 DNA methylation

From the specifically chemical point of view, DNA methylation is the process of the modification of certain DNA nucleotide bases by the addition of the methyl group ($-CH_3$). Methylation of DNA in mammals only occurs in position 5 of the cytosine pyrimidine ring and is catalyzed by the family of enzymes known as DNA methyltransferases, resulting in 5-methylcytosine. Methyl groups play a crucial role in DNA synthesis, stability, and integrity. The mammalian DNA methylation process is composed of the DNA methyltransferases and the methyl CpG binding proteins, which are indispensable for "reading" methylation marks (Robertson, 2005). CpG dinucleotides or islands are genomic regions characterized by a high GC content. The DNA methylation process plays a number of important roles in cellular homeostasis and normal mammalian development through regulating gene expression and cell differentiation. In normal somatic cells, only 1–4% of all cytosine residues are methylated, depending on the cell or tissue type (Choi & Friso, 2010). Interestingly, among all tissues in the body, the brain contains the highest DNA methylation rate. Importantly, DNA methylation in different genomic regions may exert different influences on gene activities based on the underlying genetic sequence. One of the most important roles of the DNA methylation process is to inhibit the expression of potentially harmful genetic elements in the genome, such as transposable and viral elements or endogenous retroviruses (Moore *et al.*, 2013). In the available literature, there is a lot of evidence that DNA methylation is associated with many disorders or diseases: altered patterns of DNA methylation are observed in psychiatric patients with bipolar disorders and schizophrenia (Mill *et al.*, 2008), and in different types of cancer, diabetes, neurodegenerative diseases (Alzheimer's disease [AD], Parkinson's disease [PD], Huntington's disease [HD], and amyotrophic lateral sclerosis [ALS]) (Lu *et al.*, 2013), and autoimmune

diseases (Gupta & Hawkins, 2015). Increasing evidence shows that DNA methylation is labile in response to nutritional and environmental influences. Global hypomethylation can result in chromosome instability, while hypermethylation has been associated with the silencing of cancer suppressor genes (Anderson *et al.*, 2012). The influence of environmental factors, especially the diet, on the DNA methylation process will be described in Section 23.4.

23.3.2 Histone modifications

Histones are among the most evolutionary conserved proteins in nature (Budhavarapu *et al.*, 2013). The term "histones" describes a family of proteins that are associated with DNA in the nucleus and help to condense it into chromatin. Figure 23.2 shows that nuclear DNA is highly condensed and wraps around histone octamers, which consist of four different histones.

Chromatin has two forms, the less intense euchromatin and the tighter packing heterochromatin. In mammals, most of the chromatin exists in the form of heterochromatin, characterized by hypoacetylated and methylated histone that results in transcriptional silencing (a mechanism of transcriptional control where DNA is incorporated into heterochromatin in order to make it permanently inaccessible for future transcription) (Weitzman, 2002). Euchromatin contains histone with modifications that promote gene expression. Heterochromatin has important functions associated with gene regulation as well as chromosome integrity protection. Posttranslational histone modifications are crucial for chromatin structure. In contrast to DNA that is modified only by methylation, histone modifications include: methylation, acetylation, ubiquitination, and sumoylation of lysines, and phosphorylation of serine and threonine (Kim *et al.*, 2009). These

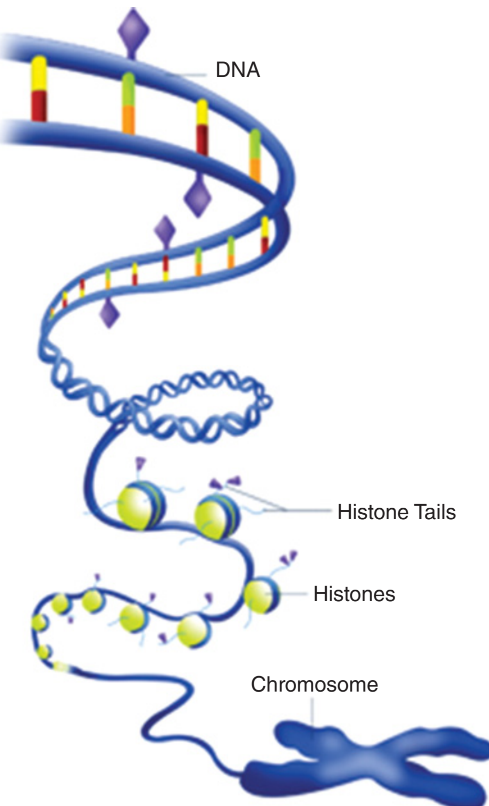

Figure 23.2 Structure of DNA and histones. *Source*: Courtesy of National Institute of General Medical Sciences, USA.

modifications are performed on N-terminal tails of histone proteins. Next to DNA methylation, histone acetylation and histone methylation are the most well-characterized epigenetic marks (Bartova *et al.*, 2008). Histone acetylation is catalyzed by histone acetyltransferase (HAT) and histone deacetylase (HDAC) enzymes, while histone methylation is catalyzed by histone methyltransferases and histone demethylases. HDAC inhibitors have been recognized as potential cancer therapeutic agents, because they induce cell cycle arrest and apoptosis by enhancing the expression of certain proapoptotic genes and genes that induce cell cycle arrest (Choi & Friso, 2010). There are many studies that have investigated the inhibitory effect of food components, especially biologically active compounds, on HDAC and HAT activity (Rajendran *et al.*, 2011; Vanden Berghe, 2012). Acetylation of histone lysine residues is associated with euchromatin characterized by less condense chromatin and facilitated DNA transcription. Histone methylation can be either gene silencing or activating, depending on the location of the methylated residue (Allis *et al.*, 2007). There is a correlation between DNA methylation and histone modification. If genes are methylated, chromatin is usually deacetylated and inactive (Herranz & Esteller, 2007). Thus, DNA methylation determines histone acetylation status.

23.3.3 MicroRNAs

MicroRNAs are short (22 nucleotides), noncoding RNAs that regulate gene expression posttranscriptionally. Interestingly, they represent only 1% of the entire genome, but they target 30% of genes (Lewis *et al.*, 2005). Currently, there are more than 460 known human microRNAs (Chuang & Jones, 2007). MicroRNAs are involved in the control of the DNA methylation process and histone modification (Choi & Friso, 2010). The roles of microRNAs in cancer have been extensively investigated in recent years. Although some authors have considered that microRNAs play an important role in tumor suppression, cell apoptosis, and cell proliferation (Kanherkar *et al.*, 2014), it is important to point out that microRNAs, depending on cancer type, can act as either oncogenes or cancer suppressors (Chuang & Jones, 2007). In addition, microRNAs play a role in virus infection and defense (Saetrom *et al.*, 2007). Recent evidence suggests that bioactive dietary compounds can affect various oncogenic or tumor-suppressive microRNAs, altering the gene expression in cancer prevention (Kala *et al.*, 2013). Research on microRNAs and their epigenetic-like potential is still at the beginning. However, there are promising findings on natural bioactive components and their influence on microRNA expression. *Olea europaea* leaf extract modulates the expression of some microRNAs involved in anticancer activity in human glioblastoma cells (Tunca *et al.*, 2012). Monounsaturated oleic acid from olive oil restored the expression level of microRNAs that was affected in mouse myoblasts by palmitic acid (Li *et al.*, 2011b). Olive oil phenols modulate microRNA profiles in mouse brain (Luceri *et al.*, 2013).

23.4 Environmental factors and epigenetics (the role of the diet)

All living organisms need *homeostasis* – a dynamic, very vulnerable and healthy balance between the environmental factors and the organism. Maintenance of homeostasis is essential to the maintenance of good health and prevention or delay of diseases. The major challenge of nutrition is to effectively retain this balance.

Epigenetic variations depend on the genotype (intrinsic factor) as well as environmental factors (extrinsic factors) (Aguilera *et al.*, 2010). Environmental factors include: physical, chemical, biological, and nutritional aspects (Ho & Domann, 2015). Major environmental factors that act as epigenetic and genetic modulators in the human body are shown in Figure 23.3. There are two crucial facts concerning the complex relation between epigenetic changes and environmental factors: (a) the organism is more sensitive to environmental factors during certain phases of life, such as the prenatal period, early postnatal period, and slow growth period (the time before the start of puberty); and (b) the impact of the environment has been observed to extend over multiple generations (known as a "transgenerational epigenetic effect").

Results of scientific studies provided evidence that the effects of a mother's early environment can be passed on to the next generation (Roth *et al.*, 2009; Arai *et al.*, 2012). The precise mechanism of the environmental effect on the epigenome is still far from being explained, as well as the mechanisms of epigenetic inheritance. One of the most important properties of this epigenetic memory is its dynamic

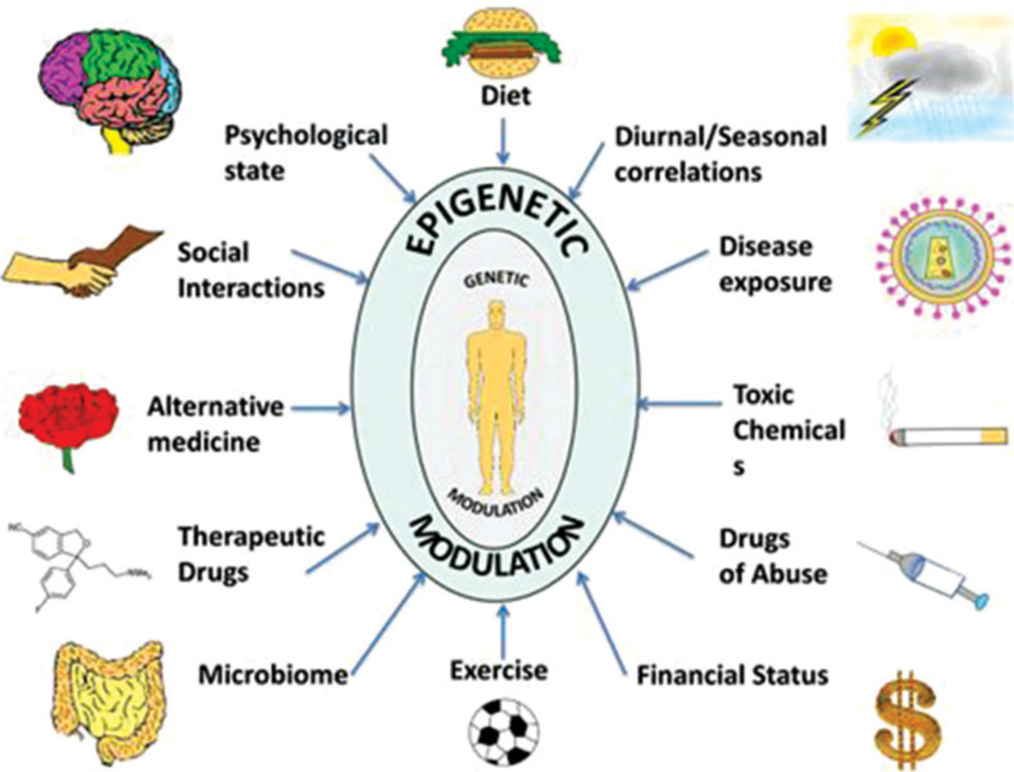

Figure 23.3 Environmental factors as epigenetic and genetic modulators in the human body. *Source*: Kanherkar *et al.* (2014), http://journal.frontiersin.org/article/10.3389/fcell.2014.00049/full. Used under Creative Commons License.

nature and particularly its ability to be erased. During the prenatal period, intensive cell differentiation occurs, and the fetus is more susceptible to environmental factors that can easily cross the placental barrier and come into the intrauterine environment (Ho & Domann, 2015). There are numerous studies on the impact of different environmental factors on epigenetic changes in humans and animals. High doses of ionizing radiation result in epigenetic modification in adult mice (Tawa *et al.*, 1998). Early life stress in humans is linked with gene expression changes for a polymorphic form of serotonin receptor (Caspi *et al.*, 2003). Rats show persistent DNA methylation changes of the glucocorticoid receptor in the hippocampus in response to high or low levels of maternal care during the first week of life (Weaver *et al.*, 2004; McGowan *et al.*, 2011). Adult humans abused in early life display increased DNA methylation at the NR3C1 glucocorticoid receptor promoter in the hippocampus (McGowan *et al.*, 2009). In mice, stress from maternal separation results in depressive behavior resulting in changed DNA methylation mechanisms in a number of genes (Franklin *et al.*, 2010). A maternal antioxidant-rich diet during pregnancy and the lactation period decreased the impact of radiation exposure on DNA methylation mechanisms (Bernal *et al.*, 2013). Fetal exposure to chemicals results in adverse health effects that can persist into adulthood (Ho & Domann, 2015). Chemicals such as bisphenol A (BPA), phthalates, pesticides, and persistent organic pollutants are associated with detrimental processes in the organism such as neurodegenerative problems, the decrease or loss of reproductive function, carcinogenesis, obesity, and immunological problems (Ho & Domann, 2015). BPA has an effect on DNA methylation mechanisms (Zhang *et al.*, 2012). Persistent organic pollutants such as organochlorine pesticides, dioxins, and hexachlorobenzene have been found to accumulate in biological systems, and early exposures to these chemicals have been associated with delayed cognitive development, delayed growth, and immune-related disease (Guo *et al.*, 2004; Ho & Domann, 2015). Pesticides exposure interferes with epigenetic mechanisms, leading to adverse health effects such as neurodegenerative diseases

like Alzheimer's and Parkinson's, reproductive function problems, behavioral disorders, cancer, and diabetes (Ho & Domann, 2015). Their effect is related to not only DNA methylation mechanisms but also microRNAs (Wang, 2010). In addition, exposure to metals or metal-containing compounds has been shown to interact with the epigenome (Cheng *et al.*, 2012). Smoking also affects epigenetic mechanisms in the body. The sons of men who had smoked during their slow growth period were significantly more overweight (Northstone *et al.*, 2014). Maternal smoking has been associated with asthma in children (Pingsheng, 2012). The impacts of bacteria, viruses, or parasites and their toxins on epigenetic mechanisms include increased transcription of DNA methyltransferases resulting by hypermethylation of tumor suppressor regions, thereby decreasing the activity of tumor suppressor genes (Niller *et al.*, 2013). Furthermore, the altered control of cell cycle regulation increases the risk of unchecked cellular propagation and represents an important factor connecting infection to cancer development (Jones & Thompson, 2009). Bacteria such as *Shigella flexneri*, *Listeria monocytogenes*, *Chlamydia trachomatis*, and *Mycobacterium* spp. are capable of synthesizing proteins that modify histones and chromatin structures (Rennoll-Bankert & Dumler, 2012). Human *gut microbiota* affect epigenetic mechanisms. Microbiota consist of more than 100 trillion bacterial cells and produces multiple low-molecular-weight compounds, many of which are certainly able to interfere in genomic and epigenomic processes and in the host metabolism (Shenderov, 2012).

When analyzing the effects of various environmental factors on epigenetic mechanisms in animal and human studies, it is very important to point out the beneficial effects of olive's phytochemicals such as oleuropein and hydroxytyrosol. Oleuropein and hydroxytyrosol protect against the effects of BPA in liver and kidneys of lactating mother rats and their pups (Mahmoudi *et al.*, 2015). Olive leaf extract modulates permethrin (a synthetic chemical used as an insecticide and acaricide) genetic and oxidative damage in rats (Turkez *et al.*, 2012). Olive oil and its lipophilic and hydrophilic fractions can decrease cardiac complications and genotoxicity in the heart of rat exposed to aluminum and acrylamide (Ghorbel *et al.*, 2016). In addition, there are numerous studies that have reported antiviral, antimicrobial, and antioxidant effects of oleuropein (Omar, 2010). Oleocanthal (decarboxy methyl ligstroside aglycone), first reported in extra virgin olive oil by Montedoro and Servilli (1993), is recognized as an anti-inflammatory agent in olive oil, exhibiting anti-inflammatory mechanisms very similar to those of ibuprofen (Cicerale *et al.*, 2012; Parkinson & Keast, 2014). Hydroxytyrosol, a phenolic from olive oil, exhibits a protective activity against cancer by arresting the cell cycle and inducing apoptosis in tumor cells (Fabiani *et al.*, 2002).

23.4.1 Nutritional factors

Food comprises a complex system that, after consumption, enters into a more complex system, namely, the human body. It is very difficult to describe the precise effects of nutrients or bioactive food components on epigenetic modulations and their association with physiological and pathological processes in the body, because nutrients interact with genes, with other nutrients, with gut microbiota, with digestive enzymes in the gastrointestinal system, and with other factors. Diet-induced epigenetic alterations might also be inherited transgenerationally, thereby potentially affecting the health of future generations. A variety of maternal nutritional changes during pregnancy have been implicated in immune development and allergic risk, such as reduction in intake of omega-3 fatty acids, antioxidants, and a range of specific micronutrients such as vitamins and minerals (Calder *et al.*, 2006). Pregnant mice fed a methyl donor–enriched diet had offspring with enhanced allergic potential (O'Neill *et al.*, 2014). Higher intake of vitamin D during pregnancy was found to be associated with some protection from wheeze in five-year-old children (Litonjua, 2009). Alterations in paternal diet (a high-fat or low-protein diet) or intrauterine exposure to maternal caloric restriction can result in increased metabolic risk in offspring (Ho & Domann, 2015). An Överxalix study showed that starvation or overeating during the slow growth period would influence a risk of diabetes and heart disease in descendants (Kaati *et al.*, 2002). Interestingly, this study showed that a single environmental factor can affect descendants in a sex-specific way, because previously described observations were specific only for men, and not for women. Studies have found increased neonatal adiposity among the grandchildren of women who had been undernourished during pregnancy (Devereux *et al.*, 2007). Mice fed a low-protein diet displayed increased risk of a high-cholesterol phenotype in the next generation (Blackmore *et al.*, 2012). Biotin (vitamin H) deficiency causes abnormally low biotinylation of histones and results in aberrant gene regulation (Zempleni *et al.*, 2008). In that sense, the supply of biotin depends on diet and also on the ability of gut microbiota to synthesize biotin. Folate (vitamin B_9) is another micronutrient that is important as a mediator

for the transfer of methyl groups. It is the most extensively studied nutrient in animal and epidemiological DNA methylation studies. It is thought that folate intake and global DNA methylation are positively correlated (Crider *et al.*, 2012). Epidemiological studies indicate that inadequate dietary intake of folate increases cancer risk possibility (Ghosh *et al.*, 2008). Deficiency of folate and vitamin B_{12} is associated with various adverse consequences, including elevated blood homocysteine, intrauterine growth retardation, congenital heart defects, and Down's syndrome. In addition, folate and vitamin B_{12} deficiency are risk factors for developmental disorders such as neural tube defects (Kerek *et al.*, 2013). Epigenetic effects of isothiocyanates (hydrolyzed products of glucosinolates from Brassicaceae) have been linked to the inhibition of HAT activity and histone hyperacetylation (Ho *et al.*, 2009). Epigalocatechin-3-gallate (EGCG) was reported to inhibit enzymes involved in DNA methylation (Fang *et al.*, 2007). Tea catechins are reported to exert DNA demethylating effects *in vitro* (Yiannakopoulou, 2015). Curcumin was reported to inhibit HAT activity (Morimoto *et al.*, 2008), while resveratrol from red grapes was implicated in silencing of the BRCA-1 gene in human breast cancer cells (Papoutsis *et al.*, 2010). Isoflavones from soybean, such as genistein, impacted histone acetylation and demethylation (Zhang *et al.*, 2013). Allyl sulfide from allium species inhibited HDAC (Nian *et al.*, 2009). Diet enriched with methyl donors (folic acid, selenium, choline, folate, and diallyl sulfides) has been shown to prevent transgenerational obesity (Waterland *et al.*, 2008).

Generally, it is possible to conclude that dietary factors represent one of the most important environmental factors that exert strong effects on epigenetic and genetic modulations in the organism. Maternal dietary intake during pregnancy and the lactation period is considered to be a crucial element that can influence embryogenesis and fetal development, leading to either beneficial prevention or adverse effects. The case of agouti mouse fed with different diets that resulted in significant differences in their phenotype is shown in Figure 23.4.

Methyl-donating nutrients are found in leafy green vegetables, peas and beans, sunflower seeds, milk, eggs, citrus fruits, beef liver, fish, broccoli, Brussels sprouts, and shrimp. Within the context of dietary impact

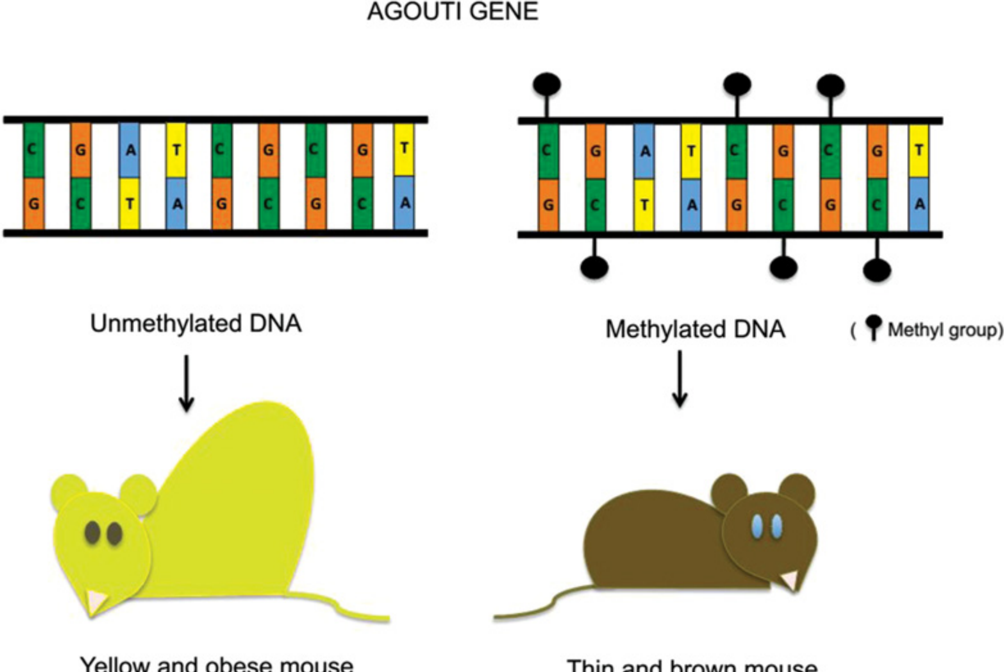

Figure 23.4 Mother's diet during pregnancy affected agouti gene expression in Avy/a mice that resulted in altered epigenetic regulation accompanied with significant change in the mouse phenotype. *Source*: Courtesy of Florean (2014). Food that shapes you: how diet can change your epigenome. Science in School, 28, http://www.scienceinschool.org/2014/issue28/epigenetics.

on epigenetic modulations, the role of olive oil's bioactive components as putative epigenetic modulators will be discussed separately.

23.5 Epigenetics and human health

The concept of a genome having an accompanying epigenome that regulates the activity of individual genomic sequences within the genome, as well as recent understanding that some heritable information may not be encoded by DNA, offers new insights into how organisms adapt to environmental change and on the important role of epigenetics in health maintenance (Ravin, 2008). The current state of knowledge on public health is very obscure. Over the last few decades, chronic diseases have been rapidly increasing worldwide. According to Oxford Health Alliance and the identification of Grand Challenges in chronic disease control, the four leading chronic diseases in the world are: heart disease, cancer, respiratory disease, and diabetes (Stuckler, 2008). Their rapid rise represents a big social and economic problem as well as a big problem for the quality of life of millions of people. According to the World Health Organization (WHO), chronic diseases account for an estimated 35 million deaths per year, representing 60% of worldwide mortality. It has been estimated that if major risk factors for chronic diseases were eliminated, at least 80% of all heart disease and type 2 diabetes would be prevented, and more than 40% of cancer cases would be prevented (WHO, 2015). Otherwise, without a right and strong strategy, chronic diseases and their risk factors can be expected to cause more harm and be costlier for society! In that sense, it is important to invest additional research efforts to understand the basis of human chronic diseases. The developmental origins of disease hypothesis postulated that chronic diseases not only are genetic but also result from early-life events, especially those occurring during the gestation period (Smith & Mill, 2011). Evidence from animal studies adds support to the role of epigenetics in development of chronic diseases. There is an association between low birth weight and greater risk of developing coronary heart disease, high blood pressure, obesity, and type 2 diabetes in later periods of life. The study found that rats born to undernourished mothers had an increased chance of developing type 2 diabetes (Godfrey & Barker, 2000). In addition, the epidemiological evidence from large independent population samples in the Netherlands and China showed that *in utero* nutritional deficiency, due to maternal exposure to severe famine during pregnancy, is associated with an increased risk of developing schizophrenia in adult life (Brown & Susser, 2008). Epidemiological studies reported on the influence of paternal age on the increased risk for several chronic diseases, schizophrenia, and autism (Smith & Mill, 2011). The epigenomic study by Mill *et al.* (2008) showed DNA methylation differences in the brain in patients suffering from schizophrenia and bipolar disorder. In addition, histone modifications in brain cells have been observed in patients suffering from schizophrenia (Ptak & Petronis, 2010). There are scientific reports on the role of dysregulated epigenetic mechanisms (especially through proinflammatory activity of hypomethylated DNA) in gene expression and immune cell proliferation that contribute to diverse autoimmune disorders such as rheumatoid arthritis and osteoarthritis (Smith & Mill, 2011). Hales and Barker (1992) proposed that environmental factors experienced in early life may enhance the risk of type 2 diabetes in later life. Physical inactivity, one of the major risk factors for type 2 diabetes, is also associated with epigenetic mechanisms (Pareja-Galeano *et al.*, 2014). There are scientific studies on the role of epigenetic mechanisms in the incidence of asthma, which represents one of the growing health problems in developing countries. Prenatal exposure to environmental tobacco smoke, especially in the last trimester of pregnancy, is associated with impaired respiratory function and disease development (Smith & Mill, 2011). Smoke alters DNA methylation, thus provoking oxidative stress that can cause oxidative damage to DNA that interferes with the binding of DNA methyltransferases, resulting in DNA hypomethylation. The relation between epigenetic mechanisms and mental diseases such as Rett syndrome, anorexia nervosa, and bulimia nervosa was observed by Ptak and Petronis (2010). A link between DNA methylation and cancer was first demonstrated in 1983, when it was shown that the genomes of cancer cells were hypomethylated (Robertson, 2005). A low level of genomic methylation is observed in the early period of carcinogenesis, and correlates with disease severity and metastatic potential in many types of cancer. However, in some types of cancer such as colon cancer, the CpG islands' hypermethylation is observed, and scientists believe that it represents a good potential biomarker for early cancer detection (Robertson, 2005). Results of the first two large-scale epigenome-wide association studies (EWASs) in Alzheimer's disease, one of the most burdensome threats to public health today, suggested that epigenetic changes contributed more to Alzheimer's

disease than expected through modifications in DNA methylation in four gene loci, not previously associated with Alzheimer's disease (Lord & Cruchaga, 2014). Studies on DNA methylation changes or histone modifications in relation to the development of obesity are numerous. Epigenetic changes have been detected at genes that regulate growth factors, adipogenesis, appetite regulation, and glucose homeostasis (Youngson & Morris, 2013). According to the results of many studies, epigenetics may represent a missing link between genetic and environmental factors and their potential interactions in the development of Parkinson's disease (Marques & Outeiro, 2013). Epigenetic processes are central to sustaining and regulating transcriptional plasticity, and the maintenance and regulation of epigenetic mechanisms represent important factors in prevention or regression of various chronic diseases. In that sense, the role of the diet is crucial. It is believed that food containing methionine, serine, glycine, histidine, choline, creatine, and B vitamins (folate, vitamin B_{12}, and vitamin B_6) may reduce the risk of epigenetic changes (Wang *et al.*, 2012). Furthermore, numerous epidemiological studies suggest that phytochemicals from plant-derived food play a role in slowing of progression of certain cancers, reduce the risks of cardiovascular disease, neurodegenerative diseases, diabetes, osteoporosis (Arts & Hollman, 2005; Scalbert *et al.*, 2005; Vauzour *et al.*, 2010; Afzal *et al.*, 2015). However, the effect of various plant food-derived biologically active compounds as putative epigenetic regulators is poorly explored and new important scientific findings should be expected in this field.

23.6 Epigenetics and aging

Aging is an inevitable process for any biological system resulting from a cascade of destructive events that lead to progressive morphologic and physiologic deterioration of the organs (Preedy, 2014). The American gerontologist Bernard Strehler indicated four postulates in order to describe the aging process as universal, intrinsic, progressive, and deleterious (Viña *et al.*, 2007). Modern biological theories of aging in human can be summarized and divided into two groups (Barja, 2004). The first group can be characterized by theories commonly named "programmed theories," which imply that aging follows a programmed biological timetable and include programmed longevity theory, endocrine theory, and immunological theory. The second group includes several theories, among which are free radical theory and somatic DNA damage theory (Jin, 2010). One the most accepted theories on aging is the oxidative stress aging theory, today known as the mitochondrial free radical theory, which explains that damage to DNA, proteins, and lipids by free radicals increases with aging and that this damage accumulates and contributes to senescence. Figure 23.5 shows the relation among oxidative stress, epigenetics, and aging.

The theory on oxidative stress was truly born in 1972, when Harman suggested that mitochondria's electron transport chain is the source of toxic free radicals such as superoxide and hydroxyl radicals, while mitochondrial membranes are their main target. Recent studies have strengthened this theory, so that it has gained the support of large numbers of professional gerontologists (de Grey, 1999). Oxidative stress, defined by Sies (1985) as disturbance in the prooxidant–antioxidant balance in favor of the former, is believed to be associated with many pathologic conditions and disease processes in elderly people, such as cardiovascular disease, diabetes, neurodegenerative diseases, immunosenescence, cancers, and endocrine dysfunction (Sies, 2015). Comparative studies of animals with different aging rates have shown that the rate of mitochondrial oxygen radical generation is directly related to the steady-state level of oxidative damage to mitochondrial DNA and inversely correlated with maximum longevity in higher vertebrates (Barja, 2004). Caloric-restricted animals also show decreased levels of both mitochondrial reactive oxygen species (ROS) production and oxidative damage in mitochondrial DNA (Pamplona and Barja, 2006). Olive oil and its phenolics are known as protective factors against oxidative stress (Kiritsakis, 1998; Nakbi *et al.*, 2010; Cicerale *et al.*, 2012).

Much evidence demonstrates that an aged genome is characterized by the global hypomethylation level, associated with a decrease in the activity of DNA methylation enzymes, as well as by specific histone modifications (Cencioni *et al.*, 2013). Thus, DNA methylation regulation plays a crucial role during aging processes. However, it is important to point out that epigenomic marks are not directional (both hyper- and hypomethylation are present at specific loci), are not uniform across the genome, and are quite variable between individuals of the same age (Issa, 2014). Studies on monozygotic twins, who share the same genotype, indicate that external environmental factors contribute to interindividual differences such as susceptibility to disease and potential to live longer (Li *et al.*, 2011a). Very recent studies indicated the multifactorial

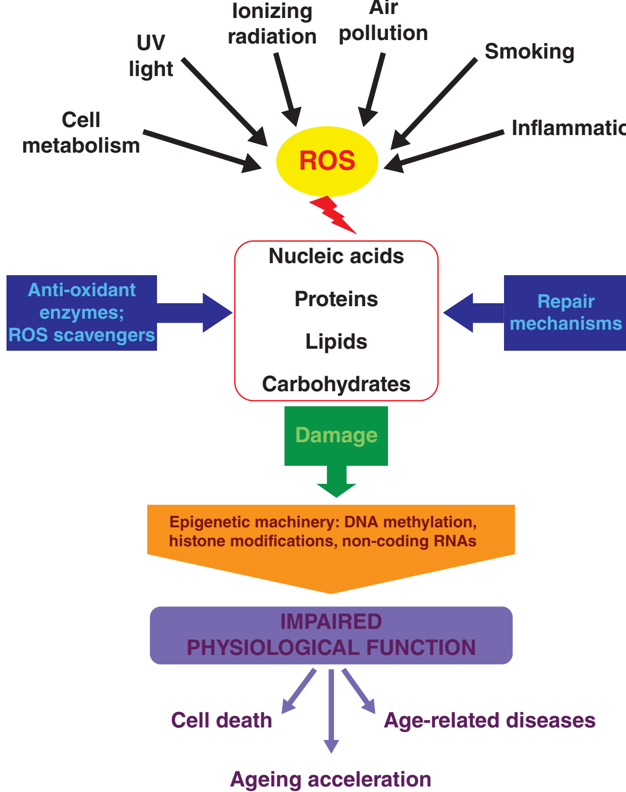

Figure 23.5 Oxidative stress, epigenetics, and aging. *Source*: MDPI (www.mdpi.org).

etiology of aging-associated diseases as related to both genetic and epigenetic changes in the genome (Cencioni *et al.*, 2013). Changes in DNA methylation patterns have been observed in association with cardiovascular diseases, inflammation, autoimmune diseases, infections, cancers, Alzheimer's disease, and brain malfunction (Bierne *et al.*, 2012; Gupta & Hawkins, 2015). Moreover, alterations in microRNA expression may be involved in the age-associated impairment of organ function often seen in elderly people, such as cardiac aging, which is characterized by cardiomyocyte cell death, hypertrophy, and fibrosis and regulated by microRNA alteration (Cencioni *et al.*, 2013).

The cardiovascular system is particularly sensitive to endogenous oxidative stress due to the high presence of mitochondria, whose metabolism represents the main source of free radicals. The oxidative balance is disturbed by the increased oxidative stress of mitochondria as well as the lipid peroxidation process that occur with aging (Preedy, 2014). The brain is highly vulnerable to oxidative stress due to its high oxygen consumption, its modest antioxidant defenses, and its lipid-rich constitution. The human brain utilizes 20% of oxygen consumed by the body, even though this organ constitutes only about 2% of the body weight. In the presence of oxidative stress, the lipid-rich constitution of the brain favors peroxidation, which results in a decrease in membrane fluidity and damage in membrane proteins, inactivating receptors, enzymes, and ion channels. As a result, oxidative stress can alter neurotransmission, neuronal function, and overall brain activity. Oxidative DNA damage is now accepted as one of the earliest deleterious changes in Alzheimer's pathogenesis. The influence of environmental factors regulated by epigenetic mechanisms in the development of Alzheimer's disease has been reported by many authors (Marquez & Outeiro, 2013; Lord & Cruchaga, 2014; Ho & Domann, 2015). Lungs are also exposed to endogenous and exogenous sources of oxidants, and in that sense the accumulation of ROS impairs the lung cells' function, resulting in posttranslational modification of histones as well as the chromatin remodeling enzymes (Cencioni *et al.*, 2013).

Cancer provides a dramatic example of the interaction between aging, epigenetic marks, and disease. Cancer cells are generally characterized by a high degree of aberrant DNA methylation (Esteller, 2005). In addition, approximately 70–80% of the DNA methylation abnormalities in cancer can be traced to aging defects (Li *et al.*, 2011a). Diet, as a major environmental factor, has a strong effect on health balance of the body, including aging-related changes. In Section 23.5 on epigenetics and human health, the beneficial role of the diet in the maintenance of human health balance was already described. Nevertheless, it is important to emphasize the beneficial role of the Mediterranean diet and regular olive oil consumption for prevention of age-related diseases. The Mediterranean diet is considered as clinically relevant for public health due to its preventive effects on major chronic diseases (Sofi *et al.*, 2008). Olive oil is the major lipid component in the Mediterranean diet, and its intake ranges from 25 to 50 mL daily (Corona *et al.*, 2009). Several studies have reported that oleocanthal has a protective effect on the development of the age-related disease, Alzheimer's disease, by altering the structure of neurotoxic proteins that contribute to disease development or by inhibiting tau fibrilization via reaction with the amino groups of tau proteins (Li *et al.*, 2009; Pitt *et al.*, 2009; Abuznait *et al.*, 2013). The treatment of old rats with oleuropein reduces the oxidative damage by increasing the antioxidant enzyme activities (Sarbishegi *et al.*, 2014). In addition, oleuropein, the major bioactive compound of *Olea europaea*, exhibits beneficial effects against neurodegeneration through preventive activity against the formation of toxic amyloid aggregation (Casamenti *et al.*, 2015). According to the results of a four-year follow-up study on cognitive functioning among the elderly population (6947 subjects, over 65 years old), carried out in Bordeaux, Montpellier, and Dijon, France, olive oil consumption is significantly associated with selective cognitive deficit and cognitive decline (Berr *et al.*, 2009). Intensive use of olive oil in the diet is associated with lower odds of cognitive deficit in visual memory and verbal fluency and decline in visual memory. In addition, an analysis of 704 participants in the population-based Italian Longitudinal Study on Aging (ILSA study) showed that high monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) intake was associated with better cognitive performance in an 8.5-year follow-up study (Solfrizzi *et al.*, 2006).

Concerning the relation between the diet and aging, the role of one additional factor, caloric restriction, was noticed. The effect of caloric restriction on aging was first defined in experimental animal models by McCay *et al.* (1935). They reported longer lifespan of rats fed a calorie-restricted diet in comparison with control rats fed a regular diet. Caloric restriction has also been shown to delay a wide range of aging-associated diseases, such as cancer, diabetes, atherosclerosis, cardiovascular diseases, and neurodegenerative disease (Heilbronn & Ravussin, 2003). Recent evidence suggests that the DNA methylation level in specific gene loci may be associated with the effect of a calorie-restricted diet and longevity (Vanden Berghe, 2012). In addition, there is a connection between the biological effects of caloric restriction and chromatin function (Vaquero & Reinberg, 2009). The Mediterranean diet, rich in natural bioactive compounds, has a long-term effect on biomarkers of aging and oxidative stress in overweight men (Esposito *et al.*, 2010).

23.7 Olive oil components as dietary epigenetic modulators

There are a large number of scientific papers on therapeutic effects of olive oil based on different types of research studies (*in vitro* and *in vivo* laboratory studies as well as various types of clinical trials). Continuous scientific and technological progress has offered new knowledge on the mechanisms of biological and therapeutic effects of olive oil constituents. Like all vegetable oils, olive oil consists of a saponifiable or acyglycerol fraction (98 and 99%) and a large spectrum of various minor components (more than 230 compounds) such as tocopherols, phenolic compounds, sterols, hydrocarbons, terpenic alcohols, pigments, and aromatic substances (Bulotta *et al.*, 2014). There is much scientific evidence about the beneficial effects of a diet rich in extra virgin olive oil on human health. Health effects of olive oil are attributed to its various components, namely, phenolic compounds, monounsaturated oleic acid, α-linoleic acid, and α-tocopherol (Kiritsakis, 1998). Compounds exhibiting antioxidant activity in olive oil are carotenoids (β-carotene and lutein), chlorophylls, hydrocarbons such as squalene, phenolic compounds (phenolic acids, tyrosol, hydroxytyrosol and derivatives, lignans, and flavonoids), and tocopherols.

The effect of olive oil on gene expression is reported in many studies. Significant change in the expression of genes involved in insulin metabolism after intake of olive oil has been reported by Konstantinidou *et al.* (2009). Oleuropein, the main olive oil polyphenol, plays an important role in regulating MDA (breast cancer)

cell metastasis by modulating the expression of genes involved in breast cancer progression (Hassan *et al.*, 2012). Olive leaf extract containing 16% oleuropein exerts beneficial effects against obesity by regulating the expression of genes involved in adipogenesis in mice fed a high-fat diet (Shen *et al.*, 2014). Supplementation with olive leaf polyphenols for 12 weeks improves glucose regulation through improvements in insulin sensitivity and pancreatic β-cell secretion capacity in a cohort of overweight middle-aged men (46 participants from New Zealand) (de Bock *et al.*, 2013). Components present in olive oil, ω-3 fatty acids and monounsaturated oleic acid, show a beneficial influence on genes (Tokunaga *et al.*, 2013). *In vivo* and *in vitro* studies have reported that a phenolic compound from olive oil, hydroxytyrosol, inhibits mammary tumor growth and proliferation rates by altering expression of genes related to apoptosis, cell cycle, proliferation, differentiation, and transformation pathways (Nan *et al.*, 2014). (−) Oleocanthal, a naturally occurring secoiridoid from extra virgin olive oil, exhibited *in vitro* (human breast cancer cell lines MDA-MB-231, MCF-7, and BT-474) and *in vivo* (mouse model) anticancer activity via G1 cell cycle arrest, apoptosis, and inhibition of the c-Met-dependent signaling pathway (Akl *et al.*, 2014). Furthermore, a randomized controlled trial showed that olive oil intake decreased plasma oxidative and inflammatory status and the gene expression related with inflammation and oxidative stress (Konstantinidou *et al.*, 2010). Inflammation influences cumulative epigenetic changes that finally result in cancer development. Furthermore, the antioxidant activity and free radical scavenging capacity of many dietary components are thought to be beneficial in blocking cancer initiation processes by enhancing the activity of antioxidant enzymes such as catalase, glutathione peroxidase, glutathione reductase, and superoxide dismutase (Ferguson *et al.*, 2015). Components from olive oil are well known as strong antioxidants and free radical scavengers (Pérez-Jiménez *et al.*, 2007; Boskou, 2009; Caramia *et al.*, 2012).

A new scientific challenge is to determine which adverse epigenomic marks in cancer inflammation are reversible or can be prevented by specific diet changes, natural phytochemicals, or lifestyle changes (Vanden Berghe, 2012). In that sense, the research on putative diet-derived inhibitors of DNA methyltransferase (DNMT); Class I, II, and IV HDAC; HAT; and Class III HDAC sirtuins (SIRT or silent information regulator), which modulate cancer inflammation, is of crucial interest. HDACs are zinc metalloproteins that need Zn^{2+} for their activity and are divided into four classes. Class III HDAC is zinc independent but nicotinamide adenine dinucleotide (NAD^+) dependent. Class I, II, and IV HDAC inhibitors contain the Zn^{2+} chelating group (Vanden Berghe, 2012).

Several studies have shown that polyphenols are able to modulate epigenetic mechanisms, including DNA methylation, histone modifications, and microRNA expression. A number of natural compounds have been identified as HDAC inhibitors (epigallocatechin galate or EGCG, curcumin, genistein, and quercetin), a HAT activator (genistein), HAT inhibitors (EGCG and curcumin), a SIRT activator (resveratrol), and a SIRT inhibitor (genistein) (Cuevas *et al.*, 2013). Polyphenols from olive oil, including flavonoids (quercetin, rutin, and luteolin) and nonflavonoids (oleuropein, hydroxytyrosol, verbascoside, and caffeic acid), exhibited *in vitro* inhibitory effects on Class I and II HDAC activity (Omar, 2010). These results showed that olive oil polyphenols could serve as promising protectors against epigenetic changes. Hydroxytyrosol can alter epigenetic mechanisms via changes in histone methylation, and it is considered a potential anticancer and chemopreventative agent (Nan *et al.*, 2014). Extra virgin olive oil *in vitro* (Caco-2 cells) and *in vivo* upregulates genes associated with human colon cancer via an epigenetic mechanism (modulation of DNA methylation as well as reduced expression of four microRNAs involved in pathogenesis of colorectal cancer) (Di Francesco *et al.*, 2015). The main tocopherol homolog found in olive oil and a strong antioxidant in lipid medium, α-tocopherol, downregulates the expression of microRNA 122 a (involved in lipid metabolism) and microRNA 125b (involved in inflammation) (Rimbach *et al.*, 2010). Besides α-tocopherol, olive oil contains tocotrienols, members of the vitamin E family found in a number of vegetable oils. Tocotrienols possess neuroprotective, antioxidant, anticancer, and cholesterol-lowering properties (Ahsan *et al.*, 2014). In addition, they may regulate microRNA expression in stroke-affected brain tissue (Park *et al.*, 2011). A tocotrienol-rich fraction prevented cellular senescence of human diploid fibroblasts via modulation of SA-miRNAs and target gene expression (Khee *et al.*, 2014).

It is important to point out that research on epigenetic changes influenced by dietary factors anticipates the stability of epigenetic changes over time. Although the stability of epigenetic changes over time has not yet been completely shown, there are strong indications that the methylation process at a wide range of gene loci is likely to be stable over long periods of time (11–20 years) (Turecki, 2014). In addition, it is shown that dietary components that affect energy metabolism or mitochondrial respiration can interfere

with epigenetic mechanisms upon changes in NAD^+ availability and SIRT (NAD^+-dependent histone deacetylase) activity (Vanden Berghe, 2012). It was demonstrated that olive oil and oleuropein optimized cardiac energy metabolism in obesity conditions in male Wistar rats (Ebaid *et al.*, 2010). Another study demonstrated that total body oxygen consumption was higher in rats fed with olive oil in comparison with other diet types (Rodriguez *et al.*, 2002).

Due to the plasticity of epigenetic changes, various dietary exposures during certain life periods are considered as crucial, including pregnancy, lactation, neonatal life, early life, and puberty. Epigenetic changes occurring during embryonic development will have a much greater influence on the global epigenetic status in the body because epigenetic changes can be transmitted over consecutive mitotic divisions. In that sense, alterations happening in a single embryonic stem cell will affect many more cells in comparison with those occurring during postnatal development (Vanden Berghe, 2012). Health effects of olive oil during pregnancy and the first year of life were shown in several studies. It was reported that olive oil, when used as the main source of oil for cooking and dressing salads during pregnancy, is associated with less (nearly 40%) wheezing of the offspring during the first year of life (Castro-Rodriguez *et al.*, 2010). Maternal hydroxytyrosol administration improved neurogenesis and cognitive function in prenatally stressed offspring rats (Zheng *et al.*, 2015). A maternal diet enriched with olive oil can regulate expression of genes involved in antioxidant and anti-inflammatory pathways and reduce the pro-oxidant and proinflammatory environment during rat early organogenesis (Higa *et al.*, 2014). An olive oil–enriched diet reduces brain oxidative damage during different periods of rats' lifetime (during pregnancy and lactation, and after weaning until pups' adulthood) (Pase *et al.*, 2015). Perinatal exposure to olive oil may have a protective effect against future development of mammary cancer in female offspring, whereas high-fat diets to pregnant and lactating rats, in particular corn oil, may be deleterious (Stark *et al.*, 2003). Oleuropein and olive oil have antiproliferative and antimetastatic effects in prostate cancer cell lines (LNCaP and DU145) and in breast cancer (Acquaviva *et al.*; 2012, Hassan *et al.*, 2012). Furthermore, (−) oleocanthal and virgin olive oil can have potential therapeutic use for the control of c-Met proto-oncogene in breast and prostate cancers (Elnagar *et al.*, 2011).

One of the important factors that may affect epigenetic mechanisms in the body is related to the role of gut microbiota. Technological progress has allowed new knowledge on the relation between human health and gut microflora. It has been estimated that the human gut contains approximately 1000 bacterial species and 100-fold more genes than are found in the human genome (Guinane & Cotter, 2013). Respecting such amazing data, it is easy to accept that gut microbiota are much more than only passive inhabitants inside the human body. In that sense, recent scientific findings suggested that disruption of the gut microbiota, the state known as dysbiosis, can be related to different pathological conditions such as obesity, diabetes type 2, chronic inflammatory diseases such as inflammatory bowel disease, ulcerative colitis and Crohn's disease, and colorectal cancer (Guinane & Cotter, 2013). The microbial communities are crucially involved in various physiological processes in the body, such as the synthesis of short-chain fatty acids, metabolism of nutrients and organic substrates, development of intestinal epithelium, protection against foreign pathogens, and maturation and development of the immune system (Flint *et al.*, 2007). Three intestinal genera are the most important – *Bacteroides*, *Bifidobacterium*, and *Eubacterium*. *Clostridium*, Enterobacteriaceae, and *Streptococcus* are also important but less numerous (Conlon & Bird, 2015). Gut microbiota produce multiple low-molecular-weight substances (proteins, peptides, polysaccharides, endotoxins, simple biochemical groups and compounds, various enzymes, and cofactors) that act as potential modulators of host epigenomic mechanisms (Shenderov, 2012). Diet, as one of the most important environmental factors, modulates the composition and activity of human gut microbiota. However, little is known regarding the effects of specific diets on microbiota composition. It is possible that dietary components that modulate gut microbiota composition and activity indirectly affect the epigenetic mechanisms in the human body. Studies on extra virgin olive oil's effects on gut microbiota showed its beneficial activity on mouse gut microbiota environment (Hidalgo *et al.*, 2014). In addition, the unsaponifiable fraction of extra virgin olive oil possesses anti-inflammatory activity and exerts preventative effects in murine models of inflammatory bowel disease (Cardeno *et al.*, 2014). It is considered that disturbance in gut microbiota composition may facilitate persistent bacterial infections that promote chronic inflammation, which finally may provoke cancer. The study on male ApcMin mice fed a diet high in olive oil showed a reduction of intestinal adenomas (Mai *et al.*, 2003). Furthermore, diet supplemented with olive oil modulated the composition of the intestinal microbiota in ApcMin mice toward a composition that might be more beneficial (Mai *et al.*, 2007).

Dietary polyphenols represent an important factor in the maintenance of gut microbiota balance due to their ability and the ability of their metabolites to inhibit harmful bacteria and stimulate beneficial bacteria. The antimicrobial activity of the main phenolics from olive oil, oleuropein, hydroxytyrosol, and tyrosol, is well documented (Tranter *et al.*, 1993; Bisignano *et al.*, 1999; Keceli & Robertson, 2002; Brenes *et al.*, 2007; Medina *et al.*, 2007; Romero *et al.*, 2007). Although an *in vitro* study by Romero *et al.* (2007) showed significant antimicrobial activity of olive oil polyphenols against *Helicobater pylori*, which is linked to a majority of peptic ulcers and to some types of gastric cancer, results of an intervention study showed moderate effectiveness of virgin olive oil in eradicating *H. pylori* (Castro *et al.*, 2012). Nevertheless, olive oil polyphenols may modulate human intestinal microflora toward a health-promoting condition for the host (Zampa *et al.*, 2006) and prevent epigenetic changes.

23.8 Conclusion

An unambiguously healthy diet such as the Mediterranean diet as well as food rich in natural bioactive components, such as virgin olive oil, are able to modulate epigenetic mechanisms in the human body. The effect of olive oil on epigenetic mechanisms is associated with its strong antioxidant, radical-scavenging, antimicrobial, anti-inflammatory, pro-apoptotic, and antiproliferative activity on cancer cells and/or with its ability to regulate gene expression. Although olive oil has been used as a functional food since ancient times, a huge level of scientific and technological progress will allow us to define the detailed mechanisms of its bioactive component in the maintenance of a healthy balance between the body and the environment.

References

Abuznait, A.H., Qosa, H., Busnena, B.A., El Sayed, K.A., & Kaddoumi, A. (2013) Olive oil derived oleocanthal enhances β-amyloid clearance as a potential neuroprotective mechanism against Alzheimer's disease: *in vitro* and *in vivo* studies. ACS Chemical Neuroscience 4, 973–982.

Acquaviva, R., Di Giacomo, C., Sorrenti, V., Galvano, F., Santangelo, R., Cardile, V., Gangia, S., D'Orazio, N., Abraham, N.G., & Vanella, L. (2012) Antiproliferative effect of oleuropein in prostate cell lines. International Journal of Oncology 41, 31–38.

Afzal, M, Safer, A.M., & Menon, M. (2015) Green tea polyphenols and their potential role in health and disease. Inflammopharmacology 23, 151–161.

Aguilera, O., Fernandez, A.F., Muñoz, A., & Fraga, M.F. (2010) Epigenetics and environment: a complex relationship. Journal of Applied Physiology 109, 243–251.

Ahsan, H., Ahad, A., Iqbal, J., & Siddiqui, W.A. (2014) Pharmacological potential of tocotrienols: a review. Nutrition & Metabolism 11, 52. DOI:10.1186/1743–7075

Akl, M.R., Ayoub, N.M., Mohyeldin, M.M., Busnena, B.A., Foudah, A.I., Liu, Y.Y., & Sayed, K.A. (2014) Olive phenolics as c-Met inhibitors: (−) oleocanthal attenuates cell proliferation, invasiveness, and tumor growth in breast cancer models. PLoS One 21,9, e97622.

Allis, C.D., Jenuwein, T., & Reinberg, D. (2007) Epigenetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 23–62.

Anderson, O.S., Sant, K.E., & Dolinoy, D.C. (2012) Nutrition and epigenetics: an interplay of dietary methyl donors, one-carbon metabolism and DNA methylation. Journal of Nutritional Biochemistry 23, 853–859.

Arai, J.A., Shaomin, L., Hartley, D.M., & Feig, L.A. (2012) Transgenerational rescue of a genetic defect in long-term potentiation and memory formation by juvenile enrichment. Journal of Neuroscience 29, 1496–1502.

Arts, I.C., & Hollman, P.C. (2005) Polyphenols and disease risk in epidemiologic studies. American Journal of Clinical Nutrition 81, 317–325.

Barja, G. (2004) Free radicals and aging. Trends in Neuroscience 27, 595–600.

Bartova, E., Krejci, J., Harnicarova, A., Galiova, G., & Kozubek, S. (2008) Histone modifications and nuclear architecture: a review. Journal of Histochemistry & Cytochemistry 56, 711–721.

Berger, S.L., Kouzarides, T., Shiekhattar, R., & Silatifard, A. (2009) An operational definition of epigenetics. Genes & Development 23, 781–783.

Bernal, A.J., Dolinoy, D.C., Huang, D., Skaar, D.A., Weinhouse, C., & Jirtle, R.L. (2013) Adaptive radiation-induced epigenetic alterations mitigated by antioxidants. FASEB Journal 27, 665–671.

Berr, C., Portet, F., Carriere, I., Akbaraly, T., Feart, C., Gourlet, V., Combe, N., Barberger-Gateau, P., & Ritchie, K. (2009) Olive oil and cognition: results from the three-city study. Dementia and Geriatric Cognitive Disorders 28, 357–364.

Bierne, H., Hamon, M., & Cossart, P. (2012) Epigenetics and bacterial infections. Cold Spring Harbor Perspectives in Medicine 2, a010272.

Bisignano, G., Tomaino, A., Lo Cascio, R., Crisafi, G., Uccele, N., & Saija, A. (1999) On the in-vitro antimicrobial activity of oleuropein and hydroxytyrosol. Journal of Pharmacy and Pharmacology 51, 971–974.

Blackmore, H.L., Piekarz, A.V., Fernandez-Twinn, D.S, Mercer, J.R., Figg, N., Bennett, M., & Ozanne, S.E. (2012) Poor maternal nutrition programmes a pro-atherosclerotic phenotype in $ApoE^{-/-}$ mice. Clinical Science 123, 251–257.

Boskou, D. (2009) Olive oil and health. CRC Press, Taylor & Francis Group, Boca Raton, FL, 11–45.

Brenes, M., Medina, E., Romero, C., & De Catro, A. (2007) Antimicrobial activity of olive oil. Agro FOOD Industry High Tech 4, 6–8.

Brown, A.S., & Susser, E.S. (2008) Prenatal nutritional deficiency and risk of adult schizophrenia. Schizophrenia Bulletin 34, 1054–1063.

Budhavarapu, V.N., Chavez, M., & Tyler, J.K. (2013) How is epigenetic information maintained through DNA replication? Epigenetics and Chromatin 6, 1–7.

Bulotta, S., Celano, M., Lepore, S.M., Montalcini, T., Puija, A., & Russo, D. (2014) Beneficial effects of the olive oil phenolics components oleuropein and hydroxytyrosol: focus on protection against cardiovascular and metabolic diseases. Journal of Translational Medicine 219, 2–9.

Calder, P., Prescott, S., & Caplan. M. (2006) Scientific review: the role of nutrients in immune function of infants and young children. Mead Johnson Nutrition. Available from https://www.meadjohnson.com/pediatrics/us-en/clinical-support/lcpufas-and-the-immune-system

Caramia, G., Gori, A., Valli, E., & Cerretani, L. (2012) Virgin olive oil in preventive medicine: From legends to epigenetics. European Journal of Lipid Science and Technology 114, 375–388.

Cardeno, A., Sanchez-Hidalgo, M., Aparicio-Soto, M., & Alarcón-de-la-Lastra, C. (2014) Unsaponifiable fraction from extra virgin olive oil inhibits the inflammatory response in LPS-activated murine macrophages. Food Chemistry 147, 117–123.

Casamenti, F., Grossi, C., Rigacci, S., Pantano, D., Luccarini, I., & Stefani, M. (2015) Oleuropein agylcone: a possible drug against degenerative conditions. In vivo evidence of its effectiveness against Alzheimer's disease. Journal of Alzheimer's Disease 45, 679–688.

Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harrington, H., McClay, J., Martin, J., Braithwaite, A., & Poulton, R. (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301, 386–389.

Castro, M., Romero, C., de Castro, V., Vargas, J., Medina, E., Millán, R., & Brenes, M. (2012) Assessment of *Helicobacter pylori* eradication by virgin olive oil. Helicobacter 17, 305–311.

Castro-Rodriguez, J.A., Garcia-Marcos, L., Sanhez-Solis, M., Pérez-Fernández, V., Martinez-Torres, A., & Mallol, H. (2010) Olive oil during pregnancy is associated with reduced wheezing during the first life of the offspring. Pediatric Pulmonology 45, 395–402.

Cencioni, C., Spallotta, F., Martelli, F., Valente, S., Mai, A., Zeiher, A.M., & Gaetano, C. (2013) Oxidative stress and epigenetic regulation in ageing and age-related diseases. International Journal of Molecular Sciences 14, 17643–17663.

Cheng, T.F., Choudhuri, S., & Muldoon-Jacobs, K. (2012) Epigenetic targets of some toxicologically relevant metals: a review of the literature. Journal of Applied Toxicology 32, 643–653.

Choi, S.W., & Friso, S. (2009) Nutrients and epigenetics. CRC Press, Taylor & Francis Group, Boca Raton, FL, 7–37.

Choi, S.W., & Friso, S. (2010) Epigenetics: a new bridge between nutrition and health. Advances in Nutrition 1, 8–16.

Chuang, J.C., & Jones, P.A. (2007) Epigenetics and microRNAs. Pediatric Research 61, 24–29.

Cicerale, S., Lucas, L.J., & Keast, R.S.J. (2012) Oleocanthal: a naturally occurring anti-inflammatory agent in virgin olive oil. In: Olive oil: constituents, quality, health properties and bioconversions, ed. D. Boskou. InTech, Rijeka, Croatia.

Collins, F.S., Green, E.D., Guttmacher, A.E., & Guyer, M. (2003) A vision for the future of genomics research. Nature 422, 835–847.

Conlon, M.C., & Bird, A.R. (2015) The impact of diet and lifestyle on gut microbiota and human health. Nutrients 7, 17–44.

Corona, G., Spencer, J.P.E., & Dessi, M.A. (2009) Extra virgin olive oil phenolics: absorption, metabolism, and biological activities in GI tract. Toxicology and Industrial Health 25, 285–293.

Crider, K.S., Yang, T.P., Berry, R.J., & Bailey, L.B. (2012) Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. Advances in Nutrition 3, 21–38.

Cuevas, A., Saavedra, N., Salazar, L.A., & Abdalla, D.S. (2013) Modulation of immune function by polyphenols: possible contribution of epigenetic factors. Nutrients 5, 2314–2332.

de Bock, M., Derraik, J.G.B., Brennan, C.M., Biggs, J.B., Morgan, P.E., Hodgkinson, S.C., Hofman, P.L., & Cutfield, W.S. (2013) Olive (*Olea europaea* L.) leaf polyphenols improve insulin sensitivity in middle-aged overweight men: a randomized, placebo-controlled, crossover trial. PLoS One 3, 1–8, e57622.

de Grey, A.D.N.J. (1999) The mitochondrial free radical theory of aging. RGL Landes Company, Austin, TX, 53–68.

Devereux, G., Litonjua, A.A., Turner, S.T., Craig, L.C.A., McNeill, G., Martindale, S., Helms, P., Seaton, A., & Weiss, S.T. (2007) Maternal vitamin D intake during pregnancy and early childhood wheeze. American Journal of Clinical Nutrition 85, 853–859.

Dias, B.G., & Ressler, K.J. (2014) Parental olfactory experience influences behaviour and neural structure in subsequent generations. Nature Neuroscience 17, 89–96.

Di Francesco, A., Falconi, A. Di Germanio, C., Micioni Di Bonaventura, M.V., Costa, A., Caramuta, S., Del Carlo, M., Compagnone, D., Dainese, E., Cifani, C., Maccarrone, M., & D'Addario, C. (2015) Extra virgin olive oil up-regulates CB1 tumor suppressor gene in human colon cancer cells and in rat colon via epigenetic mechanims. Journal of Nutritional Biochemistry 26, 250–258.

Ebaid, G.M., Seiva, F.R., Rocha, K.K., Souza, G.A., & Novelli, E.L. (2010) Effects of olive oil and its minor phenolic constituents on obesity induced cardiac metabolic changes. Nutrition Journal 9, 46. Available from http://www.nutritionj.com/content

Elnagar, A., Sylvester, P.W., & El Sayed, K.A. (2011) (−) Oleocanthal as a c-Met inhibitor for the control of metastatic breast and prostate cancers. Planta Medica 77, 1013–1019.

Esposito, K., Di Palo, C., Maiorino, M.I., Petrizzo, M., Bellastella, G., Siniscalchi, I., & Giugliano, D. (2010) Long-term effect of Mediterranean-style diet and calorie restriction on biomarkers of longevity and oxidative stress in overweight men. Cardiology Research and Practice 2011, 293916.

Esteller, M. (2005) Aberrant DNA methylation as a cancer-inducing mechanism. Annual Review of Pharmacology and Toxicology 45, 629–656.

Fabiani, R., De Bartolomeo, A., Rosignoli, P., Servilli, M., Montedoro, G.F., & Morozzi, G. (2002) Cancer chemoprevention by hydroxytyrosol isolated from virgin olive oil through G1 cell cycle arrest and apoptosis. European Journal of Cancer Prevention 11, 351–358.

Fang, M., Chen, D., & Yang, C.S. (2007) Dietary polyphenols may affect DNA methylation. Journal of Nutrition 137, 223–228.

Ferguson, L.R., Chen, H., Collins, A.R., Connell, M., Damia, G., Dasgupta, S., Malhotra, M., Meeker, A.K., Amedei, A., Amin, A., Ashraf, S.S., Aquilano, K., Azmi, A.S., Bhakta, D., Bilsland, A., Boosani, C.S., Chen, S., Ciriolo, M.R., Fujii, H., Guha, G., Halicka, D., Helferich, W.G., Keith, W.N., Mohammed, S.I., Niccolai, E., Yang, X., Honoki, K., Parslow, V.R., Prakash, S., Rezazadeh, S., Shackelford, R.E., Sidransky, D., Tran, P.T., Yang, E.S., & Maxwell, C.A. (2015) Genomic instability in human cancer: molecular insights and opportunities for therapeutic attack and prevention through diet and nutrition. Seminars in Cancer Biology 35 (suppl.), S5–S34. doi:10.1016/j.semcancer.2015.03.005

Flint, H.J., Duncan, S.H., Scott, K.P., & Louis, P. (2007) Interactions and competition within the microbial community of the human colon: links between diet and health. Environmental Microbiology 9, 1101–1111.

Florean, C. (2014) Food that shapes you: how diet can change your epigenome. Science in School 28, http://www.scienceinschool.org/2014/issue28/epigenetics

Franklin, T. B., Russig, H., Weiss, I.C., Gräff, J., Linder, N., Michalon, A., Vizi, S., & Mansuy, I.M. (2010) Epigenetic transmission of the impact of early stress across generations. Biological Psychiatry 68, 408–415.

Ghosh, C., Baker, J.A., Moysich, K.B., Rivera, R., Brasure, J.R., & McCann, S.E. (2008) Dietary intakes of selected nutrients and food groups and risk of cervical cancer. Nutrition and Cancer 60, 331–341.

Godfrey, K.M., & Barker, D.J. (2000) Fetal nutrition and adult disease. American Journal of Clinical Nutrition 71, 1344–1352.

Ghorbel, I., Khemakhem, M., Boudawara, O., Marrekchi, R., Jamoussi, K., Ben Amar, R., Boudawara, T., Zeghal, N., & Kamoun, N.G. (2016) Effects of dietary extra virgin olive oil and its fractions on antioxidants status and DNA damage in the heart of rats co-exposed to aluminium and acrylamide. Food & Function, 23, 19397–19408.

Guinane, M.C., & Cotter, P.D. (2013) Role of the gut microbiota in health and chronic gastrointestinal disease: understanding a hidden metabolic organ. Therapeutic Advances in Gastroenterology 6, 295–308.

Guo, Y., Lambert, G.H., Hsu, C.C., & Hsu, M.M. (2004) Yucheng: health effects of prenatal exposure to polychlorinated biphenyls and dibenzofurans. International Archives of Occupational and Environmental Health 77, 153–158.

Gupta, B., & Hawkins, R.D. (2015) Epigenomics and autoimmune diseases. Immunology and Cell Biology 93, 271–276.

Haig, D. (2004) The (dual) origin of epigenetics. Cold Spring Harbor Symposia on Quantitative Biology 69, 67–70.

Hales, C.N., & Barker, D.J.P. (1992) Type 2 (non-insulin-dependent) *Diabetes mellitus*: the thrifty phenotype hypothesis. Diabetologia 35, 595–601.

Harman, D. (1972) A biological clock: the mitochondria? Journal of the American Geriatrics Society 20, 145–147.

Hassan, Z.K., Elamin, M.H., Daghestani, M.H., Omer, S.A., Al-Olayan, E.M., Elobeid, M.A., Virk, P., & Mohammed, O.B. (2012) Oleuropein induces anti-metastatic effects in breast cancer. Asian Pacific Journal of Cancer Prevention 13, 4555–4559.

Heilbronn, L.K., & Ravussin, E. (2003) Calorie restriction and aging: review of the literature and implications for studies in humans. American Journal of Clinical Nutrition 78, 361–369.

Herranz, M., & Esteller, M. (2007) DNA methylation and histone modifications in patients with cancer potential prognostic and their therapeutic targets. Methods in Molecular Biology 361, 25–62.

Hidalgo, M., Prieto, I., Abriouel, H., Cobo, A., Benomar, N., Gavlez, A., & Martinez-Cañamero, M. (2014) Effect of virgin and refined olive oil consumption on gut microbiota: comparison to butter. Food Research International 64, 553–559.

Higa, R., Roberti, S.L., Musikant, D., Mazzucco, M.B., White, V., & Jawerbaum, A. (2014) Effects of maternal dietary olive oil on pathways involved in diabetic embryopathy. Reproductive Toxicology 49C, 185–195.

Ho, E., Clarke, J.D., & Dashwood, R.H. (2009) Dietary sulforaphane, a histone deacetylase inhibitor for cancer prevention. Journal of Nutrition 139, 2393–2396.

Ho, E., & Domann, H. (2015) Nutrition and epigenetics. CRC Press, Taylor & Francis Group, Boca Raton, FL, 3–53.

Issa, J.P. (2014) Aging and epigenetic drift: a vicious cycle. Journal of Clinical Investigation 124, 24–29.

Jablonka, E., & Lamb, M.J. (2002) The changing concept of epigenetics. Annals of the New York Academy of Sciences 981, 82–96.

Jin, K. (2010) Modern biological theories of aging. Aging and Disease 1, 72–74.

Jones, R.G., & Thompson, C.B. (2009) Tumor suppressor and cell metabolism: a recipe for cancer growth. Genes & Development 23, 537–548.

Kaati, G., Gygren, L.O., & Edvinsson, S. (2002) Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. European Journal of Human Genetics 10, 682–688.

Kala, R., Peek, G.W., Hardy, T.M., & Tollefsbol, T.O. (2013) MicroRNAs: an emerging science in cancer epigenetics. Journal of Clinical Bioinformatics 3, 1–8.

Kanherkar, R.R., Bathia-Dey, N., & Csoka, A.B. (2014) Epigenetics across the human lifespan. Frontiers in Cell and Developmental Biology 2, 1–19.

Keceli, T., & Robinson, R.K. (2002) Antimicrobial activity of phenolic extracts from virgin olive oil. Milchwissenschaft 57, 436–440.

Keller, E.F. (2011) Genes, genomes and genomics. Biological Theory 6, 132–140.

Kerek, R., Geoffroy, A., Bison, A., Martin, N., Akchiche, N., Pourié, G., Helle, D., Guéant, L., Bossenmeyer-Pourié, C., & Daval, J.L. (2013) Early methyl donor deficiency may induce persistent brain defects by reducing Stat3 signalling targeted by miR-124. Cell Death and Disease 4, e755, 1–11.

Khee, S.G., Yusof, Y.A., & Makpol, S. (2014) Expression of senescence-associated microRNAs and target genes in cellular aging and modulation by tocotrienol-rich fraction. Oxidative Medicine and Cellular Longevity 2014, 725929.

Kim, J.K., Samaranayake, M., & Pradhan, S. (2009) Epigenetic mechanisms in mammals. Cellular and Molecular Life Sciences 66, 596–612.

Kiritsakis, A.K. (1998) Olive oil: from the tree to the table, 2nd ed. Food and Nutrition Press, Trumbull, CT.

Konstantinidou, V., Covas, M.I., Muñoz-Aguayo, D., Khymenets, O., de la Torre, R., Saet, G., Tormos Mdel, C., Toledo, E., Marti, A., Ruiz-Gutierrez, V., Ruiz Mendez, M.V., & Fito, M. (2010) In vivo nutrigenomics effects of virgin olive oil polyphenols within the frame of the Mediterranean diet: a randomized controlled trial. FASEB Journal 24, 2456–2557.

Konstantinidou, V., Khymenets, O., Covas, M.I., de la Torre, R., Muñoz-Aguayo, D., Anglada, R., Farré, M., & Fito, M. (2009) Time course of changes in the expression of insulin sensitivity-related genes alter an acute load virgin olive oil. OMICS 13, 431–438.

Kovalchuk, I., & Kovalchuk, O. (2012) Epigenetics in health and disease. Pearson Education, Upper Saddle River, NJ, 1–19.

Lewis, B.P., Burge, C.B., & Bartel, D.P. (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120, 15–20.

Li, W., Sperry, J.B., Crowe, A., Trojanowski, J.Q., Smith, A.B., & Lee, V.M.Y. (2009) Inhibition of tau fibrilization by oleocanthal via reaction with the amino groups of tau. Journal of Neurochemistry 110, 1339–1351.

Li, Y., Daniel, M., & Tollefsbol, T.O. (2011a) Epigenetic regulation of caloric restriction in aging. BMC Medicine 9:98, 1–12.

Li, Z.Y., Na, H.M., Peng, G., Pu, J., & Liu, P. (2011b) Alteration of microRNA expression correlates to fatty acid-meditated insulin resistance in mouse myoblasts. Molecular BioSystems 7, 871–877.

Litonjua, A.A. (2009) Childhood asthma may be a consequence of vitamin D deficiency. Current Opinion in Allergy and Clinical Immunology 9, 202–207.

Lord, J., & Cruchaga, C. (2014) The epigenetic landscape of Alzheimer's disease. Nature Neuroscience 17, 1138–1140.

Lu, H., Liu, X., Deng, Y., & Qing, H. (2013) DNA methylation, a hand behind neurodegenerative diseases. Frontiers in Aging Neuroscience 5, 1–16.

Luceri, C., Bigagli, E., Pitozzi, V., Grossi, C., Casamenti, F., & Giovannelli, L. (2013) A nutrigenomics approach for the study of anti-aging interventions: olive oil phenols and the modulation of microRNA profiles in mouse brain. 36 Congresso Nazionale della Societa Italiana di Farmacologia, Torino, 23–26 October, poster P-XV.

Mahmoudi, A., Ghorbel, H., Bouallegui, Z., Marrekchi, R., Isoda, H., & Sayadi, S. (2015) Oleuropein and hydroxytyrosol protect from bisphenol A effects in livers and kidneys of lactating mother rats and their pups. Experimental and Toxicologic Pathology 67, 413–425.

Mai, V., Colbert, L.H., Berrigan, D., Perkins, S.N., Pfeiffer, R., Lavigne, J.A., Lanza, E., Haines, D.C., Schatzkin, A., & Hursting, S.D. (2003) Calorie restriction and diet composition modulate spontaneous intestinal tumorigenesis in Apc(Min) mice through different mechanisms. Cancer Research 63, 1752–1755.

Mai, V., Colbert, L.H., Perkins, S.N., Schatzkin, A., & Hursting, H.D. (2007) Intestinal microbiota: a potential diet-responsive prevention target in APC^{Min} Mice. Molecular Carcinogenesis 46, 42–48.

Marques, S., & Outeiro, T.F. (2013) Epigenetics in Parkinson's and Alzheimer's diseases. Subcellular Biochemistry 61, 507–525.

McCay, C.M., Crowell, M.F., & Maynard, L.A. (1935) The effect of retarded growth upon the length of life span and upon the ultimate body size. Nutrition 5, 155–171.

McGowan, P.O., Sasaki, A., D'Alessio, A.C., Dymov, S., Labonté, B., Szyf, M., Turecki, G., & Meaney, M.J. (2009) Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nature Neuroscience 12, 342–348.

McGowan, P.O., Suderman, M., Sasaki, A., Huang, T.C.T., Hallett, M., Meaney, M.J., & Szyf, M. (2011) Broad epigenetic signature of maternal care in the brain of adult rats. PLoS One 6(2), e14739.

Medina, E., Romero, C., Brenes, M., & de Castro, A. (2007) Antimicrobial activity of olive oil, vinegar and various beverages against foodborne pathogens. Journal of Food Protection 70, 1194–1199.

Meloni, M. (2014) The social brain meets the reactive genome: neuroscience, epigenetics and the new social biology. Frontiers in Human Neuroscience 8, 1–12.

Mill, J., Tang, T., Kaminsky, Z., Khare, T., Yazdanpanah, S., & Bouchard, L., Jia, P., Assadzadeh, A., Flanagan, J., Schumacher, A., Wang, S.C., & Petronis, A. (2008) Epigenomic profiling reveals DNA-methylation changes associated with major psychosis. American Journal of Human Genetics 82, 696–711.

Monk, M. (1995) Epigenetic programming of differential gene expression in development and evolution. Developmental Genetics 17, 188–197.

Montedoro, G., & Servilli, M. (1993) Simple and hydrolysable compounds in virgin olive oil. Spectroscopic characterizations of the secoiridoid derivatives. Journal of Agricultural and Food Chemistry 41, 2228–2234.

Moore, L.D., Le, T., & Fan, G. (2013) DNA methylation and its basic function. Neurophysopharmacology Reviews 38, 23–38.

Morimoto, T., Sungawa, Y., Kawamura, T., Takaya, T., Wada, H., Nagasawa, A., Komeda, M., Fujita, M., Shimatsu, A., Kita, T., & Hasegawa, K. (2008) The dietary compound curcumin inhibits p300 histone acetyltransferase activity and prevents heart failure in rats. Journal of Clinical Investigation 118, 868–878.

Nakbi, A., Tayeb, W., Grissa, A., Issaoui, M., Dabbou, S., Chargui, I., Ellouz, M., Miled, A., & Hammami, M. (2010) Effects of olive oil and its fractions on oxidative stress and the liver's fatty acid composition in 2,4-dichlorophenoxyacetic acid-treated rats. Nutrition & Metabolism 80, 1–11.

Nan, J.N., Ververis, K., Bollu, S., Rodd, A.L., Swarup, O., & Karagiannis, T.C. (2014) Biological effects of the olive polyphenol, hydroxytyrosol: An extra view from genome-wide transcriptome analysis. Hellenic Journal of Nuclear Medicine 17, 62–69.

Nanney, D.L. (1958) Epigenetic control system. Proceedings of the National Academy of Sciences of the USA 44, 712–717.

Nian, H., Delage, B., Ho, E., & Dashwood, R.H. (2009) Modulation of histone deacetylase activity by dietary isothiocyanates and allyl sulphides: studies with sulforaphane and garlic organosulfur compounds. Environmental and Molecular Mutagenesis 50, 213–221.

Niller, H.H., Banati, F., Nagy, K., Buzas, K., & Minarovits, J. (2013) Update on microbe-induced changes: bacterial effectors and viral oncoproteins as epigenetic dysregulators. Future Virology 8, 1111–1126.

Northstone, K., Golding, J., Smith, G.D., Miller, L.L., & Pembrey, M. (2014) Prepubertal start of father's smoking and increased body fat in his sons: further characterization of paternal transgenerational responses. European Journal of Human Genetics 22, 1382–1386.

Omar, S.H. (2010) Oleuropein in olive and its pharmacological effects. Scientia Pharmaceutica 78, 133–154.

O'Neill, R.J., Vrana, P.B., & Rosenfeled, C.S. (2014) Maternal methyl supplemented diets and effects on offspring health. Frontiers in Genetics 5(89), 1–10.

Pamplona, R., & Barja, G. (2006) Mitochondrial oxidative stress, aging and caloric restriction: the protein and methionine connection. Biochimica and Biophysica Acta (BBA) – Bioenergetics 1757, 496–508.

Papoutsis, A., Lamore, S.D., Wondrak, G.T., Selmin, O.I., & Romagnolo, D.F. (2010) Resveratrol prevents epigenetic silencing of BRCA-1 by the aromatic hydrocarbon receptor in human breast cancer cells. Journal of Nutrition 140, 1607–1614.

Pareja-Galeano, H., Sanchis-Gomar, F., & Garcia-Gimenez, J.L. (2014) Physical exercise and epigenetic modulation: elucidating intricate mechanisms. Sports Medicine 44, 429–436.

Park, H.A., Kubicki, N., Gnyawali, S., Chan, Y.C., Roy, S., Khanna, S., & Sen, C.K. (2011) Natural vitamin E α-tocopherol protects against ischemic stroke by induction of multidrug resistance-associated protein 1. Stroke 42, 2308–2314.

Parkinson, L., & Keast, R. (2014) Oleocanthal, a phenolic derived from virgin olive oil: a review of the beneficial effects on inflammatory disease. International Journal of Molecular Sciences 15, 12323–12334.

Pase, C.S., Teixeira, A.M., Roverski, K., Dias, V.T., Calabrese, F., Molteni, R., Franchi, S., Panerai, A.E., Riva, M.A., & Burger, M.E. (2015) Olive oil-enriched diet reduces brain oxidative damages and ameliorates neurotrophic factor gene expression in different life stages of rats. Journal of Nutritional Biochemistry. DOI:http://dx.doi.org/10.1016/j.jnutbio.2015.05.013.

Peltonen, L., & McKusick, V.A. (2001) Genomics and medicine: dissecting human disease in the postgenomic era. Science 291, 1224–1229.

Pérez-Jiménez, F., Lista, J.D., Pérez-Martínez, P., López-Segura, F., Fuentes, F., Cortés, B., Lozano, A., & López-Miranda, J. (2007) Olive oil and haemostasis: a review on its healthy effects. Public Health Nutrition 9, 1083–1088.

Pingsheng, W. (2012) Maternal smoking during pregnancy and its effect on childhood asthma: understanding the puzzle. American Journal of Respiratory and Critical Care Medicine 186, 941–942.

Pitt, J., Roth, W., Lacor, P., Smith, A.B., Blankeship, M., Velasco, P., De Felice, F., Breslin, P., & Klein, W.L. (2009) Alzheimer's-associated Abeta oligomers show altered structure, immunoreactivity and synaptotoxicity with low doses of oleocanthal. Toxicology and Applied Pharmacology 240, 189–197.

Preedy, V.R. (2014) Aging: oxidative stress and dietary antioxidants. Academic Press, Elsevier, Amsterdam, 3–14.

Ptak, C., & Petronis, A. (2010) Epigenetic approaches to psychiatric disorders. Dialogues in Clinical Neuroscience 12, 25–35.

Rajendran, P., Ho, E., Williams, D.E., & Dashwood, R.H. (2011) Dietary phytochemicals, HDAC inhibition, and DNA damage/repair defects in cancer cells. Clinical Epigenetics 3, 1–23.

Ravin, D. (2008) Epigenetics and human disease. Australian Biochemist 39, 1–22.

Rennoll-Bankert, K.E., & Dumler, J.S. (2012) Lessons from *Anaplasma phagocytophilum:* chromatin remodeling by bacterial effectors. Infectious Disorders Drug Targets 12, 380–387.

Rimbach, G., Moehring. J., Huebbe, P., & Lodge, J.K. (2010) Gene-regulatory activity of α-tocopherol. Molecules 15, 1746–1761.

Robertson, K.D. (2005) DNA methylation and human disease. Nature Reviews: Genetics 6, 597–610.

Rodriguez, V.M., Portillo, M.P, Piċo, C., Macarulla, M.T., & Palou, A. (2002) Olive oil feeding up-regulates uncoupling protein genes in rat brown adipose tissue and skeletal muscle. American Journal of Clinical Nutrition 75, 213–220.

Romero, C., Medina, E., Vargas, J. Brenes, M., & De Castro, A. (2007) In vitro activity of olive oil polyphenols against *Helicobacter pylori*. Journal of Agricultural and Food Chemistry 55, 680–686.

Roth, T.L., Lubin, F.D., Funk, A.J, & Sweatt, J.D. (2009) Lasting epigenetic influence of early-life adversity on the BDNF gene. Biological Psychiatry 65, 760–769.

Saetrom, P., Snøve, O.J., & Rossi, J.J. (2007) Epigenetics and microRNAs. Pediatric Research 61, 17–23.

Sarbishegi, M., Mehraein, F., & Soleimani, M. (2014) Antioxidant role of oleuropein on midbrain and dopaminergic neurons of substantia nigra in aged rats. Iranian Biomedical Journal 18, 16–22.

Scalbert, A., Johnson, I.T., & Saltmarsh, M. (2005) Polyphenols: antioxidants and beyond. American Journal of Clinical Nutrition 81 25–217.

Shen, Y., Song, S.J., Keum, N., & Park, T. (2014) Olive leaf extract attenuates obesity in high-fat-diet-fed mice by modulating the expression of molecules involved in adipogenesis and thermogenesis. Evidence-Based Complementary and Alternative Medicine 2014:971890.

Shenderov, B.A. (2012) Gut indigenous microbiota and epigenetics. Microbial Ecology in Health and Disease 23, 1–6.

Sies, H. (1985) Oxidative stress. Academic Press, London.

Sies, H. (2015) Oxidative stress: a concept in redox biology and medicine. Redox Biology 4, 180–183.

Smith, R., & Mill, J. (2011) Epigenetics and chronic diseases: an overview. In: Epigenetic aspects of chronic diseases, ed. H.I. Roach, F. Bronner, & R.O.C. Oreffo. Springer, Berlin, 1–20.

Sofi, F. Cesari, F., Abbate, R., Gensini, G.F., & Casini, A. (2008) Adherence to Mediterranean diet and health status: meta-analysis. BMJ 337, 1–7.

Solfrizzi, V., Colacicco, A.M., D'Introno, A., Capurso, C., Torres, F., Rizzo, C., Capurso, A., & Panza, F. (2006) Dietary intake of unsaturated fatty acids and age-related cognitive decline: a 8.5-year follow-up of the Italian Longitudinal Study on Aging. Neurobiology of Aging 27, 1694–1704.

Stark, A.H., Kossoy, G., Zusman, I., Yarden, G., & Madar, Z. (2003) Olive oil consumption during pregnancy and lactation in rats influences mammary cancer development in female offspring. Nutrition and Cancer 46, 59–65.

Stuckler, D. (2008) Population causes and consequences of leading chronic diseases: a comparative analysis of prevailing explanations. Milbank Quarterly 86, 273–326.

Tawa, R., Kimura, Y., Komura, J., Miyamura, Y., Kurishita, A., Sasaki, M.S., Sakurai, H., & Ono, T. (1998) Effects of x-ray irradiation on genomic DNA methylation levels in mouse tissues. Journal of Radiation Research 39: 271–278.

Tokunaga, M., Takahashi, T., Singh, R.B., De Meester, F., & Wilson, D.W. (2013) Nutrition and epigenetics. Medical Epigenetics 1, 70–77.

Tranter, H.S., Tassou, S.C., & Nychas, G.J. (1993) The effect of olive oil phenolic compound, oleuropein, on growth and enterotoxin B production by *Staphylococcus aureus*. Journal of Applied Microbiology 74, 253–259.

Tunca, B., Tezcan, G., Cecener, G., Egeli, U., Ak, S., Malyer, H., Tumen, G., & Bilir, A. (2012) *Olea europaea* leaf extract alters microRNA expression in human glioblastoma cells. Journal of Cancer Research in Clinical Oncology 138, 1831–1844.

Turecki, G. (2014) The molecular bases of the suicidal brain. Nature Reviews Neuroscience 15, 802–816.

Turkez, H., Togar, B., & Polat, E. (2012) Olive leaf extract modulates permethrin induced genetic and oxidative damage in rats. Cytotechnology 64, 459–464.

Vanden Berghe, W. (2012) Epigenetic impact of dietary polyphenols in cancer chemoprevention: Lifelong remodelling our epigenomes. Pharmacologial Research 65, 565–576.

Vaquero, A., & Reinberg, D. (2009) Calorie restriction and the exercise of chromatin. Genes & Development 23, 1849–1869.

Vauzour, D., Rodriguez-Mateos, A., Corona, G., Oruna-Concha, M.J., & Spencer, J.P.E. (2010) Polyphenols and human health: prevention of disease and mechanism of action. Nutrients 2, 1106–1131.

Viña, J., Borras, C., & Miguel, J. (2007) Theories of ageing. IUMBM Life, 59, 249–254.

Waddington, C.H. (1942) The epigenotype. Endeavour 1, 18–20.

Wang, J., Wu, Z., Li, D., Li, N., Dindot, S.V., Sattefield, M.C., Bazer, W.B., & Wu, G. (2012) Nutrition, epigenetics, and metabolic syndrome. Antioxidants & Redox Signalling 17, 282–301.

Wang, X. (2010) Effect of triazophos, fipronil and their mixture on miRNA expression in adult zebrafish. Journal of Environmental Science and Health Part B, Pesticides, Food Contaminants, and Agricultural Wastes 45, 648–657.

Waterland, R.A., Travisano, M., Tahiliani, K.G., Rached, M.T., & Mirza, S. (2008) Methyl donor supplementation prevents transgenerational amplification of obesity. International Journal of Obesity 32, 1373–1379.

Weaver, I.C., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R., Dymov, S., Szyf, M., & Meaney, M. (2004) Epigenetic programming by maternal behavior. Nature Neuroscience 7, 847–854.

Weitzman, J.B. (2002) Histone modification in heterochromatin. Genome Biology 3, 91–102.

World Health Organization (WHO) (2015) Global status report on noncommunicable diseases 2014. WHO, Geneva.

Yiannakopoulou, E.C. (2015) Targeting DNA methylation with green tea catechins. Pharmacology 95, 111–116.

Youngson, N.A., & Morris, M.J. (2013) What obesity research tells us about epigenetic mechanisms. Philosophical Transactions of the Royal Society B: Biological Sciences 368(1609): 20110337.

Zampa, A., Silvi, S., Servili, M., Montedoro, G., Orpianesi, C., & Cresci, A. (2006) *In vitro* modulatory effects of colonic microflora by olive oil iridoids. Microbial Ecology in Health and Disease 18, 147–153.

Zempleni, J., Chew, Y.C., Hassan, Y.I., & Wijeratne, S.K. (2008) Epigenetic regulation of chromatin structure and gene function by biotin. Nutrition Review 66, 46–48.

Zhang, X.F., Zhang, L.J., Feng, Y.N., Chen, B., Feng, Y.M., Liang, G.J., Li., L., & Shen, W. (2012) Bisphenol A exposure modifies DNA methylation of imprint genes in mouse fetal germ cells. Molecular Biology Reports 39, 8621–8628.

Zhang, Y., Li, Q., Zhou, D., & Chen, H. (2013) Genistein, a soya isoflavone, prevents azoxymethane-induced up-regulation of WNT/beta-catenin signalling and reduces colon pre-neoplasia in rats. British Journal of Nutrition 109, 33–42.

Zheng, A., Li, H., Cao, K., Xu, J., Zou, X., Li, Y., Chen, C., Liu, J., & Feng, Z. (2015) Maternal hydroxytyrosol administration improves neurogenesis and cognitive function in prenatally stressed offspring. Journal of Nutritional Biochemistry 26, 190–199.

24 Phenolic compounds of olives and olive oil and their bioavailability

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24.1 Introduction

The olive tree (*Olea europaea* L.) is a member of Oleaceae family and is grown in tropical and warm regions of the world. The fruit of this tree is olive, which is commercially important in the Mediterranean region since it serves as a prime source of olive oil (Ben Othman *et al.*, 2008; Ghanbari *et al.*, 2012; Keceli, 2013). Phenolic compounds are an important class of natural antioxidants. The term "phenolic compound" includes a large number of secondary plant metabolites that differ in their chemical structures and reactivity, ranging from simple compounds to highly polymerized molecules. When present in small amounts in food, phenolic compounds are capable of preventing/retarding and/or reducing the oxidation that can lead to a decrease in both nutritional value and sensory quality (Lozano-Sanchez *et al.*, 2011).

Antioxidants are agents that, in one way or another, restrict the deleterious effects of these oxidant reactions, either by scavenging free radicals or by preventing radical formation (Fernandez-Orozco *et al.*, 2011). Bioactive compounds are intensively examined in order to evaluate their effects on health, including antioxidant, antiallergic, antimicrobial, antithrombotic, antiatherogenic, hypoglycemic, anti-inflammatory, antitumor, cytostatic, and immunosuppressive properties, and protective activities.

Extra virgin olive oil (EVOO) is directly obtained from olive fruit by mechanical means without any refining process. Among the other vegetable oils, EVOO is characterized by its unique taste and flavor, and is of particular interest due to its nutritional properties and beneficial implications for human health. Studies have shown that olive oil may play a role in the prevention of coronary heart disease and cognitive impairment (e.g., Alzheimer's disease), and has protective effects against colon, breast, and ovary cancer; diabetes accompanied by hyperglycemia; and inflammatory and autoimmune diseases, such as rheumatoid arthritis (Cioffi *et al.*, 2010).

The growing interest in EVOO is mainly attributed to its high oxidative stability due to its chemical composition that includes an unsaponifiable fraction, monounsaturated and polyunsaturated fatty acids, and a fraction composed of natural antioxidants such as carotenoids, phytosterols, flavonoids, α-tocopherol, and other phenolic compounds (Keceli & Gordon, 2001; Armaforte *et al.*, 2007; Aturki *et al.*, 2008; Oliveras-Lopez *et al.*, 2014; Ramos-Escudero *et al.*, 2015). The antioxidant quality of phenolic compounds in olives and olive oil is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers (Keceli & Gordon, 2002; Cioffi *et al.*, 2010). It is very well known that phenolic compounds of olive oil have numerous antioxidant effects, but little is known about their bioavailability at real-life doses (Tuck & Hayball, 2002; Christophoridou & Dais, 2009). Accumulating evidence suggests that virgin olive oil may have health benefits; it can be considered as an example of a functional food containing a variety of components that may contribute to its overall therapeutic characteristics. To explore and determine the mechanisms of action of olive and olive oil polyphenols and their role in disease, many studies are in progress or have been done already.

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This chapter reviews the current knowledge on the phenolic compounds present in olive and olive oil as well as the bioavailability of these compounds.

24.2 Phenolic compounds of olives and olive oil

24.2.1 Phenolic compounds in olives

Phenolic compounds have some nutritional benefits; however, they are also responsible for the bitter taste and astringency of olive fruit and make olives inedible without processing. The distribution and structure of the chemical constituents of olive fruit are complex and dependent on parameters such as cultivar, cultivation practices, geographical origin, and the maturation stage (Ghanbari *et al.*, 2012). Phenolic compounds in olives are present as 1–2% of the fresh weight of the fruit. As already noted, the most important classes of phenolic compounds in olive fruits include phenolic acids, phenolic alcohols, flavonoids, and secoiridoids. A detailed description of the main classes of olive phenolics is given at Table 24.1 (Servili & Montedoro, 2002).

The phenolic acids of olives are caffeic, *p*-coumaric, protocatechuic, vanillic, *p*-hydroxyphenylacetic, *p*-hydroxybenzoic, 3,4-dihydroxyphenylacetic, and ferulic acids. The phenolic alcohols are tyrosol and hydroxytyrosol, which may exist in glucosides and/or acetates. The predominant secoiridoids of olive fruit pulp are oleuropein and ligustroside. Some oleuropein derivatives have also been described, namely, demethyloleuropein, oleuropein aglycone, and elenolic acid. Oleuropein is the main bitter component in olives. It is a heterosidic ester of elenolic acid and hydroxytyrosol. The oleuropein concentration decreases during maturation while hydroxytyrosol concentration increases (Ben Othman *et al.*, 2008). Both lipophilic and hydrophilic phenolics are distributed in olive fruit. Lipophilic phenols are cresols, while the major hydrophilic phenols include phenolic acids, phenolic alcohols, flavonoids, and secoiridoids; they are present in almost all parts of the plant (Keceli & Gordon, 2001; Servili & Montedoro, 2002; Tripoli *et al.*, 2005; Aturki *et al.*, 2008; Ghanbari *et al.*, 2012; Keceli, 2013). Hydroxytyrosol-4-β-D-glucoside, also reported in olive flesh, has recently been proposed as the main polyphenol compound in natural black olives. Besides these compounds, other natural phenols in olives are flavonols such as rutin, luteolin-7-glucoside, and apigenin-7-glucoside, and several anthocyanin pigments. The latter are responsible for the purple color of natural black olives. Cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside are the main anthocyanins in natural black olive fruits. Other minor anthocyanin pigments, such as peonidin-3-glucoside acylated with *p*-coumaric acid, cyanindin-3-glucoside acylated with *p*-coumaric acid, cyanidin-3-O-rutinoside acylated with caffeic acid, and delphinidin-3-rhamnoglucoside, were also detected in olives (Keceli & Gordon, 2001; Romero *et al.*, 2002). Oleuropein is removed during table olive processing since the bitter taste of the olive

Table 24.1 The main phenolic compounds present in olives.

Phenolic group	Compounds
Anthocyanins	Cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3-caffeyglucoside, cyanidin-3-caffeylrutinoside, delphinidin 3-rhamosylglucoside-7-xyloside
Flavonols and flavones	Apigenin-7-glucoside, luteolin-7-rutinoside, luteolin-7-glucoside, luteolin-5-glucoside, quercetin-3-rutinoside
Phenolic acids	Chlorogenic acid, caffeic acid, *p*-hydroxybenzoic acid, protocatechuic acid, vanillic acid, syringic acid, *p*-coumaric acid, *o*-coumaric acid, ferulic acid, sinapic acid, benzoic acid, cinnamic acid, gallic acid
Phenolic alcohols	(3,4-Dihydroxyphenyl) ethanol (3,4-DHPEA), (*p*-hydroxyphenyl) ethanol (*p*-HPEA)
Secoiridoids	oleuropein, demethyloteuropein, ligstroside, nuzhenide
Hydroxycinnamic acid derivatives	Verbascoside

Adapted from Servili and Montedoro (2002) and Ghanbari *et al.* (2012).

fruit is mainly attributed to this compound. The alkali (NaOH) treatment hydrolyzes oleuropein into β-(3,4-dihydroxyphenyl) ethanol, oleoside 11-methylester, and oleside, making the olive delicious (Ghanbari *et al.*, 2012). Table olives are good sources of phenolic compounds since the consumption of 50 g of table olives provides about 56 mg of polyphenols (Ben Othman *et al.*, 2008).

24.2.2 Phenolic compounds in olive oil

Olive oil, the main lipid source in the Mediterranean diet, is a functional food that has a high level of monounsaturated fatty acids (MUFAs) and contains several minor components with biological properties. The protective and antioxidant effects of olive oil are attributed to its high content of MUFAs and to the presence of minor components, including pheolic compounds, α-tocopherol, phytosterols, carotenoids, chlorophylls, and squalene, which may be present up to 2% by weight (Keceli & Gordon, 2002; Servili & Montedoro, 2002; Tuck & Hayball, 2002; De la Torre *et al.*, 2004; Keceli, 2013; Ramos-Escudero *et al.*, 2015). EVOO contains a high amount of phenolic compounds that are crucial for its nutritional and organoleptic properties, as well as for the high oxidative stability, in comparison with other edible oils. Additionally, the level of these substances is a very important parameter for the quality of EVOO, mainly its organoleptic characteristics including flavor, astringency, pungency, and bitterness (Keceli & Gordon, 2002; Franco *et al.*, 2014). The main antioxidants of EVOO are carotenoids, tocopherols, and other phenolic compounds, both lipophilic and hydrophilic. The lipophilics include tocopherols, while the hydrophilics include flavonoids, phenolic alcohols and acids, secoiridoids, and their metabolites (Tripoli *et al.*, 2005; Garcia-Villalba *et al.*, 2011).

Several factors influence the phenolic compounds composition of EVOO, and these include: the cultivar of the olive fruit, agricultural techniques used to cultivate the olive tree, maturity of the olive fruit at harvest time, olive oil extraction, processing, and storage conditions (Kiritsakis 1998; Kiritsakis *et al.*, 2010). Consequently, the phenolic content can be a unique characteristic of EVOOs and a very important parameter for quality monitoring purposes (Garcia-Villalba *et al.*, 2011). Remarkably, the European Food Safety Authority (EFSA) has recently claimed that "the consumption of olive oil rich in polyphenols (hydroxytyrosol, 5 mg/day) contributes to the protection of oxidative damage to lipids in blood" (del Pilar Godoy-Caballero *et al.*, 2012).

EVOO contains different classes of phenolic compounds such as phenolic acids, phenolic alcohols, hydroxyisochromans, secoiridoids, lignans, and flavonoids that affect its sensory, oxidative, and antioxidant properties. Phenolic compounds present in olive oil are listed in Table 24.2 (Servili & Montedoro, 2002; Ghanbari *et al.*, 2012).

Table 24.2 Phenolic compounds in olive oil.

Phenolic group	Compounds
Flavones	Apigenin, luteolin
Phenolic acids and derivatives	Benzoic acid, caffeic acid, cinnamic acid, *p*-coumaric acid, *o*-coumaric acid, ferulic acid, gallic acid (halleridone), homovanilic acid, *p*-hydroxybeenzoic acid, protocatechuic acid, sinapic acid, syringic, vanillic acid, 4-(acetoxyethil)-1,2-dihydroxybenzene
Phenolic alcohols	Hydroxytyrosol (3,4-dihdroxyphenyl) ethanol (3,4-DHPEA) tyrosol (*p*-hydroxyphenil) ethanol (*p*-HPEA) (3,4-dihdroxyphenyl) ethanol-glucoside
Secoiridoids	Dialdehydic form of elenolic acid linked to 3,4-DHPEA (3,4-DHPEA-EDA) Dialdehydic form of elenolic acid linked to *p*-HPEA (p-HPEA-EDA) elenolic acid, elenolic acid glucoside, oleuropein aglycon (3,4-DHPEA-EA), ligstroside aglycon oleuropein, *p*-HPEA-derivative
Lignans	(+)-1-Acetoxypinoresinol (+)-Pinoresinol (+)-1-Hydroxyypinoresinol

Adapted from Servili and Montedoro (2002) and Ghanbari *et al.* (2012).

Phenolic acids and phenolic alcohols, which include 3,4-dihydroxyphenyl ethanol (3,4-DHPEA or hydroxytyrosol) and *p*-hydroxyphenyl ethanol (*p*-HPEA or tyrosol), as well as flavonoids are present in small amounts in EVOO, while secoiridoids and lignans are the most concentrated phenolic compounds of this oil (Selvaggini *et al.*, 2006). The most abundant secoiridoids of EVOO are the dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-dihydroxyphenyl ethanol or (*p*-hydroxypheny1) ethanol (3,4-DHPEA-EDA or *p*-HPEA-EDA) and an isomer of the oleuropein aglycon (3,4-DHPEA-EA). Oleuropein, oleuropein and ligstroside aglycons, and their dialdehydic forms were also detected. Flavonoids such as luteolin and apigenin were also reported as phenolic components of this oil. The last group of phenols found in EVOOs are lignans such as (+)-1-acetoxypinoresinol and (+)-pinoresinol as the most concentrated lignans in Italian and Spanish oils, respectively (Selvaggini *et al.*, 2006; Carrasco-Pancorbo *et al.*, 2007). Additionally, secoiridoids (including the aglycon derivatives of oleuropein, demethyloleuropein, and ligustroside) are the compounds responsible for the "pungent" and "bitter" taste of the oil (Servili *et al.*, 2008; Perez-Trujillo *et al.*, 2010).

24.3 Bioavailability of olive and olive oil phenolics

The term "bioavailability" was defined as the fraction of an ingested nutrient or compound that reaches the systemic circulation and the specific sites where it can exert its biological action (D'Archivio *et al.*, 2010; Yao *et al.*, 2015). The majority of studies regarding the bioavailability of olive and olive oil phenolics have focused on three major phenolics: hydroxytyrosol, tyrosol, and oleuropein. In general, these phenolics from olive and olive oil have been demonstrated to be readily bioavailable. In this section, *in vivo* and *in vitro* studies regarding the bioavailability of olive and olive oil phenolics are discussed in detail.

24.3.1 *In vivo* studies

24.3.1.1 Human studies

Direct evidence on bioavailability of olive and olive oil phenolics has been achieved by measuring the concentration of the polyphenols and their metabolites in biological fluids, mainly plasma and urine, after ingestion of pure compounds or olive oil, either pure or enriched with the phenolics. Research conducted on humans has shown that hydroxytyrosol and tyrosol are dose-dependently absorbed after ingestion (Visioli *et al.*, 2000; Caruso *et al.*, 2001; Weinbrenner *et al.*, 2004; Covas *et al.*, 2006). A study conducted by Vissers *et al.* (2002) demonstrated that absorption of olive oil phenolics, namely hydroxytyrosol, ligstroside, oleuropein, and tyrosol, was as high as 55–66%. Another study found that even from moderate doses (25 mL/d), which is close to that used as daily intake in Mediterranean countries, approximately 98% of hydroxytyrosol was present in plasma and urine, mainly as glucuronide conjugates (Miro-Casas *et al.*, 2003). Further research by the same group (Miro-Casas *et al.*, 2001a, 2001b) as well as other groups (Garcia-Villalba *et al.*, 2010; Khymenets *et al.*, 2011) confirmed that olive oil phenolics were excreted in urine mainly as glucuronide conjugates. Later, hydroxytyrosol sulfate, vanillin sulfate (Suarez *et al.*, 2011), and hydroxytyrosol acetate sulfate (Rubio *et al.*, 2012a, 2012b) were found to be the main biological metabolites of hydroxytyrosol in plasma after olive oil ingestion. In addition, hydroxytyrosol also undergoes the action of catechol-O-methyl transferase, an enzyme involved in the catecholamine catabolism, resulting in the excretion of homovanillic alcohol and homovanillic acid in plasma and urine as shown in Figure 24.1 (Caruso *et al.*, 2001; Salvini *et al.*, 2006; Kountouri *et al.*, 2007; Gonzalez-Santiago *et al.*, 2010; Oliveras-Lopez *et al.*, 2014).

It is known that polyphenols are subjected to phase I and phase II metabolism, in which they are hydrolyzed (phase I) and conjugated (phase II) into their glucuronidated, methylated, and sulfated forms in order to be absorbed (Manach *et al.*, 2004). This is a major drawback in understanding the biological activity of hydroxytyrosol *in vivo*. The picture gets even more complicated by the fact that hydroxytyrosol is also a dopamine metabolite, and body fluid concentrations combine exogenous and endogenous sources (De la Torre, 2008). Overall, the exact absorption mechanism of olive oil phenolics in humans still remains unclear; however, the polarity of the phenolic compounds has been suggested to play an important role

Figure 24.1 Transformation of hydroxytyrosol. COMT = cathecolmethyltransferase; SULT = sulfotransferase; UDGPT = uridine diphosphoglucuronosyltransferase.

in the absorption of these compounds (Vissers *et al.*, 2002). Further details of the human studies on the bioavailability of olive and olive oil phenolics are presented in Table 24.3.

24.3.1.2 Animal studies

These studies, just like human investigations, showed that phenolic compounds were present in plasma and urine as hydroxytyrosol and tyrosol, mainly as glucuronide and sulfate conjugates, and in methylated form as homovanillic alcohol and homovanillic acid (Tuck *et al.*, 2002; Del Boccio *et al.*, 2003; Bazoti *et al.*, 2005, 2010). On the other hand, free forms of some phenolic compounds were also reported, such as oleuropein derivative in plasma and brain; luteolin in the kidney, testicle, brain, and heart; or hydroxytyrosol in the plasma, kidney, and testicle (Serra *et al.*, 2012). Furthermore, a recent study demonstrated that the changes in the metabolic disposition of hydroxytyrosol in rats were dose-dependent. In particular, following the treatment of 1 mg/kg of hydroxytyrosol, approximately 25–30% of the compounds present in urine were glucuronides, whereas lower recoveries were observed for sulfate conjugates (14%). However, at higher doses (10–100 mg hydroxytyrosol/kg), sulfates were predominant (57–75%) (Kotronoulas *et al.*, 2013). In addition, the bioavailability of phenolics of olive oil is shown to depend on the carrier by which they are administered (oil or water), as well as on the administration route (intravenous or oral) (Tuck *et al.*, 2001). As mentioned in this chapter, the exact absorption mechanism of olive oil phenolics remains uncertain. Nevertheless, it is proposed that oleuropein–glycoside may diffuse through lipid bilayers of the epithelial cell membrane and be absorbed via a glucose transporter. Two additional absorption mechanisms for oleuropein–glycoside are the paracellular route or transcellular passive diffusion (Edgecombe *et al.*, 2000). Table 24.4 shows animal studies regarding the bioavailability of olive and olive oil phenolics that were largely conducted on rats.

24.3.2 *In vitro* studies

24.3.2.1 Gastrointestinal digestion studies

In vitro methods for simulating the human digestive tract are being extensively used at present since they are rapid, are safe, and do not have the same ethical restrictions as *in vivo* methods (Kamiloglu *et al.*, 2014). Table 24.5 includes *in vitro* gastrointestinal digestion studies on olive and olive oil phenolics, which show that polyphenols might be partially modified in the acidic environment of the stomach.

The effect of gastric juice on olive oil phenolics has been examined *in vitro* by incubating the samples at 37 °C in simulated gastric conditions and during normal physiological time frames (Corona *et al.*, 2006; Dinnella *et al.*, 2007; Soler *et al.*, 2010; Pinto *et al.*, 2011). Although hydrolysis takes place, releasing free phenolics, some fractions of the conjugated forms were still present unhydrolyzed and, thus, entered the

Table 24.3 Human studies on the bioavailability of olive and olive oil phenolics.

Treatment	Subjects	Dose of phenolics	Design	Results	Reference
4 VOO (A, B, C, D)	6 male healthy volunteers (27–33 years)	50 mL samples A: 487.5 mg/L B: 975 mg/L C: 1462.5 mg/L D: 1950 mg/L	4 single doses Urine sampling for 24 h	– HT and T were dose-dependently absorbed and excreted in urine mainly as glucuronide conjugates. Only 30–60% of HT and 20–22% of T excreted in urine. – Urinary excretion of HT metabolites, homovanillic alcohol and homovanillic acid, correlated with the dose of administered HT.	Visioli *et al.* (2000) Caruso *et al.* (2001)
EVOO	8 healthy volunteers (3 women and 5 men) (25–52 years)	50 mL sample 1650 μg T/50 mL	Single dose Urine sampling for 24 h	– T was excreted mainly in its conjugated form. Only 6–11% of T in urine was in its free form. Recovery of T at 24 h was 17–43%.	Miro-Casas *et al.* (2001a)
VOO	11 healthy volunteers (6 women and 5 men) (25–65 years)	50 mL sample 1055 μg HT/50 mL 655 μg T/50 mL	Single dose Urine sampling for 24 h	– HT and T are mainly excreted in conjugated form; only 5.9% of HT and 13.8% of T were in free form. Recovery of HT and T at 24 h was 32–98 and 12–32%, respectively.	Miro-Casas *et al.* (2001b)
3 supplements containing olive oil phenolics	20 healthy volunteers (12 women and 8 men) (20–75 years)	100 mg of samples with HT, L, OE, T	3 single doses Ileostomy effluent and urine sampling for 24 h	– 55–66% of ingested olive oil phenolics are absorbed, and 5–16% is excreted as HT and T in urine.	Vissers *et al.* (2002)
VOO	6 healthy volunteers (3 women and 3 men) (25–47 years)	25 mL sample 49.3 mg HT/L	Single dose Plasma sampling for 8 h Urine sampling for 12 h	– ≈98% of HT was present in plasma and urine in conjugated forms, mainly glucuronoconjugates.	Miro-Casas *et al.* (2003)
3 VOO (L, M, H)	12 male healthy volunteers (20–22 years)	40 mL samples L: 10 mg/kg M: 133 mg/kg H: 486 mg/kg	3 single doses Urine sampling for 24 h	– HT and T were dose-dependently absorbed.	Weinbrenner *et al.* (2004)
3 VOO (L, M, H)	12 male healthy volunteers (20–22 years)	40 mL samples L: 2.7 mg/kg M: 164 mg/kg H: 366 mg/kg	3 single doses Plasma sampling for 24 h	– HT and T were dose-dependently absorbed.	Covas *et al.* (2006)

2 EVOO (L, H)	10 postmenopausal women (47–67 years)	50 g samples L: 147 mg/kg H: 592 mg/kg	8 weeks crossover 50 g per day Urine sampling for 24 h	– Urinary excretion of HT and its metabolite homovanillyl alcohol were significantly increased in subjects consuming high EVOO.	Salvini *et al.* (2006)
20 olives	8 male healthy volunteers (30–40 years)	≈100 g sample 76.73 mg HT/100 g 19.48 mg T/100 g	Single dose Blood sampling for 4 h Urine sampling for 24 h	– HT and T appeared in plasma and urine, mainly as glucuronides.	Kountouri *et al.* (2007)
EVOO	10 healthy volunteers (5 women and 5 men) (24–35 years)	50 mL sample	Single dose Urine sampling for 6 h	– Tentative identification of more than 60 metabolites of olive oil polyphenols was achieved.	Garcia-Villalba *et al.* (2010)
HT supplement	10 healthy volunteers (2 women and 8 men) (22–34 years)	2.5 mg HT/kg weight	Single dose Blood sampling for 120 min Urine sampling for 24 h	– HVA was detected in plasma. – The main metabolites in urine were HVA (31%), DHPA glucuronide (22.7%), and 5% free or conjugated HT.	Gonzalez-Santiago *et al.* (2010)
VOO	11 healthy volunteers (5 women and 6 men) (20–44 years)	50 mL sample 21.96 µmol HT/50 mL 15.20 µmol T/50 mL 0.27 µmol HVA/50 mL	Single dose Plasma and urine sampling for 24 h	– 13% of the olive oil polyphenols were recovered in urine, where 75% of them were glucuronides and 25% as free compounds.	Khymenets *et al.* (2011)
VOO vs. PEVOO	13 healthy volunteers (6 women and 7 men) (25–69 years)	30 mL samples VOO: 288.89 mg/kg PEVOO: 961.17 mg/kg	2 single doses Blood sampling for 300 min	– 24 compounds detected, mostly secoiridoids and phenol alcohol groups in sulfated and glucuronidated forms. 14 out of 24 compounds were higher in samples of PEVOO compared to VOO.	Suarez *et al.* (2011)
3 PEVOO (L, M, H)	12 healthy volunteers (6 women and 6 men) (22–60 years)	30 mL samples L: 250 mg/kg M: 500 mg/kg H: 750 mg/kg	3 single doses Plasma sampling for 360 min	– Dose-dependent responses of phenol conjugates were obtained in plasma. – Hydroxytyrosol acetate sulfate was identified as the main biological metabolite of HT from olive oil ingestion.	Rubio *et al.* (2012a, 2012b)
EVOO	45 healthy volunteers (34 women and 11 men) (21–45 years)	50 mL sample 509.72 mg/L	30 days crossover Twice (30+20 mL)/day Blood sampling on days 0 and 30.	– HT and its 3 metabolites, and T and its 2 metabolites, were identified in the plasma.	Oliveras-Lopez *et al.* (2014)

Note: DHPA = 3,4-dihydroxyphenylacetic acid; EVOO = extra virgin olive oil; HT = hydroxytyrosol; HVA = homovanillic alcohol; L = ligstroside; OE = oleuropein; POVOO = phenol-enriched virgin olive oil; T = tyrosol; VOO = virgin olive oil.

Table 24.4 Animal studies on the bioavailability of olive and olive oil phenolics.

Treatment	Species	Dose of phenolics	Design	Results	Reference
HT and T dissolved in oil or water	Male Sprague-Dawley rats	225 mg samples 23.5 mg HT/1300 mg oil 14.7 mg T/1300 mg oil 25.5 mg HT/1300 mg water 14.4 mg T/1300 mg water	Single-dose Urine sampling for 24 h	– Bioavailability of HT and T was higher when administered as oil solution (98–99%) compared to aqueous solution (71–75%). – HT and its metabolites (monosulfate conjugate, 3-*O*-glucuronide conjugate, and homovanillic acid) were excreted in urine.	Tuck *et al.* (2001, 2002)
OE	CD-COBS rats (14-week-old)	100 mg sample/kg rat	Single dose Plasma and urine sampling for 300 min	– In plasma, OE and small amount of HT were present, whereas in urine, both compounds were mainly found as glucuronides.	Del Boccio *et al.* (2003)
OE or EVOO	Female Wistar rats	0.15 mg OE/kg rat 50 g EVOO/kg rat	80 days crossover Urine sampling	– HT, T, and metabolites (glucuronidic forms) and sulfate derivatives are identified in urine samples.	Bazoti *et al.* (2005)
OE or EVOO	Female Wistar rats	0.33 mg OE/kg rat 1.1 g EVOO/kg rat	80 days crossover Plasma sampling	– OE was rapidly metabolized and eliminated. HT was metabolized to HVA.	Bazoti *et al.* (2010)
PEOC	Male Wistar rats (3-month-old)	3 g sample/kg rat 101 mg/g sample	Single dose Plasma and tissue (liver, kidney, testicle, brain, and spleen) sampling for 4 h	– Sulfate conjugates of HT and T were the main metabolites quantified in the plasma and tissues. Free phenolics, such as OED in the plasma and brain; LT in the kidney, testicle, brain, and heart; or HT in the plasma, kidney, and testicle were also present.	Serra *et al.* (2012)
3 dose of HT	Male and female Sprague-Dawley rats	1 mg HT/kg rat 10 mg HT/kg rat 100 mg HT/kg rat	Single dose Urine sampling	– At the dose of 1 mg HT/kg, glucuronidation was most relevant pathway (25–30%), while lower recoveries were observed for sulfate conjugates (14%). At the dose of 10 mg HT/kg, sulfation was more relevant (57%), while lower recoveries of glucuronides were observed (15%). For 100 mg HT dose, 3-SulfHT was the most prevalent metabolite (75%).	Kotronoulas *et al.* (2013)

Note: EVOO = extra virgin olive oil; HT = hydroxytyrosol; HVA = homovanillyl alcohol; LT = luteolin; OE = oleuropein; OED = oleuropein derivative; PEOC = phenolic extract from olive cake; T = tyrosol; 3-SulfHT = 3-sulfate hydroxytyrosol acetate.

Table 24.5 *In vitro* gastrointestinal digestion studies on the bioavailability of olive and olive oil phenolics.

Treatment	Dose of phenolics	Design	Results	References
OE	1 mmol OE/L	*ID*: In situ intestinal perfusion, 37 °C, 40 min	– *ID*: OE was poorly absorbed from isolated rat intestine.	Edgecombe *et al.* (2000)
EVOO-PE, HT, T, OE	*GD*: 1 mg/mL EVOO-PE *ID*: 54 μM HT, 62.5 μM T, 100 μM OE, 18 mg/L EVOO-PE *CD*: 1 mM OE	*GD*: 37 °C, 30 min–4 h, pH:2 *ID*: Rat segments of jejunum and ileum, 37 °C, 80 min *CD*: 37 °C, 48 h, pH:7	– *GD*: After 4 h of incubation, the amount of HT and T increased (7 and 5 times, respectively), whereas some fractions of the conjugated forms were still present unhydrolyzed. – *ID*: HT and T are absorbed and metabolized to HVA and glucuronide conjugates. OE was not transported. – *CD*: OE degradation resulted in formation of three new metabolites, one of which is HT.	Corona *et al.* (2006)
EVOO	10 g sample	*GD*: 37 °C, 2 h, pH:2 *ID*: 37 °C, 2 h, pH:7	– *ID*: 37–90% of the phenolics were found to be bioaccessible.	Dinnella *et al.* (2007)
VOO	15 g sample	*GD*: 37 °C, 2 h, pH:2 *ID*: 37 °C, 2 h, pH:6.5	– *GD*: All the major olive oil phenolics showed good stability. – *ID*: Only 10% of the secoridoids were recovered. The flavonoids were also unstable, whereas lignans were quite stable.	Soler *et al.* (2010)
OE aglycon, OE aglycon dialdehyde	1 mM samples	*GD*: 37 °C, 1–4 h, pH:2 *ID*: Rat segments of jejunum and ileum, 37 °C, 80 min	– *GD*: Although some hydrolysis takes place releasing free HT from both compounds, a large amount of both compounds (67–78%) remained intact. – *ID*: Glucuronides represented the major intestinal metabolites entering the portal blood.	Pinto *et al.* (2011)
T, HT, HTA, OE	500 μM samples	*CD*: 37 °C, 48 h, pH:7	– *CD*: An increase in phenolic acids, the stability of HT and T, and the high degradation of HTA and OE were observed.	Mosele *et al.* (2014)

Note: CD = colonic digestion; EVOO-PE = extra virgin olive oil phenolic extract; GD = gastric digestion; HT = hydroxytyrosol; HTA = hydroxytyrosol acetate; HVA = homovanillic alcohol; ID = intestinal digestion; OE = oleuropein; T = tyrosol; VOO = virgin olive oil.

small intestine unmodified (Corona *et al.*, 2006). The resistance of these phenolics to the acidic conditions of the stomach may be related with the conditions they have encountered during the oil extraction process. Indeed, the major forms of phenolics in the olive fruits are glycosides, and the aglycones (oleuropein, ligstroside, flavonoids, and lignans) that are the predominant forms of phenolics in the olive oil are formed as a consequence of the acidic conditions and the presence of β-glucosidase activity when the olives are pasted during olive oil production (Soler *et al.*, 2010). Intestinal digestion of the phenolic compounds from olives and olive oil was simulated by using either enzymatic (Dinnela *et al.*, 2007; Soler *et al.*, 2010) or isolated perfused rat intestines models (Edgecombe *et al.*, 2000; Corona *et al.*, 2006; Pinto *et al.*, 2011). Research showed that 37–90% of the olive oil phenolics were bioaccessible (Dinnela *et al.*, 2007). On the other hand,

Table 24.6 Cell culture studies on the bioavailability of olive and olive oil phenolics.

Treatment	Cell type	Dose of phenolics	Design	Results	References
HT	Caco-2	50–500 μM sample	Incubation at 37 and 4 °C for 2 min	– HT was transported via a bidirectional passive diffusion mechanism. The only metabolite identified in the culture medium was HVA (10%).	Manna *et al.* (2000)
HT, T, HTA	HepG2	100 μM samples	Incubation at 37 °C for 2–18 h	– Extensive uptake and metabolism of HT and HTA were observed with scarce metabolism of T. Main metabolites formed were glucuronidated and methylated conjugates.	Mateos *et al.* (2005)
HT, T, OE	Caco-2	10 μM HT 50 μM T 100 μM OE	Incubation at 37 °C for 2 h	– HT and T were transported. Major metabolites were an *O*-methylated derivative of HT, glucuronides of HT and T, and a glutathionylated conjugate of HT. OE was not absorbed.	Corona *et al.* (2006)
HT, T, PCA, PR, LT	Caco-2/TC7	40–100 μM samples	Incubation at 37 °C for 1–24 h	– Major metabolites were methylated conjugates. Time-dependent transport of various free and conjugated compounds was observed.	Soler *et al.* (2010)
HT, HTA	Caco-2/TC7	50 μM samples	Incubation at 37 °C for 1–4 h	– HVA was detected as a result of HT metabolism (20%). HTA was largely converted into free HT (38%) and subsequently metabolized into HVA (7%).	Mateos *et al.* (2011)
OE aglycon, OE aglycon dialdehyde	Caco-2	50–200 μM samples	Incubation at 37 °C for 2 h	– Caco-2 cells expressed limited metabolic activity. Only HT and HVA were formed.	Pinto *et al.* (2011)

Note: HT = hydroxytyrosol; HTA = hydroxytyrosol acetate; HVA = homovanillic alcohol; LT = luteolin; OE = oleuropein; PCA = *p*-coumaric acid; PR = pinoresinol; T = tyrosol.

oleuropein as well as its aglycone and dialdehydic form are not well absorbed as such in the small intestine (Edgecombe *et al.*, 2000; Corona *et al.*, 2006; Soler *et al.*, 2010; Pinto *et al.*, 2011); the major metabolites detected using the perfused rat intestine model were the glucuronide conjugates of the reduced form of these compounds (Pinto *et al.*, 2011). It is also quite likely that oleuropein reaches the large intestine, where it is subjected to degradation by the colonic microflora (Corona *et al.*, 2006). In fact, *in vitro* colon fermentation of the olive oil phenolic compounds revealed an increase in phenolic acids, the stability of hydroxytyrosol and tyrosol, as well as high degradation of hydroxytyrosol acetate and oleuropein (Mosele *et al.*, 2014). Nevertheless, *in vitro* gastrointestinal digestion models can be considered as novel techniques, and therefore further studies should be conducted to support the present findings.

24.3.2.2 Cell culture studies

The most widely used cell line to study the bioavailability of olive and olive oil phenolics *in vitro* is Caco-2, which is a human colon carcinoma cell line, representing the human intestinal epithelium. Table 24.6 shows these culture studies on the bioavailability of olive and olive oil phenolics.

Studies carried out on Caco-2 cells demonstrated that olive oil phenolics, including hydroxytyrosol, hydroxytyrosol acetate, and tyrosol, are absorbed and metabolized to their methylated and glucuronated conjugates (Manna *et al.*, 2000; Corona *et al.*, 2006; Soler *et al.*, 2010; Mateos *et al.*, 2011). On the other hand, Caco-2 cells expressed limited metabolic activity toward oleuropein and its aglycon (Corona *et al.*, 2006; Pinto *et al.*, 2011). A study conducted using HepG2 cells as a hepatic model system suggested that olive oil phenols can be metabolized by the liver (Mateos *et al.*, 2005). When the bioavailability of polyphenols is assessed by using cultured cells as tissue models, in almost all studies cells are treated with aglycones or polyphenol-rich extracts derived from plants and foods, in this case from olive oil, and data are reported at concentrations that elicited a response. However, plasma and tissues are not exposed *in vivo* to polyphenols in these forms. Also, the polyphenol concentrations tested should be of the same order as the maximum plasma concentrations attained after a polyphenol-rich meal, which are in the range of 0.1–10 µmol/L (Kroon *et al.*, 2004).

24.4 Conclusion

Several studies have been applied *in vivo* (to humans and animals) and *in vitro* (via gastrointestinal digestion studies and cell culture studies) to determine the bioavailability of olives and olive oil phenolics. More studies, however, should be taken into account, and the methodologies should be adopted accordingly.

References

Armaforte, E., Mancebo-Campos, V., Bendini, A., Desamparados Salvador, M., Fregapane, G., & Cerretani, L. (2007) Retention effects of oxidized polyphenols during analytical extraction of phenolic compounds of virgin olive oil. Journal of Separation Science 30, 2401–2406.

Aturki, Z., Fanali, S., D'Orazio, G., Rocco, A., & Rosati, C. (2008) Analysis of phenolic compounds in extra virgin olive oil by using reversed-phase capillary electrochromatography. Electrophoresis 29, 1643–1650.

Bazoti, F.N., Gikas, E., Puel, C., Coxam, V., & Tsarbopoulos, A. (2005) Development of a sensitive and specific solid phase extraction-gas chromatography-tandem mass spectrometry method for the determination of elenolic acid, hydroxytyrosol, and tyrosol in rat urine. Journal of Agricultural and Food Chemistry 53, 6213–6221.

Bazoti, F.N., Gikas, E., & Tsarbopoulos, A. (2010) Simultaneous quantification of oleuropein and its metabolites in rat plasma by liquid chromatography electrospray ionization tandem mass spectrometry. Biomedical Chromatography 24, 506–515.

Ben Othman, N., Roblain, D., Thonart, P., & Hamdi, M. (2008) Tunisian table olive phenolic compounds and their antioxidant capacity. Journal of Food Science 73, C235–C240.

Carrasco-Pancorbo, A., Neususs, C., Pelzing, M., Segura-Carretero, A., & Fernandez-Gutierrez, A. (2007) CE-and HPLC-TOF-MS for the characterization of phenolic compounds in olive oil. Electrophoresis 28, 806–821.

Caruso, D., Visioli, F., Patelli, R., Galli, C., & Galli, G. (2001) Urinary excretion of olive oil phenols and their metabolites in humans. Metabolism 50, 1426–1428.

Christophoridou, S., & Dais, P. (2009) Detection and quantification of phenolic compounds in olive oil by high resolution 1 H nuclear magnetic resonance spectroscopy. Analytica Chimica Acta 633, 283–292.

Cioffi, G., Pesca, M.S., De Caprariis, P., Braca, A., Severino, L., & De Tommasi, N. (2010) Phenolic compounds in olive oil and olive pomace from Cilento (Campania, Italy) and their antioxidant activity. Food Chemistry 121, 105–111.

Corona, G., Tzounis, X., Assunta Dessi, M., Deiana, M., Debnam, E.S., Visioli, F., & Spencer, J.P. (2006) The fate of olive oil polyphenols in the gastrointestinal tract: implications of gastric and colonic microflora-dependent biotransformation. Free Radical Research 40, 647–658.

Covas, M.I., De la Torre, K., Farre-Albaladejo, M., Kaikkonen, J., Fito, M., Lopez-Sabater, C., Pujadas-Bastardes, M.A., Joglar, J., Weinbrenner, T., Lamuela-Raventos, R.M., & De la Torre, R. (2006) Postprandial LDL phenolic content and LDL oxidation are modulated by olive oil phenolic compounds in humans. Free Radical Biology and Medicine 40, 608–616.

D'Archivio, M., Filesi, C., Varì, R., Scazzocchio, B., & Masella, R. (2010) Bioavailability of the polyphenols: status and controversies. International Journal of Molecular Sciences 11, 1321–1342.

De la Torre, R. (2008) Bioavailability of olive oil phenolic compounds in humans. Inflammopharmacology 16, 245–247.

De la Torre, R., Fito, M., Farre-Albadalejo, M., Marrugat, J., & Covas, M.I. (2004) Bioavailability and biological effects of olive oil phenolic compounds in humans. Chemistry and Physics of Lipids 130, 11–12.

Del Boccio, P., Di Deo, A., De Curtis, A., Celli, N., Iacoviello, L., & Rotilio, D. (2003) Liquid chromatography–tandem mass spectrometry analysis of oleuropein and its metabolite hydroxytyrosol in rat plasma and urine after oral administration. Journal of Chromatography B 785, 47–56.

Del Pilar Godoy-Caballero, M., Galeano-Diaz, T., & Isabel Acedo-Valenzuela, M. (2012) Simple and fast determination of phenolic compounds from different varieties of olive oil by nonaqueous capillary electrophoresis with UV-visible and fluorescence detection. Journal of Separation Science 35, 3529–3539.

Dinnella, C., Minichino, P., D'Andrea, A.M., & Monteleone, E. (2007) Bioaccessibility and antioxidant activity stability of phenolic compounds from extra-virgin olive oils during in vitro digestion. Journal of Agricultural and Food Chemistry 55, 8423–8429.

Edgecombe, S.C., Stretch, G.L., & Hayball, P.J. (2000) Oleuropein, an antioxidant polyphenol from olive oil, is poorly absorbed from isolated perfused rat intestine. The Journal of Nutrition 130, 2996–3002.

Fernandez-Orozco, R., Roca, M., Gandul-Rojas, B., & Gallardo-Guerrero, L. (2011) DPPH-scavenging capacity of chloroplastic pigments and phenolic compounds of olive fruits (cv. Arbequina) during ripening. Journal of Food Composition and Analysis 24, 858–864.

Franco, M.N., Galeano-Diaz, T., Lopez, O., Fernandez-Bolanos, J.G., Sanchez, J., De Miguel, C., Gil, M.V. & Martin-Vertedor, D. (2014) Phenolic compounds and antioxidant capacity of virgin olive oil. Food Chemistry 163, 289–298.

Garcia-Villalba, R., Carrasco-Pancorbo, A., Nevedomskaya, E., Mayboroda, O.A., Deelder, A.M., Segura-Carretero, A., & Fernandez-Gutierrez, A. (2010) Exploratory analysis of human urine by LC–ESI-TOF MS after high intake of olive oil: understanding the metabolism of polyphenols. Analytical and Bioanalytical Chemistry 398, 463–475.

Garcia-Villalba, R., Pacchiarotta, T., Carrasco-Pancorbo, A., Segura-Carretero, A., Fernandez-Gutierrez, A., Deelder, A.M., & Mayboroda, O.A. (2011) Gas chromatography–atmospheric pressure chemical ionization-time of flight mass spectrometry for profiling of phenolic compounds in extra virgin olive oil. Journal of Chromatography A 1218, 959–971.

Ghanbari, R., Anwar, F., Alkharfy, K.M., Gilani, A.H., & Saari, N. (2012) Valuable nutrients and functional bioactives in different parts of olive (*Olea europaea* L.): a review. International Journal of Molecular Sciences 13, 3291–3340.

Gonzalez-Santiago, M., Fonolla, J., & Lopez-Huertas, E. (2010) Human absorption of a supplement containing purified hydroxytyrosol, a natural antioxidant from olive oil, and evidence for its transient association with low-density lipoproteins. Pharmacological Research 61, 364–370.

Kamiloglu, S., Demirci, M., Selen, S., Toydemir, G., Boyacioglu, D., & Capanoglu, E. (2014) Home processing of tomatoes (*Solanum lycopersicum*): effects on *in vitro* bioaccessibility of total lycopene, phenolics, flavonoids, and antioxidant capacity. Journal of the Science of Food and Agriculture 94, 2225–2233.

Keceli, T., & Gordon, M.H. (2001) The antioxidant activity and stability of the phenolic fraction of green olives and extra virgin olive oil. Journal of the Science of Food and Agriculture 81, 1391–1396.

Keceli, T., & Gordon, M.H. (2002) Ferric ions reduce the antioxidant activity of the phenolic fraction of virgin olive oil. Journal of Food Science 67, 943–947.

Keceli, T.M. (2013) Some properties and antioxidant potential of olives and their corresponding extra virgin olive oils in Turkey. Asian Journal of Chemistry 25, 9401–9406.

Khymenets, O., Farre, M., Pujadas, M., Ortiz, E., Joglar, J., Covas, M.I., & De la Torre, R. (2011) Direct analysis of glucuronidated metabolites of main olive oil phenols in human urine after dietary consumption of virgin olive oil. Food Chemistry 126, 306–314.

Kiritsakis, A.K. (1998) Olive oil: from the tree to the table, 2nd ed. Food & Nutrition Press, Trumbull, CT.

Kiritsakis, K., Kontominas, M.G., Kontogiorgis, C., Hadjipavlou-Litina, D., Moustakas, A., & Kiritsakis, A. (2010) Composition and antioxidant activity of olive leaf extracts from Greek olive cultivars. Journal of the American Oil Chemists' Society 87, 369–376.

Kotronoulas, A., Pizarro, N., Serra, A., Robledo, P., Joglar, J., Rubio, L., Hernaez, A., Tormos, C., Motilva, M.J., Fito, M., Covas, M.I., Sola, R., Farre, M., Saez, G., & De la Torre, R. (2013) Dose-dependent metabolic disposition of hydroxytyrosol and formation of mercapturates in rats. Pharmacological Research 77, 47–56.

Kountouri, A.M., Mylona, A., Kaliora, A.C., & Andrikopoulos, N.K. (2007) Bioavailability of the phenolic compounds of the fruits (drupes) of *Olea europaea* (olives): impact on plasma antioxidant status in humans. Phytomedicine 14, 659–667.

Kroon, P.A., Clifford, M.N., Crozier, A., Day, A.J., Donovan, J.L., Manach, C., & Williamson, G. (2004) How should we assess the effects of exposure to dietary polyphenols in vitro? The American Journal of Clinical Nutrition 80, 15–21.

Lozano-Sanchez, J., Giambanelli, E., Quirantes-Pine, R., Cerretani, L., Bendini, A., Segura-Carretero, A., & Fernandez-Gutierrez, A. (2011) Wastes generated during the storage of extra virgin olive oil as a natural source of phenolic compounds. Journal of Agricultural and Food Chemistry 59, 11491–11500.

Manach, C., Scalbert, A., Morand, C., Remesy, C., & Jimenez, L. (2004) Polyphenols: food sources and bioavailability. The American Journal of Clinical Nutrition 79, 727–747.

Manna, C., Galletti, P., Maisto, G., Cucciolla, V., D'Angelo, S., & Zappia, V. (2000) Transport mechanism and metabolism of olive oil hydroxytyrosol in Caco-2 cells. FEBS letters 470, 341–344.

Mateos, R., Goya, L., & Bravo, L. (2005) Metabolism of the olive oil phenols hydroxytyrosol, tyrosol, and hydroxytyrosyl acetate by human hepatoma HepG2 cells. Journal of Agricultural and Food Chemistry 53, 9897–9905.

Mateos, R., Pereira-Caro, G., Saha, S., Cert, R., Redondo-Horcajo, M., Bravo, L., & Kroon, P.A. (2011) Acetylation of hydroxytyrosol enhances its transport across differentiated Caco-2 cell monolayers. Food Chemistry 125, 865–872.

Miro-Casas, E., Albaladejo, M.F., Covas, M.I., Rodriguez, J.O., Colomer, E.M., Raventos, R. M.L., & De la Torre, R. (2001a) Capillary gas chromatography–mass spectrometry quantitative determination of hydroxytyrosol and tyrosol in human urine after olive oil intake. Analytical Biochemistry 294, 63–72.

Miro-Casas, E., Albadalejo, M.F., Planells, M.I.C., Colomer, M.F., Raventos, R.M.L., & De la Torre, R. (2001b) Tyrosol bioavailability in humans after ingestion of virgin olive oil. Clinical Chemistry 47, 341–343.

Miro-Casas, E., Covas, M.I., Farre, M., Fito, M., Ortuno, J., Weinbrenner, T., Roset, P., & de la Torre, R. (2003) Hydroxytyrosol disposition in humans. Clinical Chemistry 49, 945–952.

Mosele, J.I., Martin-Pelaez, S., Macia, A., Farras, M., Valls, R.M., Catalan, U., & Motilva, M.J. (2014) Faecal microbial metabolism of olive oil phenolic compounds: ın vitro and in vivo approaches. Molecular Nutrition & Food Research 58, 1809–1819.

Oliveras-Lopez, M.J., Berna, G., Jurado-Ruiz, E., De la Serrana, H.L.G., & Martin, F. (2014) Consumption of extra-virgin olive oil rich in phenolic compounds has beneficial antioxidant effects in healthy human adults. Journal of Functional Foods 10, 475–484.

Perez-Trujillo, M., Gomez-Caravaca, A.M., Segura-Carretero, A., Fernandez-Gutierrez, A., & Parella, T. (2010) Separation and identification of phenolic compounds of extra virgin olive oil from *Olea europaea* L. by HPLC-DAD-SPE-NMR/MS: identification of a new diastereoisomer of the aldehydic form of oleuropein aglycone. Journal of Agricultural and Food Chemistry 58, 9129–9136.

Pinto, J., Paiva-Martins, F., Corona, G., Debnam, E.S., Jose Oruna-Concha, M., Vauzour, D., Gordon, M.H., & Spencer, J.P. (2011) Absorption and metabolism of olive oil secoiridoids in the small intestine. British Journal of Nutrition 105, 1607–1618.

Ramos-Escudero, F., Morales, M.T., & Asuero, A.G. (2015) Characterization of bioactive compounds from monovarietal virgin olive oils: relationship between phenolic compounds-antioxidant capacities. International Journal of Food Properties 18, 348–358.

Romero, C., Garcia, P., Brenes, M., Garcia, A., & Garrido, A. (2002) Phenolic compounds in natural black Spanish olive varieties. European Food Research and Technology 215, 489–496.

Rubio, L., Macia, A., Valls, R.M., Pedret, A., Romero, M.P., Sola, R., & Motilva, M.J. (2012a) A new hydroxytyrosol metabolite identified in human plasma: hydroxytyrosol acetate sulphate. Food Chemistry 134, 1132–1136.

Rubio, L., Valls, R.M., Macia, A., Pedret, A., Giralt, M., Romero, M.P., De la Torre, R., Covas, M.I., Sola, R., & Motilva, M.J. (2012b) Impact of olive oil phenolic concentration on human plasmatic phenolic metabolites. Food Chemistry 135, 2922–2929.

Salvini, S., Sera, F., Caruso, D., Giovannelli, L., Visioli, F., Saieva, C., Masala, G., Ceroti, M., Giovacchini, V., Pitozzi, V., Galli, C., Romani, A., Mulinacci, N., Bortolomeazzi, R., Dolara, P., & Palli, D. (2006) Daily consumption of a high-phenol extra-virgin olive oil reduces oxidative DNA damage in postmenopausal women. British Journal of Nutrition 95, 742–751.

Selvaggini, R., Servili, M., Urbani, S., Esposto, S., Taticchi, A., & Montedoro, G. (2006) Evaluation of phenolic compounds in virgin olive oil by direct injection in high-performance liquid chromatography with fluorometric detection. Journal of Agricultural and Food Chemistry 54, 2832–2838.

Serra, A., Rubio, L., Borras, X., Macia, A., Romero, M.P., & Motilva, M.J. (2012) Distribution of olive oil phenolic compounds in rat tissues after administration of a phenolic extract from olive cake. Molecular Nutrition & Food Research 56, 486–496.

Servili, M., & Montedoro, G. (2002) Contribution of phenolic compounds to virgin olive oil quality. European Journal of Lipid Science and Technology 104, 602–613.

Servili, M., Taticchi, A., Esposto, S., Urbani, S., Selvaggini, R., & Montedoro, G. (2008) Influence of the decrease in oxygen during malaxation of olive paste on the composition of volatiles and phenolic compounds in virgin olive oil. Journal of Agricultural and Food Chemistry 56, 10048–10055.

Soler, A., Romero, M.P., Macia, A., Saha, S., Furniss, C.S., Kroon, P.A., & Motilva, M.J. (2010) Digestion stability and evaluation of the metabolism and transport of olive oil phenols in the human small-intestinal epithelial Caco-2/TC7 cell line. Food Chemistry 119, 703–714.

Suarez, M., Valls, R.M., Romero, M.P., Macia, A., Fernandez, S., Giralt, M., Sola, R., & Motilva, M.J. (2011) Bioavailability of phenols from a phenol-enriched olive oil. British Journal of Nutrition 106, 1691–1701.

Tripoli, E., Giammanco, M., Tabacchi, G., Di Majo, D., Giammanco, S., & La Guardia, M. (2005) The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. Nutrition Research Reviews 18, 98–112.

Tuck, K.L., Freeman, M.P., Hayball, P.J., Stretch, G.L., & Stupans, I. (2001) The in vivo fate of hydroxytyrosol and tyrosol, antioxidant phenolic constituents of olive oil, after intravenous and oral dosing of labeled compounds to rats. The Journal of Nutrition 131, 1993–1996.

Tuck, K.L., & Hayball, P.J. (2002) Major phenolic compounds in olive oil: metabolism and health effects. The Journal of Nutritional Biochemistry 13, 636–644.

Tuck, K.L., Hayball, P.J., & Stupans, I. (2002) Structural characterization of the metabolites of hydroxytyrosol, the principal phenolic component in olive oil, in rats. Journal of Agricultural and Food Chemistry 50, 2404–2409.

Visioli, F., Galli, C., Bornet, F., Mattei, A., Patelli, R., Galli, G., & Caruso, D. (2000) Olive oil phenolics are dose-dependently absorbed in humans. FEBS Letters 468, 159–160.

Vissers, M.N., Zock, P.L., Roodenburg, A.J., Leenen, R., & Katan, M.B. (2002) Olive oil phenols are absorbed in humans. The Journal of Nutrition 132, 409–417.

Weinbrenner, T., Fito, M., Farre, A.M., Saez, G.T., Rijken, P., Tormos, C., Coolen, S., De la Torre, R., & Covas, M.I. (2003) Bioavailability of phenolic compounds from olive oil and oxidative/antioxidant status at postprandial state in healthy humans. Drugs under Experimental and Clinical Research 30, 207–212.

Yao, M., McClements, D.J., & Xiao, H. (2015) Improving oral bioavailability of nutraceuticals by engineered nanoparticle-based delivery systems. Current Opinion in Food Science 2, 14–19.

25 Antiatherogenic properties of olive oil glycolipids

Haralabos C. Karantonis

25.1 Introduction

Virgin olive oil (VOO) is the primary lipid source in the Mediterranean diet. Its consumption has been linked with positive health benefits like reduced systolic blood pressure, a strong trend toward increased plasma high-density lipoprotein cholesterol (HDL-C), and an enhancing effect on T-cell-mediated function (Rozati *et al.*, 2015; Sikand *et al.*, 2015). The beneficial effects of VOO on human health have previously been attributed to its high concentration of monounsaturated fatty acids (MUFAs). However, since other foods such as seed oils do not have similar health benefits to VOO despite the fact that they also contain high amounts of MUFAs (Aguilera *et al.*, 2004), the attention of research has moved to an effort to identify and study other compounds in VOO. A group of bioactive minor compounds in VOO that has been extensively studied is that of the phenolic compounds (Claro *et al.*, 2015; Hohmann *et al.*, 2015). Moreover, lipid compounds that act as inhibitors of the lipid thrombotic and inflammatory mediator known as platelet activating factor (PAF) have attracted scientific interest due to their unique structures and activities (Demopoulos *et al.*, 2003).

The purpose of this chapter is to highlight the studies on lipid functional components of olive oil that may modulate the activity or production of PAF, contributing in this way to the prevention against atherosclerosis development.

25.2 The role of inflammation in the development of chronic diseases

From the very beginning of human life, the ability of the human body to defend against pathogens and recover from infections and injuries was always and still remains a critical vital issue (Kotas & Medzhitov, 2015).

The human body encounters a huge number of stimuli on a daily basis. The etiologies of many of the injuries or infections in the human body are such stimuli that may eventually lead to lesion development in various organs, such as arteries. The human body uses inflammation as a mechanistic response to overcome pathogen infections and injuries. That is, the inflammatory response is recognized as a body promoting an immediate acute response to assist with its repair. Upon inflammatory response, a series of complex physiological repair pathways, catalyzed by specific enzymes, take place at the molecular level. The complex repair pathways of inflammation are finely regulated. Figure 25.1 shows that regulated inflammation after infection and injury may restore body homeostasis (Serhan *et al.*, 2015).

Any dysregulation to tissue homeostasis activates the innate immune cells, such as leukocytes, mast cells, and dendrite cells. Secreted cytokines such as tumor necrosis factor-α (TNFα) induce the chemotactic molecules of chemokines to attract these immune cells to the site of injury and infection, setting in this

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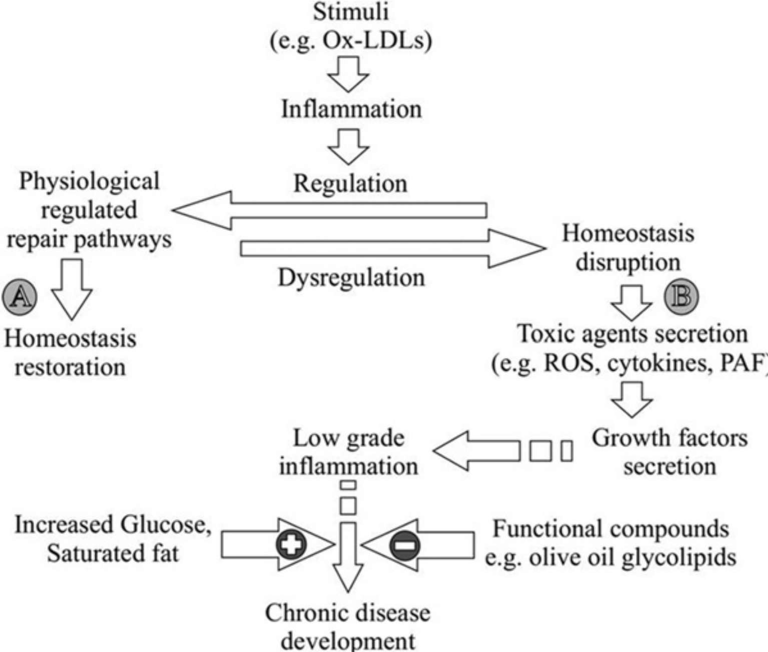

Figure 25.1 Schematic representation of (A) body homeostasis restoration by strict regulated inflammation or (B) chronic disease development by dysregulated inflammatory response; and the effects of various food components to promote or retard the progress of those diseases' development.

way a toxic inflammatory condition. Upon activation, immune cells produce various toxic agents in order to stop the cause of injury or infection. These agents include reactive oxygen species (ROS), reactive nitrogen species (RNS), specific proteases such as elastases and cathepsins, and lipid inflammatory mediators like PAF (Demopoulos *et al.*, 2003; Yost *et al.*, 2010).

Toxic agents produced by activated immune cells are also able to damage host tissues. For this reason, the rate of inflammation resolution is very critical in order to avoid or limit adverse effects (Nathan & Ding, 2010). Any delay or dysregulation of inflammation resolution may lead to the initiation of a chronic inflammatory condition characterized by cell recruitment, mainly macrophages (Kotas & Medzhitov, 2015). As shown in Figure 25.1, upon dysregulation, cells such as macrophages not only fail to assist in the regulatory functions of a homeostatic state, but instead secrete increased amounts or different types of growth factors and signals. These factors and signals cause, in turn, more immune cells to recruit in the site of inflammation, producing a toxic microenvironment that promotes tissue malfunction (Davies & Taylor, 2015).

Obviously, mediators of inflammation play an important role in maintaining a balance in an acute inflammatory response. If the initial acute response does not resolve the causative factor, then the inflammatory response will continue and the subsequent inflammatory microenvironment will disrupt tissue homeostasis, causing a low-grade chronic inflammatory condition that, as shown in Figure 25.1, would be the initiation of a chronic inflammatory disease (Libby *et al.*, 2014).

During recent decades, reports have shown that inflammation is linked with the pathophysiology of atherosclerosis and many other chronic disease states, such as type 2 diabetes mellitus, Alzheimer's disease, and osteoporosis (Bessueille & Magne, 2015). One of the most common treatments of inflammation has been the usage of nonsteroidal anti-inflammatory drugs (NSAIDs) that inhibit the synthesis of lipid compounds that exert diverse hormone-like effects in organisms known as prostaglandins. The critical issue on NSAIDs usage is that by inhibiting prostaglandin synthesis, they only alleviate inflammatory symptoms such as pain and edema, but they do not eliminate the causative factor, which would lead to inflammation resolution. For this reason, the inflammatory process continues and develops into a chronic state, rendering NSAIDs noncurative substances (Kohli *et al.*, 2014).

25.3 The role of diet in inflammation

Studies in recent decades have revealed a link between inflammation and nutrition (Minihane *et al.*, 2015; Mlakar *et al.*, 2015). It has been shown that an excessive intake of macronutrients such as glucose and saturated fat promotes inflammatory responses in terms of increment of inflammatory transcription factors and inflammatory cytokine activity (Jansen *et al.*, 2013; Shrestha *et al.*, 2013). Such inflammatory responses on a daily basis are believed to contribute to the pathogenesis of chronic inflammatory diseases by establishing a continuous low-grade inflammatory state (Bessueille & Magne, 2015).

On the other hand, as shown in Figure 25.1, specific dietary habits may alleviate inflammatory processes through functional compounds that various foods contain. The Seven Countries Study showed that the Mediterranean diet, a diet characterized by naturally derived foods, such as vegetables, fruits, nuts, fish, and grains, exerted beneficial effects in terms of prevention of coronary heart disease (Keys, 1995). Those results have been confirmed by more recent studies showing improvement of cardiovascular function by adherence to a Mediterranean-style diet (Lee *et al.*, 2015; Widmer *et al.*, 2015).

25.4 PAF and its metabolism as a searching tool for functional components with antiatherogenic activity

PAF has been characterized as the glycerylether phospholipid of 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (Demopoulos *et al.*, 1979). It is produced and released by various stimulated cells such as platelets, leukocytes, monocytes, macrophages, and foam and endothelial cells, and it represents a highly active lipid mediator in many critical mechanisms taking place in chronic disease development. Figure 25.2 shows that PAF promotes through its receptor a wide range of biological responses such as oxidation, inflammation, and thrombosis that have been linked to the pathogenesis of atherosclerosis and other chronic diseases (Demopoulos *et al.*, 2003).

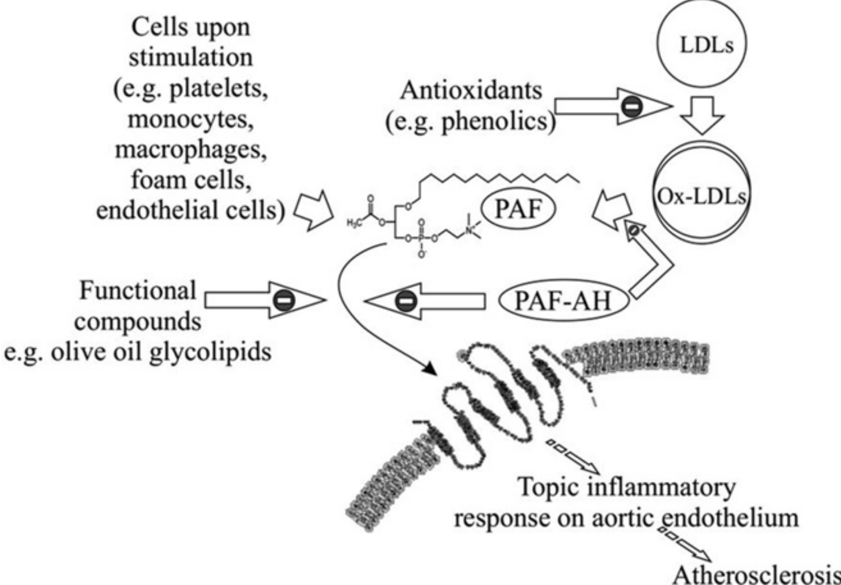

Figure 25.2 Schematic representation of the inflammatory response and atherosclerosis development mediated by platelet activating factor (PAF), along with the inhibitory activity of olive oil functional compounds on this biochemical procedure.

PAF levels are under strict metabolic control, and its action is mediated through specific PAF-receptors (PAFRs), which are members of the seven-transmembrane domain-receptor family coupled to G-proteins (Ishii & Shimizu, 2000). The biosynthesis of PAF is accomplished through two distinctive enzymatic pathways. These are the *de novo* pathway, catalyzed by a specific dithiothreitol-insensitive CDP-choline, 1-alkyl-2-acetyl-*sn*-glycerol cholinephosphotransferase (PAF-cholinephosphotransferase [PAF-CPT], EC 2.7.8.16), which converts 1-O-alkyl-2-acetyl-glycerol to PAF; and the remodeling pathway, catalyzed by lyso-PAF:acetyl-CoA acetyltransferase (lyso-PAF-acetyltransferase [lyso-PAFAT], EC 2.3.1.67) (Snyder, 1995), which acetylates lyso-PAF. On the other hand, the catabolism of PAF to its biologically inactive form is catalyzed by a PAF-specific acetylhydrolase (PAF-AH, EC 3.1.1.47) whose plasma form is known as lipoprotein-associated phospholipase A2 (Lp-PLA2) (Stafforini, 2009). Apart from hydrolysis of the *sn*-2 acetyl group of PAF, PAF-AH also cleaves short-chain acyl chains from the *sn*-2 position of oxidized phospholipids known as PAF-like lipids due to their PAF-like activities (Demopoulos *et al.*, 2003).

The critical role of PAF in atherosclerosis is indicated by biological activities such as cell-mediated stimulation of active oxygen species production, induction of platelet aggregation, activation of platelets and vascular cells, and growth of smooth muscle cells. PAF also mediates increase of endothelium permeability, promotes angiogenesis, and stimulates the cellular release of chemokines, cytokines, and growth factors; and its activity is critical to cell–cell interactions upon inflammation. Moreover, PAF is produced during LDL oxidation, and at the same time the activity of PAF-acetylhydrolase – its main catabolic enzyme – exhibits reduced activity in oxidized LDL (ox-LDL) compared to unmodified LDL. Interestingly, PAF-AH in high-density lipoprotein (HDL) protects against the production and activity of Ox-LDLs by facilitating hydrolysis of PAF-like lipids, and it is able to suppress atherogenesis development (Demopoulos *et al.*, 2003).

The above indicates that dysregulation of PAF metabolism may develop an inflammatory microenvironment that may initiate atherosclerosis development (Demopoulos *et al.*, 2003; Ninio, 2005). As shown in Figure 25.2, any compound that is able to inhibit or antagonize the production and/or activity of PAF may be characterized as a functional compound with at least antiatherogenic properties.

25.5 Functional components of olive oil with antiatherogenic properties

The amount of minor components in VOO depends on various conditions, such as climate, degree of olive fruit ripeness, and postharvest olive fruit handling. These minor compounds are in higher quantities in VOOs compared to other oils that have been refined or are characterized by higher acidity (Karantonis *et al.*, 2002).

The majority of studies concerning olive oil have focused mostly on its fatty acid composition and phenolics, while other minor components such as tocopherols, hydroxyterpenic acids, phytosterols, glycolipids, and phospholipids are less studied.

The protective effect of olive oil has been mainly attributed to oleic acid and its phenolic antioxidant properties. Until now, only olive oil polyphenols have been recognized by the European Food Safety Authority (EFSA) as beneficial to human health due to their antioxidant activity, which protects LDL particles from oxidative damage (EFSA, 2011). More specifically, hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) have been shown as the functional compounds of olive oil with such activity. In order to bear the health claim, olive oil should provide 5 mg of these compounds daily, which may not be the case for all olive oils in the market when someone consumes olive oil in the context of a balanced diet (EFSA, 2011).

Evidence accumulated to date on the functional properties of foods demonstrates that this functionality of foods is not just the sum of its components. Studies have shown that the presence of phenolic compounds exerting antioxidant activities *in vitro* and *in vivo* (Claro *et al.*, 2015; Hohmann *et al.*, 2015) explains only partially the beneficial effects of VOO consumption.

25.5.1 Glycolipids of olive oil as functional components with antiatherogenic properties

The critical role of PAF in atherosclerosis and the well-accepted beneficial effects of VOO on human health have been taken into consideration in an effort to isolate and identify bioactive minor compounds in VOO

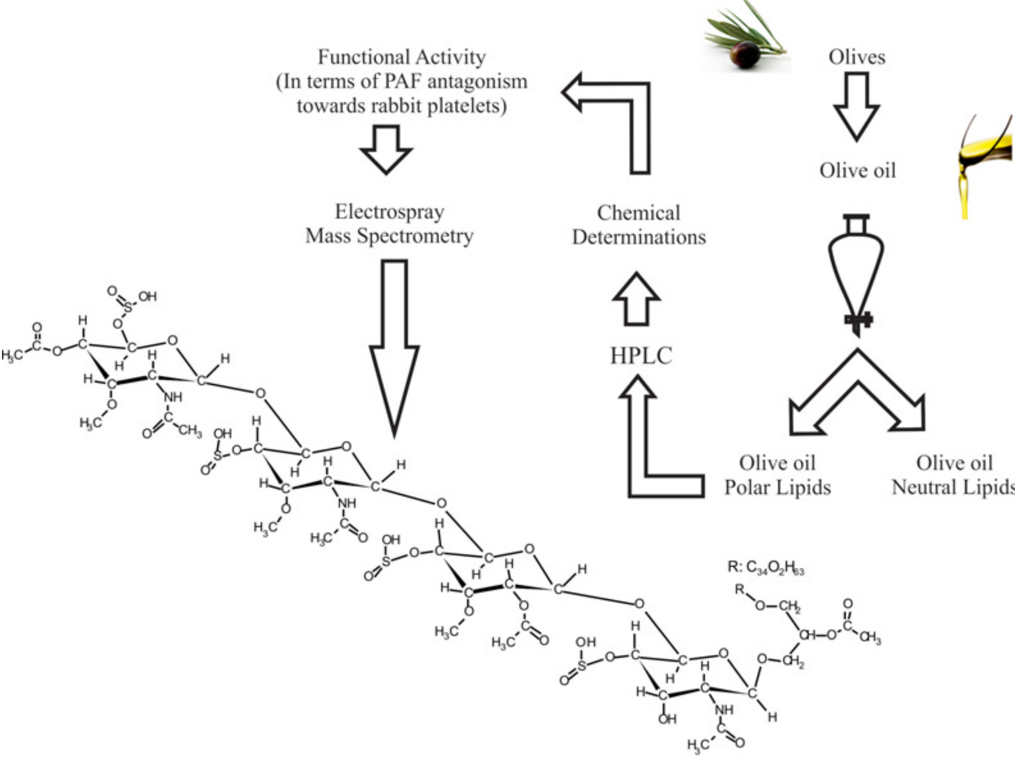

Figure 25.3 Working flowchart for the identification of olive oil functional polar lipids; and the proposed structure of the most active functional glycolipid in the olive oil polar lipid class, identified as glycerol ether glycolipid, that exerts antiatherogenic activities in terms of PAF antagonism in an *in vitro* rabbit platelet aggregation assay.

other than MUFAs and phenolic compounds and to elucidate mechanisms through which they exert health-beneficial effects against atherosclerosis.

A specific PAF antagonist, namely BN 52021, has been shown to exert a protective effect in diet-induced atherosclerosis in rabbis (Feliste *et al.*, 1989). Later, it was noted for the first time that polar minor compounds of VOO act as PAF antagonists (Koussissis *et al.*, 1993). Based on the aforementioned research, it was proposed as a working hypothesis that VOO may contain minor components that could slow down atherosclerosis development by antagonizing PAF (Antonopoulou & Demopoulos, 1997).

The interest in research on the ability of polar lipid extracts from VOO to antagonize inflammatory mediators such as PAF and the possible increased nutritional value of these dietary compounds in the prevention of inflammatory chronic diseases prompted further research. A modification of a countercurrent distribution extraction procedure was developed to explore the polar lipid class of virgin oil by separating VOO into its two major lipid classes, namely polar lipids and neutral lipids (Karantonis *et al.*, 2002). Moreover, a new high-performance liquid chromatographic (HPLC) system was also developed to efficiently separate VOO polar lipids constituents within 60 min (Nomikos *et al.*, 2002). Using the above methodology in 12 different samples of VOOs originating from various Greek areas and various extraction procedures, it was confirmed that their bioactivity was mainly attributed to their polar lipid class, whereas their neutral lipid class exerted lower bioactivity in terms of PAF inhibition and/or antagonism. It was proposed that total lipid bioactivity could serve as a nutritional index in VOOs.

Separation by HPLC, chemical determinations, biological tests, and electrospray mass spectrometry showed a glycerol ether glycolipid, shown in Figure 25.3, with a molecular weight equal to m/z 1789 as the most active functional compound in the polar lipid fraction of VOO (Karantonis *et al.*, 2002).

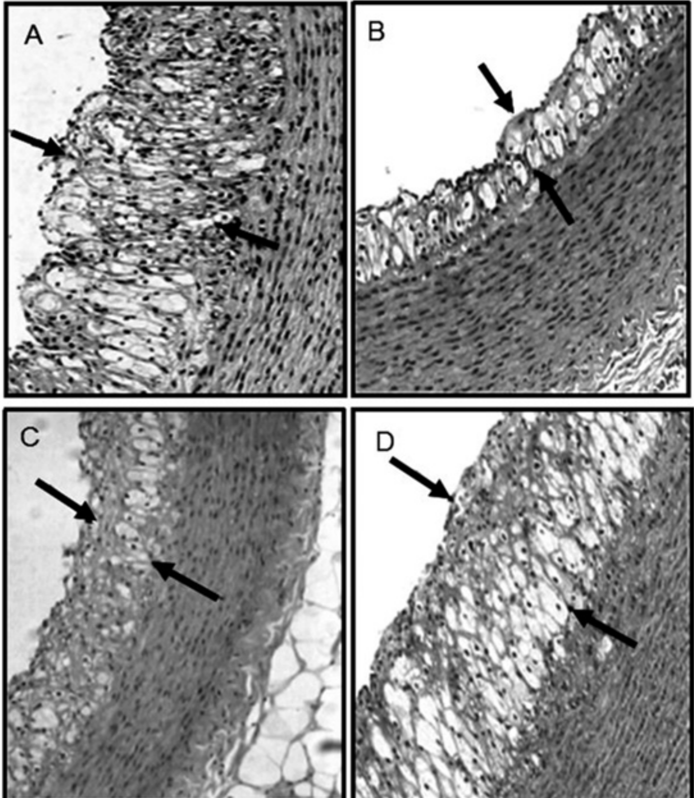

Figure 25.4 Representative optic micrographs of aortic wall sections stained with hematoxylin and eosin. Atherosclerotic lesions appear as foam cells where black arrows indicate thickness of atherosclerotic lesions. Atherosclerotic lesions in rabbit aortas on: (A) atherogenic diet; (B) atherogenic diet supplemented with olive oil; (C) atherogenic diet supplemented with polar lipids from olive oil; and (D) atherogenic diet supplemented with neutral lipids from olive oil.

The effect of VOO polar lipids in diet-induced atherosclerosis development has been studied *in vivo* in male white New Zealand rabbits (Karantonis *et al.*, 2006a). Figure 25.4 shows that in this study, supplementation in diet-induced hyperchlosterolemic rabbits with either 15% (w/w) VOO in food or polar lipid extracts that were procured from the same volume of VOO led to reduction of early atherosclerosis lesions compared to the group that received only an atherogenic diet. On the other hand, rabbits that received neutral lipid extracts from the corresponding VOO did not exhibit reduction of atherosclerosis lesions compared to the control group. The reduced lesions from polar VOO lipids were in agreement with a maintenance in aortic wall elasticity to values near to those of rabbits that consumed only a typical diet. This effect was not observed in rabbits that consumed neutral VOO extract in their diet. The results revealed for the first time that in *in vivo* conditions, the nutritional value of VOO in terms of antiatherogenic activities in early lesion development is mainly attributed to the polar lipid class of VOO. In this study, it was also observed that PAF metabolism is modulated during atherosclerosis development, since plasma PAF-AH activity was increased as a response to blood PAF levels, indicating a countervailing protective mechanism.

Those results, in agreement with previous studies in *in vitro* conditions (Karantonis *et al.*, 2002), reinforce the view that *in vitro* activity against PAF toward platelets could be used as a nutritional value index. It is worth mentioning that the usage of VOO in preparing meals, as a source of PAF inhibitors and/or antagonists, may provide more healthy choices for consumers in terms of prevention against chronic disease development or progression (Karantonis *et al.*, 2006b; Aronis *et al.*, 2007).

Interestingly enough, it has been shown that polar lipids that inhibit or antagonize PAF exist also in by-products of VOO during production (Karantonis *et al.*, 2008), rendering VOO by-products candidate material for isolation of functional minor constituents that could be used to enrich foodstuffs in order to produce new functional foods with increased nutritional value in terms of health impact comparable to that of VOO. Extraction and further fractionation of total polar lipids from olive oil, pomace, pomace oil, and waste by-products showed the existence of bioactive polar lipids from all but the refined pomace olive oil sample, indicating that a significant amount of functional minor compounds are drawn away during the refining procedure to obtain olive oil. The most potent bioactive compound present in olive pomace (glycerol ether glycolipid) has been shown to share common bioactivity and structure with VOO, and the authors concluded that such compounds could be useful in producing healthy functional foods with antiatherogenic activities (Karantonis *et al.*, 2008).

The bioactivity of olive pomace polar lipids in comparison to those of VOO in *in vivo* conditions was further investigated by supplementing the diet of induced hypercholesterolemic rabbits with either olive pomace polar lipids or VOO polar lipids (Tsantila *et al.*, 2007). Both extracts inhibited PAF activity toward washed rabbit platelet aggregation as well as its specific binding on its receptor on rabbit platelets. Results from the *in vivo* dietary intervention showed a decrease in platelet sensitivity of rabbits that consumed polar lipids either from VOO or from olive pomace, determined by their increased EC_{50} values for PAF-induced aggregation in their platelet-rich plasma. In all groups, blood PAF levels increased upon atherogenesis. Blood PAF level increase was accompanied by an increased activity of plasma PAF-AH in all groups as a countervailing protective mechanism.

Morphometric assessment concerning early atherosclerosis lesions showed that consumption of polar lipids from VOO or olive pomace led to lower early atherosclerosis lesion development compared to the control group that consumed only an atherogenic diet, confirming their beneficial effects against inflammatory disease development.

In a further study (Tsantila *et al.*, 2010), olive pomace polar lipids were studied for their activities in preventing the progression of established atherogenic lesions in diet-induced hypercholesterolemic rabbits. As shown in Figure 25.5, it was observed that diet-induced atherogenesis continued to progress after replacing an atherogenic diet with a typical diet, while the replacement of an atherogenic diet with a typical diet supplemented with olive pomace polar lipids in rabbits with established early atherosclerotic lesions led to stabilization and regression of those lesions. In that study, it was also shown that the protective effect of olive pomace polar lipids was comparable to that of simvastatin, a known drug of the statin group that act as 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase inhibitors, which is used to lower cholesterol and thus to lower the risk of stroke, heart attack, and other heart complications in people with diabetes, coronary heart disease, or other risk factors (Tsantila *et al.*, 2010).

Other studies indicate that olive pomace polar lipids, as PAF inhibitors and/or antagonists, also have protective effects on other disease models. The presence of olive pomace polar lipids in cultured human mesangial cells has been shown to significantly decrease the biosynthetic enzyme of PAF-CPT activity and increase the catabolic enzyme of PAF-AH activity, reducing in this way PAF levels and its subsequent proinflammatory manifestations. In addition, the implication of PAF in cancer and the beneficial role of PAF inhibitors and antioxidants in its growth and metastasis have also been described. These studies show that polar lipids from VOO and olive pomace may protect from various chronic diseases that share common mechanisms at the molecular level, including oxidation, inflammation, and thrombosis (Tsoupras *et al.*, 2009, 2011).

It is worth mentioning that it has been demonstrated that microporous materials are able to isolate biologically active fractions enriched in functional minor compounds that act as PAF inhibitors from olive mill wastes in a viable way that could be used for dietary supplements production with increased nutritional value (Stamatakis *et al.*, 2009).

In recent studies, fish oil in fish feed was partially substituted with olive pomace as a rich source of PAF inhibitors/antagonists, in an attempt to improve the nutritional value of the gilthead sea bream (*Sparus aurata*) and produce a functional fish that, when consumed, will exert increased protective effects against atherosclerosis development under the guidelines of EC1924/2006. The results showed that supplementation of gilthead sea bream with a diet enriched with olive pomace resulted in a fish with increased antiatherogenic properties in terms of PAF inhibition in an *in vitro* washed rabbit platelet aggregation assay compared to the fish that consumed a typical diet (Nasopoulou *et al.*, 2011, 2013, 2014).

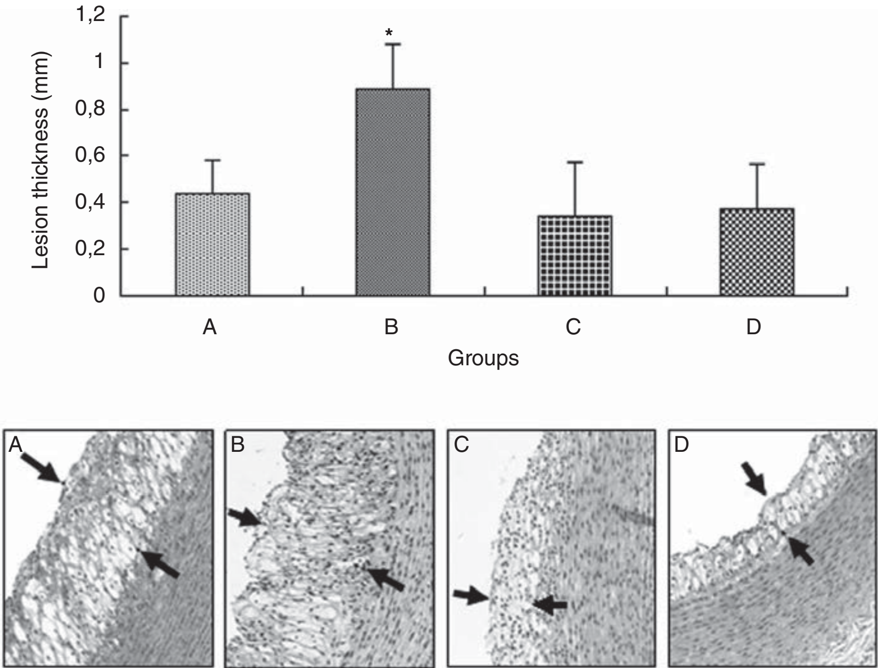

Figure 25.5 Bar chart displaying atherosclerotic lesions in rabbit aortas accompanied with representative optic micrographs of aortic wall sections stained with hematoxylin and eosin on: (A) 6-week atherogenic diet; (B) 6-week atherogenic diet and additional 3 weeks on a typical diet; (C) 6-week atherogenic diet and additional 3 weeks on a typical diet supplemented with polar lipids from olive oil; and (D) 6-week atherogenic diet and additional 3 weeks on a typical diet supplemented with simvastatin. Results are expressed as mean ± standard deviation. ∗ Denotes significant difference of B compared to A, C, and D for $p < 0.05$. In optic micrographs, atherosclerotic lesions appear as foam cells where black arrows indicate thickness of atherosclerotic lesions.

25.6 Conclusion

It has been shown that olive oil is a functional food that potentially modifies physiological body functions and prevents atherosclerosis development. Scientific evidence indicates that the protective effects of olive oil can be ascribed to its content of not only oleic acid, phenolic compounds, hydrocarbons, and triterpenes or phytosterols, but also other minor functional compounds like glycerol ether glycolipids. A number of studies demonstrate that long-term dietary consumption of VOO would deliver over time functional olive oil glycolipids that act as PAF inhibitors and/or antagonists, which may attenuate the postprandial inflammatory response in the human body, reducing in this way the risk for an atherosclerosis development. It is necessary to always consider both the absorption and the interactions of any functional olive compounds with other compounds in the same or other food in the diet. It should be underlined that in a food like olive oil, functional minor compounds belonging to different chemical groups may have complementary, additive, or even synergic effects on various physiological functions in order to exert the most of their beneficial effects. Extensive studies on the absorption, metabolism, excretion, toxicity, and efficacy for the functional compounds of olive oil are needed in order to explore and understand their mechanism of action and their role in atherosclerosis prevention and human health improvement as part of a balanced diet.

References

Aguilera, C.M., Mesa, M.D., Ramirez-Tortosa, M.C., Nestares, M.T., Ros, E., & Gil, A. (2004) Sunflower oil does not protect against LDL oxidation as virgin olive oil does in patients with peripheral vascular disease. Clinical Nutrition 23, 673–681.

Antonopoulou, S., & Demopoulos, C.A. (1997) On the Mediterranean diet. International News on Fats, Oils, Surfactants, Detergents, and Elated Materials 8, 776–777.

Aronis, P., Antonopoulou, S., Karantonis, H.C., Phenekos, C., & Tsoukatos, D.C. (2007) Effect of fast-food Mediterranean-type diet on human plasma oxidation. Journal of Medicinal Food 10, 511–520.

Bessueille, L., & Magne, D. (2015) Inflammation: a culprit for vascular calcification in atherosclerosis and diabetes. Cellular and Molecular Life Sciences 72, 2475–2489.

Claro, C., Ogalla, E., Rodriguez-Rodriguez, R., Herrera, M.D., & Alvarez de Sotomayor, M. (2015) Phenolic content of extra virgin olive oil is essential to restore endothelial dysfunction but not to prevent vascular inflammation in atherosclerotic lesions of Apo E deficient mice. Journal of Functional Foods 15, 126–136.

Davies, L.C., & Taylor, P.R. (2015) Tissue-resident macrophages: then and now. Immunology 144, 541–548.

Demopoulos, C.A., Karantonis, H.C., & Antonopoulou, S. (2003) Platelet activating factor: a molecular link between atherosclerosis theories. European Journal of Lipid Science and Technology 105, 705–716.

Demopoulos, C.A., Pinckard, R.N., & Hanahan, D.J. (1979) Platelet-activating factor: evidence for 1-0-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine as the active component (a new class of lipid chemical mediators). Journal of Biological Chemistry 254, 9355–9358.

European Food Safety Authority (EFSA). (2011) Scientific opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL cholesterol concentrations (ID 1639), maintenance of normal blood pressure (ID 3781), "antiinflammatory properties" (ID 1882), "contributes to the upper respiratory tract health" (ID 3468), "can help to maintain a normal function of gastrointestinal tract" (3779), and "contributes to body defences against external agents" (ID 3467) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 9(4), 2033. doi:10.2903/j.efsa.2011.2033.

Feliste, R., Perret, B., Braquet, P., & Chap, H. (1989) Protective effect of BN 52021: a specific antagonist of platelet-activating factor (PAF-acether) against diet-induced cholesteryl ester deposition in rabbit aorta. Atherosclerosis 78, 151–158.

Hohmann, C.D., Cramer, H., Michalsen, A., Kessler, C., Steckhan, N., Choi, K., & Dobos, G. (2015) Effects of high phenolic olive oil on cardiovascular risk factors: a systematic review and meta-analysis. Phytomedicine 22, 631–640.

Ishii, S., & Shimizu, T. (2000) Platelet-activating factor (PAF) receptor and genetically engineered PAF receptor mutant mice. Progress in Lipid Research 39, 41–82.

Jansen, F., Yang, X., Franklin, B.S., Hoelscher, M., Schmitz, T., Bedorf, J., Nickenig, G., & Werner, N. (2013) High glucose condition increases NADPH oxidase activity in endothelial microparticles that promote vascular inflammation. Cardiovascular Research 98, 94–106.

Karantonis, H.C., Antonopoulou, S., & Demopoulos, C.A. (2002) Antithrombotic lipid minor constituents from vegetable oils: comparison between olive oils and others. Journal of Agricultural and Food Chemistry 50, 1150–1160.

Karantonis, H.C., Antonopoulou, S., Perrea, D.N., Sokolis, D.P., Theocharis, S.E., Kavantzas, N., Iliopoulos, D.G., & Demopoulos, C.A. (2006a) In vivo antiatherogenic properties of olive oil and its constituent lipid classes in hyperlipidemic rabbits. Nutrition, Metabolism and Cardiovascular Diseases 16, 174–185.

Karantonis, H.C., Fragopoulou, E., Antonopoulou, S., Rementzis, J., Phenekos,C., & Demopoulos, C.A. (2006b) Effect of fast-food Mediterranean-type diet on type 2 diabetics and healthy human subjects' platelet aggregation. Diabetes Research and Clinical Practice 72, 33–41.

Karantonis, H.C., Tsantila, N., Stamatakis, G., Samiotaki, M., Panayotou, G., Antonopoulou, S., & Demopoulos, C.A. (2008) Bioactive polar lipids in olive oil, pomace and waste byproducts. Journal of Food Biochemistry 32, 443–459.

Keys, A. (1995) Mediterranean diet and public health: personal reflections. American Journal of Clinical Nutrition 61, 1321S–1323S.

Kohli, P., Steg, P.G., Cannon, C.P., Smith Jr., S.C., Eagle, K.A., Ohman, E.M., Alberts, M.J., Hoffman, E., Guo, J., Simon, T., Sorbets, E., Goto, S., & Bhatt, D.L. (2014) NSAID use and association with cardiovascular outcomes in outpatients with stable atherothrombotic disease. American Journal of Medicine 127, 53–60.

Kotas, M.E., & Medzhitov, R. (2015) Homeostasis, inflammation, and disease susceptibility. Cell 160, 816–827.

Koussissis, S.G., Semidalas, C.E., Antonopoulou, S., Kapoulas, C.A., Demopoulos, C.A., & Kalyvas, V. (1993) PAF antagonists in foods: isolation and identification of PAF in virgin olive oil. Revue Française des Corps Gras 40, 323–327.

Lee, J., Pase, M., Pipingas, A., Raubenheimer, J., Thurgood, M., Villalon, L., Macpherson, H., Gibbs, A., & Scholey, A. (2015) Switching to a 10-day Mediterranean-style diet improves mood and cardiovascular function in a controlled crossover study. Nutrition 31, 647–652.

Libby, P., Tabas, I., Fredman, G., & Fisher, E.A. (2014) Inflammation and its resolution as determinants of acute coronary syndromes. Circulation Research 114, 1867–1879.

Minihane, A.M., Vinoy, S., Russell, W.R., Baka, A., Roche, H.M., Tuohy, K.M., Teeling, J.L., Blaak, E.E., Fenech, M., Vauzour, D., McArdle, H.J., Kremer, B.H.A., Sterkman, L., Vafeiadou, K., Benedetti, M.M., Williams, C.M., & Calder, P.C. (2015) Low-grade inflammation, diet composition and health: current research evidence and its translation. British Journal of Nutrition 114, 999–1012.

Mlakar, P., Salobir, B., Čobo, N., Strašek, J., Prezelj, M., Debevc, A., Jug, B., Terčelj, M., & Šabovič, M. (2015) The effect of cardioprotective diet rich with natural antioxidants on chronic inflammation and oxidized LDL during cardiac rehabilitation in patients after acute myocardial infarction. IJC Heart and Vasculature 7, 40–48.

Nasopoulou, C., Gogaki, V., Stamatakis, G., Papaharisis, L., Demopoulos, C.A., & Zabetakis, I. (2013) Evaluation of the in vitro anti-atherogenic properties of lipid fractions of olive pomace, olive pomace enriched fish feed and gilthead sea bream (*Sparus aurata*) fed with olive pomace enriched fish feed. Marine Drugs 11, 3676–3688.

Nasopoulou, C., Smith, T., Detopoulou, M., Tsikrika, C., Papaharisis, L., Barkas, D., & Zabetakis, I. (2014) Structural elucidation of olive pomace fed sea bass (*Dicentrarchus labrax*) polar lipids with cardioprotective activities. Food Chemistry 145, 1097–1105.

Nasopoulou, C., Stamatakis, G., Demopoulos, C.A., & Zabetakis, I. (2011) Effects of olive pomace and olive pomace oil on growth performance, fatty acid composition and cardio protective properties of gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). Food Chemistry 129, 1108–1113.

Nathan, C., & Ding, A. (2010) Nonresolving inflammation. Cell 140, 871–882.

Ninio, E. (2005) Phospholipid mediators in the wall: involvement in atherosclerosis. Current Opinion in Clinical Nutrition and Metabolic Care 8, 123–131.

Nomikos, T., Karantonis, H.C., Fragopoulou, E., & Demopoulos, C.A. (2002) One-step separation system for the main phospholipids, glycolipids, and phenolics by normal phase HPLC: application to polar lipid extracts from olive and sunflower oils. Journal of Liquid Chromatography and Related Technologies 25, 137–149.

Rozati, M., Barnett, J., Wu, D., Handelman, G., Saltzman, E., Wilson, T., Li, L., Wang, J., Marcos, A., Ordovás, J.M., Lee, Y.-C., Meydani, M., & Meydani, S.N. (2015) Cardio-metabolic and immunological impacts of extra virgin olive oil consumption in overweight and obese older adults: a randomized controlled trial. Nutrition and Metabolism 12, art. no. 22.

Serhan, C.N., Chiang, N., Dalli, J., & Levy, B.D. (2015) Lipid mediators in the resolution of inflammation. Cold Spring Harbor Perspectives in Biology 7, a016311.

Shrestha, C., Ito, T., Kawahara, K.-I., Shrestha, B., Yamakuchi, M., Hashiguchi, T., & Maruyama, I. (2013) Saturated fatty acid palmitate induces extracellular release of histone H3: a possible mechanistic basis for high-fat diet-induced inflammation and thrombosis. Biochemical and Biophysical Research Communications 437, 573–578.

Sikand, G., Kris-Etherton, P., & Boulos, N.M. (2015) Impact of functional foods on prevention of cardiovascular disease and diabetes. Current Cardiology Reports 17, 16–20.

Snyder, F. (1995) Platelet-activating factor: the biosynthetic and catabolic enzymes. Biochemical Journal 308, 1039–1043.

Stafforini, D.M. (2009) Biology of platelet-activating factor acetylhydrolase (PAF-AH, lipoprotein associated phospholipase A2). Cardiovascular Drugs and Therapy 23, 73–83.

Stamatakis, G., Tsantila, N., Samiotaki, M., Panayotou, G.N., Dimopoulos, A.C., Halvadakis, C.P., & Demopoulos, C.A. (2009) Detection and isolation of antiatherogenic and antioxidant substances present in olive mill wastes by a novel filtration system. Journal of Agricultural and Food Chemistry 57, 10554–10564.

Tsantila, N., Karantonis, H.C., Perrea, D.N., Theocharis, S.E., Iliopoulos, D.G., Antonopoulou, S., & Demopoulos, C.A. (2007) Antithrombotic and antiatherosclerotic properties of olive oil and olive pomace polar extracts in rabbits. Mediators of Inflammation art. no. 36204.

Tsantila, N., Karantonis, H.C., Perrea, D.N., Theocharis, S.E., Iliopoulos, D.G., Iatrou, C., Antonopoulou, S., & Demopoulos, C.A. (2010) Atherosclerosis regression study in rabbits upon olive pomace polar lipid extract administration. Nutrition, Metabolism and Cardiovascular Diseases 20, 740–747.

Tsoupras, A.B., Iatrou, C., Frangia, C., & Demopoulos, C.A. (2009) The implication of platelet activating factor in cancer growth and metastasis: potent beneficial role of PAF-inhibitors and antioxidants. Infectious Disorders – Drug Targets 9, 390–399.

Tsoupras, A.B., Fragopoulou, E., Iatrou, C., & Demopoulos, C.A. (2011) In vitro protective effects of olive pomace polar lipids towards platelet activating factor metabolism in human renal cells. Current Topics in Nutraceutical Research 9, 105–110.

Widmer, R.J., Flammer, A.J., Lerman, L.O., & Lerman, A. (2015) The Mediterranean diet, its components, and cardiovascular disease. American Journal of Medicine 128, 229–238.

Yost, C.C., Weyrich, A.S., & Zimmerman, G.A. (2010) The platelet activating factor (PAF) signaling cascade in systemic inflammatory responses. Biochimie 92, 692–697.

26 Nutritional and health aspects of olive oil and diseases

Elizabeth Lenart, Apostolos Kiritsakis, and Walter Willett

26.1 Introduction

Many comparisons of health status in various populations include assessment of the dietary pattern of people living in the area of the Mediterranean Sea. Briefly, this pattern refers to traditional foods consumed in southern European and the northern areas of African countries over several hundred years. Often, these examinations are based on diets consumed up to the 1960s and 1970s. Prior to that, many people living in these areas consumed relatively low levels of animal products and saturated fat. Hydrogenated oil consumption was also low, with most likely coming from the small amount that is naturally occurring in the fat of ruminants (Uauy *et al.*, 2009).

Consumption of olive oil, fruits, seeds, vegetables, legumes, whole grains, and cereals was also plentiful, and these foods along with seafood made up much of the traditional diet. This pattern began to shift once incomes rose and it became possible to procure foods outside local regions inexpensively, leading to the influx of modern, more processed foods. While the diet has changed in some profound ways over time, even today, there is a cultural tradition that includes strong ties to certain foods in this area of the world (Donini *et al.*, 2015). Clearly, the good health that these populations have traditionally exhibited, is multifactorial. However, as time goes on, more and more evidence accrues supporting the contribution of diet and some specific components of diet to this good health. Olive oil was and is a substantial contributor to the diets of people living in the Mediterranean area.

The “traditional Mediterranean diet” that we often think of probably included what was available locally to those of modest means (Nowak, 2011). The idea that any particular group of foods was chosen specifically for health reasons was adopted more recently. Prior to modern transport methods, in most cultures, income, soil, weather patterns, geographic area, and length of growing season determined what you grew and ate. People of this region were, in a sense, forced by economics, weather, and circumstance to eat what we have come to call the Mediterranean diet. When researchers started examining the diets and other lifestyle habits after World War II, the average person was unable to afford large amounts of expensive foods such as meat. When the standard of living goes up, people often add more meat, dairy products, and premade foods to their diet. In countries throughout the world where incomes have gone up, access to a more diverse, but not necessarily healthier, diet becomes the norm. This happened in southern European countries such as Greece, where between 1960 and 2005 incomes rose appreciably and people are now consuming about three times as much beef and far more meat in total than they ate in the early 1960s (Garcia-Closas *et al.*, 2006; Gerbens-Leenes *et al.*, 2010). In Crete (Figure 26.1), consumption of the classic Mediterranean diet remains more prevalent than in some mainland areas of Greece and other parts of southern Europe. Still, there are some generational differences that have been noted, even when selecting from only traditional foods. Younger Cretan women reported consuming significantly higher intakes of sugar when compared to their mothers (Tsakiraki *et al.*, 2011). The increased demand for easily prepared foods and snacks is being met by industrial-level production of foods with long shelf lives. The composition of shopping venues has

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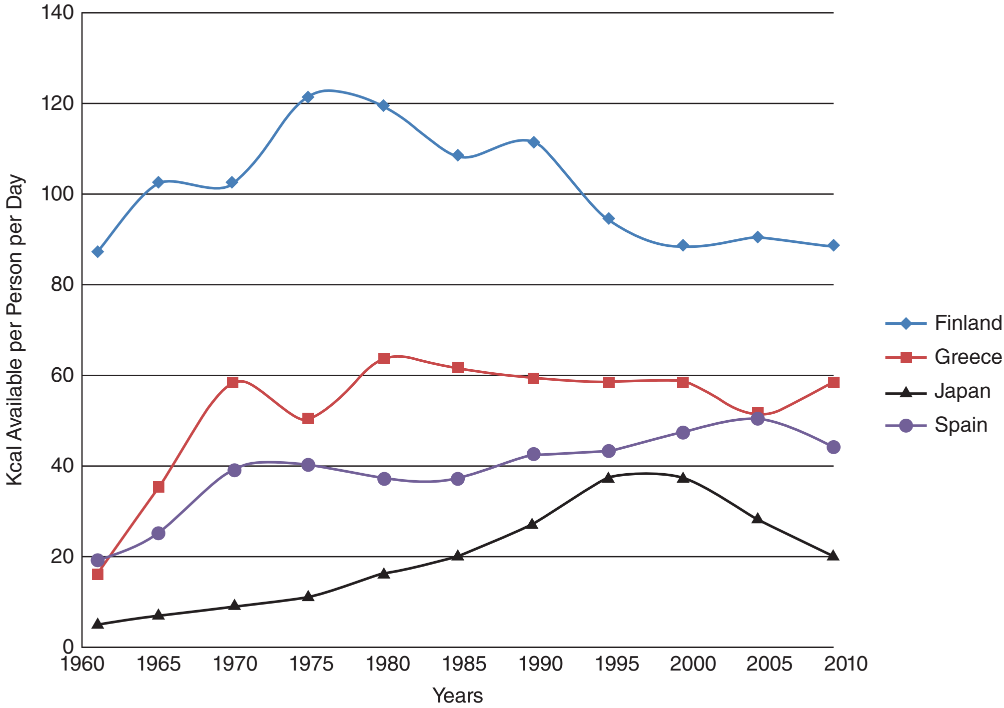

Figure 26.1 Availability of beef in the food supply, 1960–2010. *Source*: FAOSTAT (2015).

changed over time, and large supermarkets and discount megastores account for up to 90% of shopping in some major Greek cities and more urban areas (Foreign Agricultural Service [FAS], 2012). This may ultimately decrease the overall costs of packaged and prepared foods, which can be purchased economically by retailers in large quantities relative to the costs of fresh fruits, vegetables, and high-quality extra virgin olive oil (EVOO), along with other foods that are more perishable.

Fat intake has not traditionally been low in most Mediterranean countries, but rather has ranged from about 25 to 40% of energy depending on the particular location and dietary constraints (Keys, 1980; Kromhout *et al*., 1989; Fogel & Helmchen, 2002). Disease and mortality rates have typically been low. Traditional dietary patterns in the USA, Greece, Scandinavia, and Asia during the 1960s were quite different, although the portion of calories coming from total fat in Greece, Finland, and the USA was almost identical, while in Spain and Italy fat intake was somewhat lower. Japan had low fat intake at this time and still consumes less fat than many cultures (Wakai *et al*., 2014). The specific types of fat consumed in these different regions, however, was very different. Most often, the typical diet of Crete prior to about 1960 is the food pattern people are referring to when they describe a traditional Mediterranean diet (Simopoulos, 2004; Menotti & Puddu, 2015).

Historically, a common feature of Mediterranean diets was the use of olive oil as a primary fat. In other parts of Europe, the most commonly used fats would come from other vegetable sources, but also largely from dairy and other animal fats, particularly in Scandinavia. In areas such as the Pacific Islands, dietary fats traditionally came partly from coconut or palm oil and seafood. We review here the relationship between the amount and type of dietary fat and the incidence of major chronic diseases of Western countries, with a particular emphasis on the role of olive oil and monounsaturated fat. Thus, in this chapter, we provide an overview of literature related to examination of diet and in particular dietary fats and several aspects of health status, including coronary disease, several cancers, and obesity. Furthermore, we will examine the evidence regarding possible health impacts of using olive oil with its unique functional compounds as the primary source of fat, along with other foods considered part of a traditional Mediterranean diet.

26.2 Dietary lipids and cardiovascular disease

Dietary recommendations in the past promoted a reduction in the intake of total dietary fat to decrease risk of coronary heart disease (CHD). Even at the time, there was sparse evidence that all types of dietary fat consumption were strongly related to CHD (Hu *et al.*, 2001; Hu & Willett, 2002). The lack of a clear positive association between total fat intake and CHD rates in international studies was noted early on by Keys (1980). Keys (1970) and others carried out the Seven Countries Study. The two regions with the highest fat intake were Finland and the Greek island of Crete. In both areas, about 40% of total energy came from fat, but these countries demonstrated the highest and lowest rates of CHD, respectively. The percentage of energy from saturated fat intake and CHD incidence presented a different pattern (Figure 26.2). The particular type of dietary fat has long been recognized as being important in the etiology of CHD (Brown & Page, 1958; Gordon, 1988; Willett, 2012). Keys (1970) noted early on and evidence has shown that increased serum cholesterol is an important risk factor for CHD and that reduction of cholesterol results in lower risk of infarction (Law *et al.*, 1994). Serum cholesterol became one surrogate endpoint for actual CHD. Furthermore, practitioners were aware that *total* serum cholesterol not only was increased by consumption of saturated fat but also predicted coronary disease risk. We now have the benefit of years of controlled metabolic feeding studies that have examined the effects of specific dietary fats on serum cholesterol levels (Mensink & Katan, 1992; Mensink *et al.*, 2003). Many early animal feeding studies altered the proportions of saturated fat, polyunsaturated fat, monounsaturated fat, and intake of dietary cholesterol sources, showing atherosclerotic plaque buildup with high blood cholesterol levels (Wissler & Vesselinovitch, 1975). This information has contributed to the development of predictive equations, two of which were put forward by Keys (1984), Hegsted (1986), and Hegsted *et al.* (1993). Generally, saturated fat and dietary cholesterol, when substituted for an equal amount of energy (calories) from carbohydrate, increases total serum cholesterol. When levels of polyunsaturated fat are decreased, this also results in increased serum cholesterol.

When monounsaturated fat is substituted for carbohydrate, the effects on serum cholesterol appear to be relatively neutral. We have now come to appreciate that cholesterol consists of many important component subfractions. It has become apparent that increased blood levels of some subcomponents, particularly low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL), are harmful. On the other hand, increased high-density lipoprotein (HDL) cholesterol is associated with favorable heart and vascular health

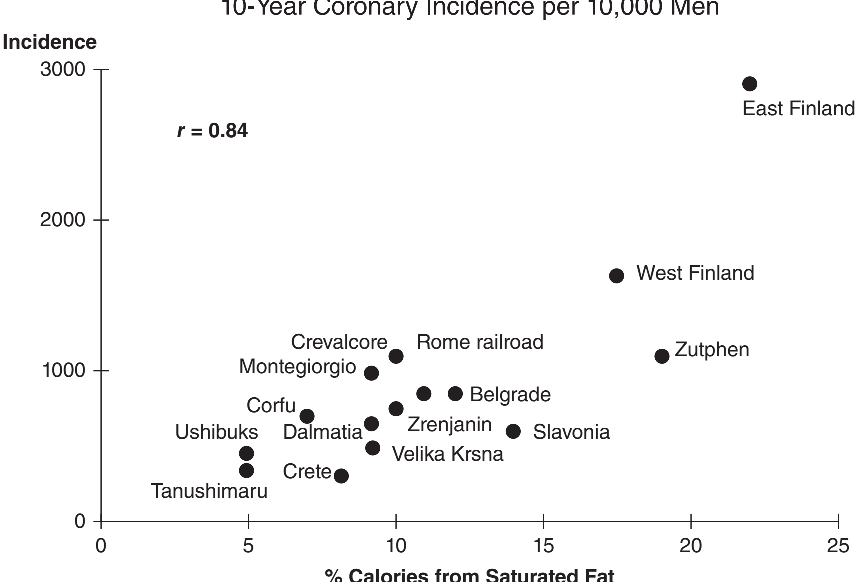

Figure 26.2 Ten-year coronary death rates of the cohorts plotted against the percentage of dietary calories supplied by saturated fatty acids. Adapted from Keys (1970).

status. This has made the use of total serum cholesterol less helpful as an endpoint to predict the effect of a dietary change on CHD risk. HDL cholesterol is a small portion of total serum cholesterol, but it is consistently inversely related to risk of CHD. Any dietary change that reduces HDL levels will also reduce total cholesterol, but this changes the ratio of HDL to total cholesterol and might be expected to have an adverse effect on CHD risk, rather than the beneficial impact predicted by the overall change in total serum cholesterol. Presently, as a general guide, the relation of plasma lipids to CHD risk appears to be well captured by the ratio of total (or LDL) cholesterol to HDL cholesterol (Castelli *et al.*, 1983; Stampfer *et al.*, 1991).

When examined relative to carbohydrates, all types of fat (except trans-fat) tend to raise HDL levels, whereas saturated fat and trans-fat also increase LDL levels (Mensink & Katan, 1992; Mensink *et al.*, 2003; Brouwer *et al.*, 2010; Ooi *et al.*, 2015; Yanai *et al.*, 2015). Metabolic studies have examined the effects of various isocaloric diets on plasma lipoprotein levels (Berry *et al.*, 1991, 1995). These diets were enriched with monounsaturated fats (from olive oil, almonds, and avocado), saturated fats (from butter and other animal fats), polyunsaturated fats (a variety of vegetable oil sources), or carbohydrate. The ratio of total cholesterol to HDL cholesterol is lowest when dietary intakes of monounsaturated or polyunsaturated fats are high, and intakes of carbohydrate and saturated fats are low. Furthermore, the ratio of total to HDL cholesterol changes little when saturated fat is substituted for carbohydrates as HDL levels decline along with total cholesterol, while triacylglycerols levels rise (Mensink & Katan, 1992). Finally, although the independent effect of triacylglycerols on CHD risk is not fully understood, the substitution of olive oil for complex carbohydrate reduces fasting levels of plasma triacylglycerols (Mensink & Katan, 1987) (Figure 26.3). These well-established metabolic studies examining serum cholesterol fractions help to explain the traditionally low CHD rates in regions where olive oil is the principal dietary fat.

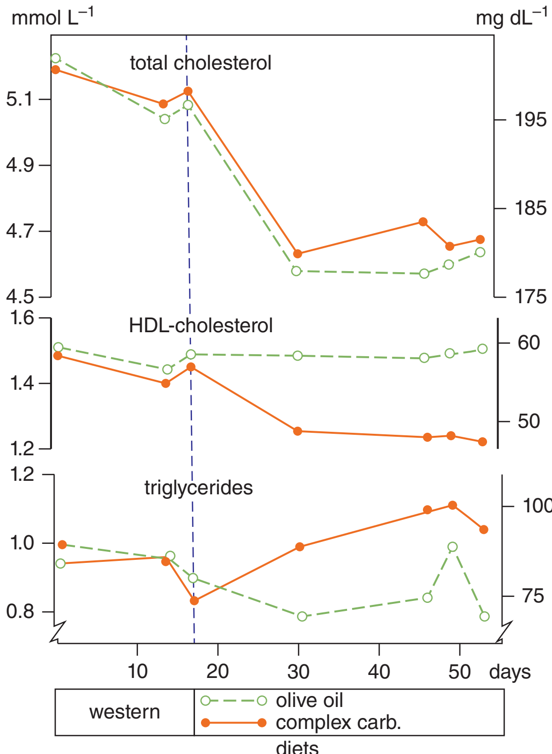

Figure 26.3 Mean serum total and HDL cholesterol and serum triglyceride concentrations throughout the experiment. All 48 subjects first received a Western-type diet high in saturated fat for 17 days. For the next 36 days, half of the subjects received an olive-oil-rich diet (dashed line), and the other half a diet low in fat and high in complex carbohydrates and fiber (solid line). *Source*: Mensink and Katan (1987). Reproduced with permission of Elsevier.

There is evidence suggesting that oxidation of LDL cholesterol substantially enhances its atherogenicity (Steinberg & Witztum, 1990). This oxidation is believed to result in the increased production of cholesterol-rich foam cells, which in turn create atherosclerotic plaques in the arterial wall (Parthasarathy *et al.*, 1992; Young & Parthasarathy, 1994). Antioxidants present in the diet, such as phenolic compounds, inhibit oxidation by scavenging oxygen radicals and stopping free-radical chain reactions (Esterbauer *et al.*, 1991). LDL formed on a diet rich in olive oil tends to be less reactive to oxidative modification (Steinberg & Witztum, 1990; Grundy, 1993). This may be partly due to the increase in size of LDL particles with higher monounsaturated fatty acid (MUFA) intake (Silva *et al.*, 2015). In studies of humans at high risk of CHD due to non-insulin-dependent diabetes mellitus, a diet high in monounsaturated fat has been shown to raise HDL and to lower LDL lipid peroxide and HDL conjugated diene formation, both indicators of reduced lipid oxidation (Reaven, 1995; Dimitriadis *et al.*, 1996). A recent small trial of diet enriched with MUFAs or n-3 polyunsaturated fatty acids (PUFAs) seemed to reduce postprandial oxidative stress when compared to a high-saturated-fat diet (Meza-Miranda *et al.*, 2014). Oxidative stress biomarkers were less evident in an EVOO-supplemented Mediterranean-type meal as compared to controls or corn oil supplementation (Carnevale *et al.*, 2014). The degree to which the balance between dietary antioxidants and unsaturation of fatty acids influences CHD risk remains to be fully explained. The relatively high levels of α-tocopherol phenols and predominance of monounsaturated bonds in olive oil may contribute to the low rates of CHD observed in Mediterranean countries, but this is not conclusive at this point.

EVOO contains the greatest amount of naturally occurring compounds with potent antioxidant properties (Kiritsakis, 1991; Franco *et al.*, 2014; Borges *et al.*, 2015). The specific compounds and characteristics of EVOO are covered extensively in Chapter 5. Briefly, potential lipid-altering effects of these antioxidants have been examined in laboratory animals supplemented with several high-fat, high-oleic-acid diets. Those selected into the EVOO group (containing hydroxytyrosol and tyrosol, phenolic compounds) had LDL blood samples most resistant to *in vitro* oxidation (Wiseman *et al.*, 1996). Importantly, virgin olive oils contain these polyphenolic antioxidants; refining processes destroy or filter out these compounds. In Mediterranean countries, such as Greece and Italy, the highest daily intakes of olive oil average between 45 and 55 grams per day (Visioli *et al.*, 1995; FAS, 2014). In the Mediterranean, much of this oil is consumed as EVOO (particularly in Greece, Italy, and Spain). As has been suggested (Visioli & Galli, 1995), the phenol intake from this olive oil alone would approach the highest levels of consumption of polyphenolic antioxidants (as flavonoids) in the Zutphen Elderly Study (Hertog *et al.*, 1993). A reduction of 60% in cardiovascular disease (CVD) risk has been observed (for the highest versus lowest quintiles of consumption) for flavanols and a 45% risk reduction for total phenolic compounds in the PREDIMED study (described further in this chapter) (Tresserra-Rimbau *et al.*, 2013). For all-cause mortality, the reduction was almost 40%. Similar benefit was not seen in a US study (Rimm *et al.*, 1996), but dietary intakes and patterns were quite different from those in the European studies. Consumption at this level was associated with a 60% reduction in risk for overall CHD mortality. In a review of both animal and human metabolic studies, the amount of phenol compounds derived from olive oil intake alone was unlikely to protect LDL against oxidation (Vissers *et al.*, 2004). The authors note that the phenolic compounds may act in concert along with many other antioxidants that are consumed in a plant-based Mediterranean diet.

Dietary fats also may influence CHD risk by mechanisms other than their effects on blood lipoproteins, but such effects are less well understood and established (Ulbricht & Southgate, 1991). For example, diets high in n-3 fatty acids reduce platelet aggregability (Leaf & Weber, 1988; Petroni *et al.*, 1995). The long-chain n-3 fatty acids are found primarily in fatty fish such as salmon and mackerel, and they can also be derived from dietary sources of linolenic acid. The n-6 fatty acids are abundant in vegetable oils, and with increased intakes, reductions in mortality from heart disease have been noted in more recent observational studies (Jakobsen *et al.*, 2009). It may be that this is in part due to reductions in inflammation, insulin resistance, and resulting metabolic syndrome (Willett, 2007; Riserus *et al.*, 2009). In general, substitution of unsaturated for saturated fats appears to have beneficial effects on platelet function (Ulbricht & Southgate, 1991). Furthermore, in several studies, inhibition of platelet aggregation has been shown in vitro (Petroni *et al*., 1995; de Roos *et al*., 2011; Rubio-Senent *et al*., 2015). An additional hypothesis is that high intakes of polyunsaturated fat may reduce sudden deaths by raising the threshold for ventricular arrhythmia (Charnock *et al.*, 1992; Leaf, 2007).

Adequate intakes of polyunsaturated fats of both the n-6 and n-3 series are essential. Some authorities have suggested that linolenic and other n-3 fatty acids should be consumed at 10–25% that of linoleic

acid (Institute of Medicine, 2005). Given a requirement of about 2–3% of energy, the amount of linoleic acid in olive oil (10–15%) would alone provide sufficient intake if olive oil constituted 25% of energy. Olive oil, however, is relatively low in n-3 fatty acids, and other sources are required. In much of the Mediterranean, fish is frequently consumed, providing ample n-3 fatty acid intakes. Some leafy green vegetables may be an important alternative source of n-3 fatty acids where seafood is unavailable (Simopoulos, 2004). In a cohort of aging men, leafy green vegetable consumption was associated with enhanced cardiac autonomic function that may, in the long term, reduce rates of sudden death (Park *et al.*, 2009). Cattle, lamb, chickens, and other animals also consume these leafy green plants and grasses. Therefore, foods such as eggs and dairy products, or items made with these ingredients, also contain higher levels of n-3 fatty acids when the animals are raised in a more traditional pastured manner than modern farm animals.

Because complex mechanisms appear to mediate the relationship between intake of specific dietary fats and CHD incidence, no single surrogate biochemical or physiological response can predict with confidence the impact of a particular dietary pattern. For this reason, examinations of the relation between specific dietary factors or patterns and CHD incidence itself are particularly valuable because such studies integrate the effects of all known and unknown mechanisms. For example, recognition of the extremely low CHD rates in countries with high consumption of olive oil suggested the benefits of substituting this fat for animal fats. This type of ecological analysis has been expanded by Jacobs *et al.* (1992), who noted that monounsaturated fat intake is inversely related to total mortality as well as CHD deaths. International studies that have examined this relationship, however, are inevitably far from definitive because of the other substantial differences in diet and lifestyle among countries.

A number of prospective studies of dietary fats and CHD risk have been conducted over the past several years. Older studies may be limited by small sample sizes, inadequate means of dietary assessment, diet not being updated over time, and lack of adjustment for the effects of energy intake (Willett, 2012). Many of these issues have now been addressed, but these studies were likely not conducted in a region where olive oil consumption was high. Data from 11 prospective cohort studies from northern Europe and the USA have been pooled and reviewed (Jakobsen *et al.*, 2009). In this examination, replacing 5% of saturated fats with polyunsaturated fats (omega-6, in particular) was predictive of a 13% reduction in risk of coronary events, in agreement with current evidence that polyunsaturated fat is associated with better heart health outcomes than was previously believed. Interestingly, monounsaturated fats were associated with an increase in risk of events. As is pointed out in an accompanying editorial, cohort participants were likely getting most of their monounsaturated fat intake from meat and dairy products, not from vegetable oils (Katan, 2009). When smoking, body mass index and physical activity levels were considered in the analyses model, the increased risk associated with monounsaturated fat intake was attenuated, suggesting that monounsaturated fat intake may have been a marker of unhealthy behavior, and polyunsaturated fat a marker of healthy behaviors in these particular populations. Analyses such as these are important because they point out the need to consider sources of dietary intake and other behaviors carefully when considering findings and how they relate to coherent public health messages.

Randomized trials that manipulate diets have provided general support for a role of dietary fat in the development of CHD, but have been less clear about the specific fats. Older trials that maintained dietary fat at about 40% of energy, but substituted vegetable oil for other fats, have produced the clearest evidence of benefit. Substitution generally resulted in polyunsaturated fat levels of about 20% energy. However, the number of documented cases of CHD was small (Dayton *et al*., 1969; Turpeinen *et al*., 1979; Law *et al*., 1994). More recently, the Women's Health Initiative trial compared one group of women consuming a low-total-fat diet with a group consuming a "usual diet" for up to eight years in almost 50,000 women. There was no effect on risk of CHD or CVD associated with the lower fat diet (Howard *et al.*, 2006). Because there was also no difference in plasma levels of triacylglycerol or HDL cholesterol, some question whether there were actual differences in the amount of fat women were consuming in either group (Willett, 2010). One study to specifically evaluate the effect of a Mediterranean-type diet on CHD recurrence compared an intervention diet high in monounsaturated fat (primarily rapeseed oil) but relatively low in n-6 polyunsaturated fats with a standard American Heart Association Step II diet among patients with unstable angina. Both groups of participants were encouraged to consume fruits, vegetables, and nuts (de Lorgeril *et al.*, 1994). This study reported a dramatically lower incidence of myocardial infarction and death among patients on the Mediterranean-type diet

(de Lorgeril *et al.*, 1999). Although the results of this study cannot be attributed to a single dietary factor, they do lend strong support to overall benefit of the Mediterranean dietary pattern on CHD risk. In a prospective study, the Greek European Prospective Investigation into Cancer (EPIC) study, 22,000 participants utilized a new Mediterranean diet score ranging from 0 to 9 constructed from typical foods and patterns of the traditional diet. This score was used in examining the adherence to a Mediterranean diet in relation to total and cardiovascular mortality (Trichopoulou *et al.*, 2003). The investigators found a 25% reduction in mortality from all causes with each incremental two-point change in diet score. Reductions in excess of 30% were seen as being at risk of cardiovascular mortality. An example of this two-point increment was described as increasing substantially the intake of monounsaturated fats in relation to saturated fats and decreasing meat consumption considerably. Others have examined the association between a Mediterranean-type diet and CVD in prospective studies and have found similar benefits with higher consumption patterns (Mitrou *et al.*, 2007; Fung *et al.*, 2009; Chiuve *et al.*, 2011). In two of the original Italian cohorts from the Seven Countries Study, a Mediterranean score of baseline intake derived from factor analyses that favored higher consumption of cereals (pasta), potatoes, vegetables, fish, and oil and lower consumption of milk, sugar, fruit, and alcoholic beverages, was associated with significantly lower CHD incidence and CHD, and CVD (and cancer) mortality, after 40 years of follow-up (Menotti *et al.*, 2012).

More recently, a randomized trial to help determine the effects of a diet supplemented with EVOO or nuts as compare to a low-fat control diet began in the early 2000s in several regions of Spain. This trial (PREvención con Dieta MEDiterránean), known as the PREDIMED study, was designed to first look at changes in cardiovascular intermediary markers and, eventually, CVD outcomes. The pilot phase recruitment and intervention took place over a three-month period in 2003. This was undertaken to be sure that participants (~770 men and women) understood and could comply with advice about dietary changes. The pilot participants, and later all participants, had either diabetes or at least three cardiovascular risk factors, such as hypertension, smoking, or elevated LDL (but no frank CVD at baseline). They were given detailed behavioral counseling and nutrition education. Two-thirds of the participants were advised to follow a Mediterranean diet (MeDiet). A low-fat diet was counseled for the controls. One of the MeDiet intervention groups received encouragement to consume ample EVOO along with a supply of 1 liter of oil per week. The second group was advised to increase nut consumption and also given a supply of 30 g/day of mixed nuts. At three months, changes in intermediate markers of cardiovascular risk, measured in pilot participants, favored the two MeDiet arms of the pilot with reductions in blood pressure, blood glucose levels, and ratio of cholesterol to HDL cholesterol, as well as increased HDL levels (Estruch *et al.*, 2006; Estruch, 2010). Full recruitment was completed in 2009 with a total of 7350 participants divided into the three study groups. Results from follow-up of the full study are very encouraging. Relative risk of several CVD related outcomes was reduced in both the EVOO- and the nut-supplemented groups as compared to the control population (Martinez-Gonzalez *et al.*, 2015) (Table 26.1).

Table 26.1 Relative risk reduction for clinical events in the PREDIMED intervention study (median follow-up = 4.8 years). Adapted from Martinez-Gonzalez (2015).

	Mediterranean diet with EVOO	**Mediterranean diet with mixed nuts**
Clinical event	**Relative risk reduction**	**Relative risk reduction**
Primary CVD endpoint	30% (8.0%; 46%)	28% (4.0%; 46%)
Type 2 diabetes	40% (15%; 57%)	18% (–10%; 39%)
Peripheral artery disease	64% (35%; 79%)	46% (8.0%; 68%)
Atrial fibrillation	38% (12%; 55%)	10% (–23%; 34%)

Note: Fully adjusted estimates for the hazard ratios from Cox regression models were used to compute the relative risks (RR). The relative risk reduction (RRR) was computed as RRR = (1 – RR)%. The absolute risk reduction (ARR) was computed, taking into account the baseline incidence of events in the control group (I0), after a median follow-up of 4.8 years and applying the estimates for the relative risks, that is, ARR = I0 (1 – RR). CVD = cardiovascular disease; EVOO = extra virgin olive oil.

26.3 Fat intake and cancer

The rates of many cancers, including those of the prostate, colorectal area, and breast, are correlated with total fat intake in international comparisons (Food and Agriculture Organization [FAO], 2015). These associations, however, have been largely limited to saturated and animal fat (Wynder, 1976; Ip, 1990; Jacobs *et al.*, 1992), and may potentially be confounded by factors other than diet. Furthermore, intakes sourced from World Health Organization (WHO) and FAO databases are, at best, meant to describe the amount of food available per capita. When compared to individual-country dietary databases, these disappearance data can over- or underestimate intake (Del Gobbo *et al.*, 2015). They do, however, provide reasonable comparisons that help for the formulation of hypotheses for further epidemiological research.

26.3.1 Prostate cancer

Prostate incidence is rising in many parts of the globe. Some of the apparent rise may be attributed to the effects of better or overdetection by PSA testing (Bray *et al.*, 2010). A relationship of dietary fat and energy to the risk of prostate cancer is also suggested by international comparisons and ecologic and migrant studies. Epidemiologic studies of prostate cancer are complicated because the disease exists in both a less aggressive, more indolent form that seems distributed similarly among countries, and an aggressive form as well; it more often takes the latter form in affluent countries where energy availability is high and physical activity levels tend to be low. Obesity is now considered to be a modifiable risk factor for many cancers, including prostate cancer (Calle *et al.*, 2003; World Cancer Research Fund, 2014). As standards of living around the world rise, the prostate cancer rate increases. The factors that influence the transition from the reasonably benign to the more aggressive form are likely quite important. Unfortunately, few studies have traditionally distinguished among these types. Some newer examinations have looked at differences in disease progression status of prostate cancer and the relationship with diet and physical activity, although disease mechanisms are still not fully understood. In a large prospective study of 51,000 men, animal fat was associated with risk of aggressive prostate cancer, but not with localized disease; vegetable fats (including those high in MUFAs) appeared to be unrelated to any risk of this disease (Giovannucci *et al.*, 1993). Consuming fish more than three times per week in the same group with longer follow-up was associated with a decreased risk, particularly for metastatic prostate cancer (Augustsson *et al.*, 2003). Consumption of marine fatty acids, high in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has been linked to tumor suppression (Rose & Connolly, 1991). When looking at specific sources of n-3 and n-6 fatty acids, alpha-linolenic acid was associated with advanced cases of prostate cancer, while EPA and DHA may be protective for both total and advanced prostate cancer (Leitzmann *et al.*, 2004). In follow-up analyses in this same prospective study, red meat and dairy product consumption was not related to overall risk, although for metastatic disease there was a weak association (Michaud *et al.*, 2001). This association was mostly attenuated when controlled for confounders and saturated fat intake. Monounsaturated fat intakes in these analyses were unrelated to any increased risk of prostate cancer. More recently in this same group of men, specific dietary fat intakes after diagnoses of prostate cancer were examined in relation to progression of cancer and total mortality (Richman *et al.*, 2013). This analyses used multivariate modeling and energy adjustment techniques for repeated dietary measurements; carbohydrates (or specific fats such as trans or saturated) are replaced with monounsaturated, polyunsaturated, or total vegetable fat. A 29% reduction in risk of lethal prostate cancer was seen when replacing 10% of postdiagnostic calories from carbohydrate with vegetable fat. For all-cause mortality, a 26% reduction was seen in those men with a similar carbohydrate/vegetable fat replacement. When animal fat was replaced by vegetable fat, the reduction in total mortality was 34%. A 5% increase in saturated fat and a 1% increase in trans-fat were associated with a 30% and 25% increase in all-cause mortality, respectively. Some case–control studies have looked at fat intake and prostate cancer deaths (Meyer *et al.*, 1999; Kim *et al.*, 2000). Saturated fat was related to higher risk of death, while marine fats were associated with a lower risk. Diet was asked after diagnoses in these relatively small case studies.

Few studies have specifically assessed the relationship of olive oil consumption to prostate cancer risk, but some have examined the relationship between a Mediterranean pattern and prostate cancer risk. In a 14-year follow-up in the Health Professionals Follow-up Study, a Mediterranean pattern diet after diagnosis was not specifically associated with risk of either advanced or lethal prostate cancer mortality (Kenfield *et al.*, 2014).

However, greater adherence to a Mediterranean pattern diet after diagnosis in those with non-metastatic prostate cancer was associated with a 22% lower overall mortality. In a case–control study carried out in Auckland, New Zealand, reporting intakes greater than 5.5 ml of MUFA-rich vegetable oils was associated with lower rates of prostate cancer, although there was no differentiation between consumption of olive, canola, or peanut oil (Norrish *et al.*, 2000). Although these studies do not focus on olive oil specifically, they do seem to show that a diet with less reliance on refined carbohydrates and more reliance on vegetable oils will help promote a healthy and protective lifestyle. Unfortunately, trials examining the role of diet in prostate cancer progression have tended to be quite small, restricted to interventions of one or two specific foods or supplements, and of short duration (Hackshaw-McGeagh *et al.*, 2015). Long-term, more robust studies would be helpful for determining the best diets to slow progression and promote survival.

26.3.2 Colorectal cancer

Older retrospective case–control studies have repeatedly identified associations between total or saturated fat and risk of colon cancer; this is not the case for prospective cohort studies (World Cancer Research Fund, 2011). Later recall of past dietary intake, as is the practice in case–control studies, likely introduces more bias. Furthermore, there are also positive associations with total energy intake in most studies. This positive association increases the possibility that dietary fat alone is not necessarily a cause of colorectal cancer, as is shown in energy adjustment studies as well as cohort studies (Willett & Stampfer, 1986, 1990; Liu *et al*., 2011). In one case–control study conducted in Switzerland, total energy was marginally positively related to colorectal cancer, as was saturated fat, but there was a 40% reduction in risk for the highest tertiles of consumption of both poly- and monounsaturated fats (Levi *et al.*, 2002). In an analyses of several case–control studies, Howe *et al.* (1997) reported that when adjustments were made for total energy intake, fat intake was no longer associated with risk. A more recent analyses utilized data from a combined series of case–control studies conducted in many centers throughout Italy between 1991 and 2007 (Bosetti *et al.*, 2009). The analysis regarding the relationship between fat intake and colorectal cancer focused on olive oil consumption in particular. These combined center studies contained over 1200 colorectal cancer cases. There was an approximately 15% lower risk of cancer for the top two tertiles of olive oil consumption as compared with the lowest category. Earlier analyses found slight nonsignificant reductions in risk for other fats (Braga *et al.*, 1998). There was also little relationship between fried foods and risk, but when the type of fat was considered, frying in olive oil did confer a very modest protective effect on colorectal cancer risk (Galeone *et al.*, 2007). In meta-analyses of 19 case–control studies by Kosti *et al.* (2011) and Psaltopoulou *et al.* (2011), a slightly reduced risk with colorectal cancer was seen with consumption of olive oil not used in cooking.

Some prospective studies have identified a positive association between animal fat consumption and risk of colorectal cancer (Willett & Stampfer, 1990), but have shown no evidence of any causal relation between most dietary fat, including vegetable fat and colorectal cancer risk (Stemmermann *et al*., 1981; Morgan *et al*., 1988; Willett & Stampfer, 1990; Giovannucci *et al*., 1994). Analyses in the Women's Health Study followed women for up to nine years after baseline completion of a food frequency questionnaire (Lin *et al.*, 2004). Neither total fat nor any specific type of fat was related to colorectal cancer risk. However, consumption of fried foods away from home was positively associated with colorectal cancer risk, with an elevated increase in risk particularly for those women in the highest compared to the lowest quintile of intake in food consumption. Other cohort studies have shown no association with dietary fat at all (Dahm *et al.*, 2010).

Although some prospective studies have shown an association between intake of red meat or processed meat and colon cancer, animal fat appears to have little association with colon cancer risk independent of meat consumption (Giovannucci *et al.*, 1994). Furthermore, in a meta-analysis of both cohort and case–control studies, any positive association with colorectal cancer seen for animal fat and protein seemed not to hold up over time (Alexander *et al.*, 2009). Furthermore, in one fiber trial study, those with the lowest percentage of energy from fat had the highest recurrence of adenomas or early cancers (Nakamura *et al.*, 2010).

Importantly, processed meats and consumption patterns were not consistently categorized across studies, making examination of processed meats impossible. This may explain some of the confusion when trying to discern potential effects of meat and fat. Rates of colon cancer were traditionally low in southern European countries. Even with diet and activity changes over the past 60 years, southern Mediterranean countries have

colorectal disease rates that are somewhat lower than those of northern Europe, although this is changing as a more Western diet and sedentary lifestyle prevail (IARC GLOBOCAN, 2012). Some colorectal cancer mortality rates have also remained lower in these countries where dietary fat intake is high, most often consumed as olive oil or other vegetable oils; but recent lifestyle, immigration and economic changes complicate interpretation. (Willett, 1994; Simopoulos, 2001; Perez-Jimenez *et al*., 2005; Lopez-Miranda *et al*., 2010).

26.3.3 Breast cancer

There was, at one time, strong support for the hypothesis that dietary fat intake was an important cause of breast cancer. This was based mostly on older international correlation studies (Hebert & Rosen, 1996), as well as rodent studies in which animals fed high-fat diets *ad libitum* developed a greater incidence of mammary tumors (Freedman *et al*., 1990; Welsch, 1992; Cowen *et al*., 2015). Animals consumed far more calories in free-feed studies and gained more weight in the high-fat group. Total energy restriction, which can come from caloric restriction or increasing physical activity levels, reduces incidence of mammary tumors even more profoundly than diet specifically. Researchers have examined animals on restricted diets and found that fat intake has had little effect (Boissonneault *et al.*, 1986; Rao, 1996). Intake did not tend to increase mammary tumor incidence among animals that were not given carcinogens (Appleton & Landers, 1986). Furthermore, in a study of rats treated with a potent carcinogen, exposure to three different levels of energetics interventions and restricted intakes resulted in carcinogenic response that was inhibited as compared to controls (Zhu *et al.*, 2012). These animals displayed lower cancer incidence, cancer multiplicity, and cancer burden as well as extended cancer latency. Several biomarkers, including insulin growth hormone-1 (IGF1), IGF3, interleukin-6 (IL6), C-reactive protein (CRP), and leptin, all associated with inflammation or fat formation, were reduced in the energetics intervention groups. Other studies have shown lower levels of circulating IGF-1, which may retard some types of tumor growth (Nogueira *et al*., 2012; Ford *et al*., 2013), as do intermittent calorie restriction and high levels of EPA intake (Mizuno *et al.*, 2013). It is important to remember that while informative, using animal models for explaining the determinants of human cancer remains complex and uncertain.

There have been many observational cohort studies over the last 30-plus years assessing the relationship between dietary fat and breast cancer in women. Few have examined olive oil only. Many recent studies have looked more closely at different types of fat and substitution of one nutrient type for another, such as replacement of carbohydrate with particular types of fat. Because the etiology of breast cancer appears to differ somewhat between women diagnosed pre- or postmenopausally, studies often look at these diagnoses separately. Premenopausal women in the Nurses' Health Study II cohort (NHSII) completed dietary questionnaires over multiple years. In an examination by Cho *et al.* (2003), there was no clear association between total fat intake during early adulthood and breast cancer risk. When considering animal fat separately, there was an elevated relative risk of breast cancer of about 30% when comparing the highest to lowest quintiles of intake in multivariate adjusted analyses that controlled for other types of fat intake. The contributors to animal fat intake were primarily red meat and high-fat dairy foods. In a later study of women in this cohort (Cho *et al.*, 2006), red meat intake alone was most strongly associated with an elevated risk of hormone receptor–positive (ER+) breast cancer. This was not attenuated when analyses were adjusted for the contribution of other food groups, including fruits, vegetables, and dairy foods. In the original NHS cohort of postmenopausal women, Western (considered least healthy) and prudent patterns of food intake were examined (Fung *et al.*, 2005). The patterns were derived from principal components analyses' factor loadings based on preselected food groups. Foods and groups that emerged for a prudent diet included leafy, cruciferous, and yellow vegetables; tomatoes; fruit and juice; legumes; fish; poultry; whole grains; low-fat dairy; salad dressing; and nuts. For the Western diet, groups included refined grains, desserts and sweets, processed and red meats, French fries and potatoes, pizza, full-fat dairy, sugar-sweetened beverages, mayonnaise, margarine, snacks, eggs, and cream soups. It must be noted that this population of women has not traditionally consumed large amounts of olive oil. When these dietary patterns were examined, there was little relationship overall between any dietary pattern and cancer risk; however, a 40% elevation in risk was seen in those women who were smokers at baseline and also consuming the Western pattern. An inverse association was observed between the prudent pattern and ER− breast cancer, with a 38% reduction in risk. There was little change in breast cancer risk per 5% energy increment of change in fat intake (Kim *et al.*, 2006). Furthermore, type of fat and estrogen or progesterone receptor status were also not related to risk when other

lifestyle and reproductive factors were controlled for. Findings were similar in a group of Swedish women, although in women over 50 years of age (the time when most diagnoses occur), risk was reduced by almost half for those in the highest quintile of MUFA and PUFA intake as compared to the lowest quintile (Lof *et al.*, 2007). In other observational studies looking at Mediterranean diet patterns, the evidence is conflicting. In a study of the Greek arm of the European Prospective Investigation into Cancer and Nutrition (EPIC), the relationship between breast cancer and consumption of foods associated with the "Mediterranean diet" was examined (Trichopoulou *et al.*, 2010). Individual foods and food groups included vegetables, legumes, fruit and nuts, dairy products, cereals, meat and meat products, fish and seafood, monounsaturated lipids (olive oil), and saturated lipids, from which compliance scores were derived. Analyses were adjusted for age at enrollment, body mass index (BMI), physical activity history, parity, and other factors. There was no significant relation between diet and cancer risk. In a detailed dietary analysis of over 60,000 postmenopausal women (also in EPIC cohorts) from Greece, Italy, and Spain, olive oil intake on its own was not related to risk of breast cancer (Buckland *et al.*, 2012). A recent study also examining diet as a composite Mediterranean score in the Swedish Women's Lifestyle and Health cohort study found no association with either pre- or postmenopausal breast cancer related to the score (Couto *et al.*, 2013). In the UK Women's Cohort Study, a nonsignificant reduction in risk was seen in the highest category of adherence to a Mediterranean pattern for premenopausal women (Cade *et al.*, 2011). In the French EPIC cohort, the alcohol/Western pattern was associated with a 20% increase in risk of breast cancer overall for those in the highest quartile, due mainly to ER+/PR+ and ER+/PR− cancer cases (Cottet *et al.*, 2009). The healthy/Mediterranean pattern was associated with a 15% reduced risk of breast cancer for all cases. When stratified by energy intake, the association with the healthy pattern remained highly significant for women with caloric intakes below the mean. In a pooled analysis of seven prospective studies conducted in four countries, little association between total fat and breast cancer risk was found (Hunter *et al.*, 1996). This was true even for the small number of women consuming less than 20% of calories from fat, an unusually low amount in Western diets.

Southern European women, whose fat intake is largely in the form of olive oil, display somewhat lower rates of breast cancer than American women, whose total fat intake is roughly similar (World Cancer Research Fund, 2012). Importantly, women in Europe have more recently reduced the number of children they have, which is known to elevate risk of breast cancer. In animal models in which fat promoted mammary tumors, olive oil was shown either to be a poor promoter of tumors or to have a protective effect (Cohen *et al.*, 1986a, 1986b; National Research Council, Committee on Diet and Health, 1989; Weisburger & Wynder, 1991). Although monounsaturated fat in the US diet largely derives from meat rather than olive oil, a highly significant inverse association was seen in the Nurses' Health Study between monounsaturated fat intake assessed early in the study and later subsequent risk of breast cancer (Willett *et al.*, 1992). Inverse associations were seen in prospective studies from Iowa (Kushi *et al.*, 1992) and the Netherlands (Van den Brandt *et al.*, 1993), but both a Canadian and Norwegian study found a positive relationship (Howe *et al.*, 1991; Gaard *et al*., 1995). An analysis of premorbid diet and long-term survival in breast cancer cases that developed while women were participating in the prospective Canadian National Breast Screening Study showed a positive relationship between saturated fat and mortality, but no significant increase in risk for oleic acid intake and mortality (Jain *et al.*, 1994). A pooled analysis of several prospective studies from North America and northern Europe showed no significant association with monounsaturated fat (Hunter *et al.*, 1996).

Some case–control studies have been conducted in areas where olive oil is a major source of fat. In case–control studies from Spain (Landa *et al.*, 1994; Martin-Moreno *et al.*, 1994) and Greece (Trichopoulou *et al.*, 1995), significant inverse associations were observed between consumption of monounsaturated fat or olive oil and breast cancer risk; an Italian case–control study found a strong positive association between saturated fat and risk of breast cancer, but no relationship with monounsaturated fat (Toniolo *et al.*, 1989). Another case–control study of breast cancer from France found a nonsignificant positive association with olive oil. This study also found a strong positive association with total caloric intake (Richardson *et al.*, 1991), however, suggesting that the cases were generally consuming more energy. In a large case–control study with almost 2600 incident breast cancer cases from six regions throughout Italy, investigators noted an inverse association between cancer risk and intakes of unsaturated fats (including oleic acid) (Franceschi *et al.*, 1996). Importantly, a positive association was found between breast cancer risk and starch-containing foods such as pasta, white bread, and other foods made with refined cereals. The authors note that these and other recent findings appear to be contrary to traditional public health messages promoting a low-fat,

high-carbohydrate diet for prevention of many diseases. Looking at intake of specific oils, La Vecchia *et al.* (1995) found an inverse association between breast cancer and both seed oils and olive oil. These authors estimated that a 30 gram (about two tablespoons) increase in daily olive oil consumption would correspond to a 10% decrease in risk. Based on these findings, they further posit the potential for a 15% reduction in breast cancer burden in Italian women by raising unsaturated fat consumption to that of the highest quintile of intake, and a 16% reduction by lowering starch consumption to that of women in the lowest quintile of intake.

The PREDIMED Study is a large Spanish randomized trial designed to examine the effect of an enhanced Mediterranean diet on CHD (Toledo *et al.*, 2015). Investigators are now examining diet and several other disease outcomes including breast cancer. For these analyses, median follow-up was 4.8 years. Randomized groups were given either extra nuts or olive oil to consume or were selected into a low-fat-diet control group. There was a significant reduction in risk for both the EVOO-supplemented group and the nut-supplemented group over the low-fat control group. The women given EVOO had a 60% reduction and the group given supplemental nuts had a 40% reduction (nonsignificant) in risk of breast cancer. The groups examined together had a 51% reduction in risk. One caveat to this finding is that case numbers are low; therefore, longer follow-up will be needed to see if this reduction in risk holds up. Furthermore, conducting other trials would be warranted to confirm the findings. The authors note that the supplemented oil group was consuming greater than 15% of total calories from olive oil, and this high amount may be a key factor in the prevention of breast cancer and could partially explain the lack of consistency in cohort study results. Perhaps some of the other studies examining a Mediterranean pattern in relation to risk have in some way not been able to adequately capture those people who report the highest levels of consumption.

26.3.4 Overall cancer rates

In addition to the cancer sites discussed above, past rates of cancer mortality have tended to be lower in Mediterranean countries. Diet has been shown to be only one component in a complex mix contributing to overall health status. As diets around the world gradually become more similar, the difference in cancer rates from country to country is changing. This change is certainly due to many factors, such as reduction in parity, decreasing levels of physical activity, and the rise in obesity. Recently, an ecological study found a positive correlation between several cancer types and the amount of energy from animal products (although not for animal fat) in data from 87 countries (Grant, 2014). It would seem prudent to replace some of this energy from animal sources with more traditional foods from the Mediterranean diet, that have in the past been associated with good health and longevity. Current rates of some cancers in Greece are now nearly identical to, or in some cases surpass, those of the USA when looking at all ages (Institute for Health Metrics and Evaluation, 2013). Certainly, disease-reporting methods, diagnoses patterns, and treatment differences, reduced rates of other diseases, and increasing lifespan all play a role in these changing rates; but clearly this upward trend is troubling. Promoting a return to a more traditional Mediterranean dietary pattern and making physical activity accessible to all will help to reverse this growing trend. Recent studies also suggest that a traditional Mediterranean diet, which would include the substitution of olive oil for other fats, may reduce risk of several cancers and appears to be associated with lower rates of cognitive decline in recent studies (Berr *et al.*, 2009; Lopez-Miranda *et al.*, 2010; Valls-Pedret *et al.*, 2015; Yannakoulia *et al.*, 2015).

26.4 Obesity and dietary fat

Overweight and obesity are fast becoming major problems throughout the globe and are leading causes of morbidity and mortality (FAO & WHO, 2003). Excess calories and more sedentary lifestyles have become the norm in many parts of the world. This is true even in countries with substantial populations that have inadequate access to healthy foods, setting up a paradox. One model for explaining weight gain and obesity has been that higher dietary fat intakes from diet lead to substantially greater accumulation of body fat (Flatt, 1985; Astrup *et al*., 1994, 2000). This idea would seem sensible when considering that each gram of fat contains 9 kcal and a gram of either carbohydrate or protein contains 4 kcal. It would seem that reducing the fat in the diet would reduce body weight. While this has been seen in some animal studies or short-term human studies (Bray & Popkin, 1998), there is little support when examining longer term studies of dietary fat

reduction for weight loss in humans (Willett, 2002). This is particularly true when other nutrients and total energy are carefully controlled for. Randomized trials of at least a 12-month duration where fat was reduced to less than 25% of calories have resulted in very small differences in body weight over time. Although the populations in developing countries generally consume less fat and display lower degrees of adiposity than do affluent countries, this relationship is obviously confounded by differences in physical activity and general availability of food. Studies that have compared populations of similar socioeconomic status have found little relation between dietary fat consumption and adiposity levels. Historically, for example, southern European populations with somewhat lower fat intake display higher levels of obesity than do northern populations with higher fat intake (Keys, 1980). In China, where fat intake historically varied from 5 to 25% of energy, no association was observed between the percentage of energy from fat and body weight (Chen *et al.*, 1990). Cross-sectional studies within populations have yielded mixed findings (Colditz *et al.*, 1990; Astrup *et al.*, 1994), but they may be confounded as a result of dieting and general health consciousness. Prospective observational examinations of dietary fat intake and weight gain have not shown a strong relationship between total fat energy in the diet and weight gain over time (Field *et al.*, 2007; Bes-Rastrollo *et al.*, 2008).

As noted above, randomized trials of fat reduction usually have observed modest short-term decreases in body weight. Many trials of one year or longer have found the majority of these reductions to be temporary and usually less than 2.0 kg (National Diet Heart Study Research Group, 1968; Boyd *et al.*, 1990; Sheppard *et al.*, 1991; Kasim *et al.*, 1993; Black *et al.*, 1994; Jeffery *et al.*, 1995). One early trial reported a substantial change in weight between groups on low-fat (17% of energy) and high-fat diets (36% of energy). They found a 3.4 kg decrease after one year, but no effect on percentage of body fat or abdominal circumference was observed (National Diet Heart Study Research Group, 1968). These changes in weight occurred early in the trial, with no later divergence among the dieting groups. In a trial of 101 overweight or obese men and women, participants were randomized into either a Mediterranean-type (goal of 35% fat) or low-fat diet (goal of 20% fat) (McManus *et al.*, 2001). Both diets were energy controlled. After 18 months, the participants in the Mediterranean moderate-fat diet had lost more weight, and were also consuming more fiber and servings of vegetables. Importantly, retention rate in this group was 54 versus 20% for the low-fat group. More recently, a two-year randomized trial was conducted in Israel (DIRECT) with the goal of comparing the weight loss potential of three diets: a low-fat, controlled-calorie diet; a Mediterranean, controlled-calorie diet; and a low-carbohydrate, *ad libitum* calorie diet (Shai *et al.*, 2008). Dietary compliance was high among the 322 mostly male participants with a mean age of 52 and BMI of 31, with an overall adherence rate of 85% at the end of two years. The largest reductions in weight were in the low-carbohydrate and the Mediterranean diet groups (5.5 and 4.6 kg, respectively, for those completing the trial), while the low-fat group lost 3.3 kg. Furthermore, for those participants on the Mediterranean diet who were diabetic, there were reductions in fasting glucose and HOMA-IR (homeostasis model assessment of insulin resistance) at 24 months. In another long-term trial of weight loss, 800 overweight and obese participants randomized to four different diets were followed for two years (Sacks *et al.*, 2009). The energy-controlled diets were designed to be either low fat (20%) with average or high protein or higher fat (40%) with average or high protein, with corresponding changes in carbohydrate. All diets were designed to have limited amounts of saturated fat. After two years, no benefit was seen in the lower fat diet for weight loss as compared to the other diets, and on average participants lost weight whatever the intervention diet goal. Lipid profiles and fasting insulin levels were improved as well (Buckland *et al.*, 2008; Esposito *et al.*, 2011). In a recent review and meta-analysis of randomized clinical trial interventions of one year or longer on weight change, when the intensity of the interventions were comparable in the comparison group, there was no benefit of a low-fat diet versus higher fat and other interventions for long-term weight loss (Tobias *et al.*, 2015).

Collectively, the longer term randomized trials provide strong evidence that the percentage of calories from fat has little influence on body fitness. Thus, high consumption of olive oil, despite concern expressed by some, will not lead to obesity if total calories and physical activity are kept in balance.

26.5 Conclusion

The traditional experience of the Mediterranean countries indicates that a diet higher in total fat, in which olive oil comprises the majority of fat intake, is compatible with lower rates of CHD, several cancers, rates

of obesity, as well as total mortality. Specifically, this experience provides strong evidence that high intakes of olive oil are unlikely to have any major adverse effects as long as total energy intake is reasonable and in accordance with physical activity levels. Furthermore, metabolic studies of blood lipids and recent evidence regarding the oxidizability of LDL cholesterol suggest that consumption of olive oil as a principal fat, as opposed to the use of animal and partially hydrogenated fats in northern Europe, India, and North America, is in part responsible for the traditionally low rates of CVD observed in Mediterranean countries. The optimal amount of monounsaturated fat in the diet is still in debate; in the southern European countries, CHD rates appear to have been lowest in Greece, where intake of monounsaturated fat was highest and total fat was approximately 40% of energy. At this point, evidence suggests that a wide range of fat intake is compatible with healthy diets if the primary fats include olive oil with its functional compounds.

References

Alexander, D.D., C.A. Cushing, K.A. Lowe, B. Sceurman, & M.A. Roberts. (2009) Meta-analysis of animal fat or animal protein intake and colorectal cancer. American Journal of Clinical Nutrition 89, 1402–1409.

Appleton, B.S., & R.E. Landers. (1986) Oil gavage effects on tumor incidence in the National Toxicology Program's 2-year carcinogenesis bioassay. Advances in Experimental Medicine and Biology 206, 99–104.

Astrup, A., B. Buemann, P. Western, S. Toubro, A. Raben, & N.J. Christensen. (1994) Obesity as an adaptation to a high-fat diet: evidence from a cross-sectional study. American Journal of Clinical Nutrition 59, 350–355.

Astrup, A., G.K. Grunwald, E.L. Melanson, W.H.M. Saris, & J.O. Hill. (2000) The role of low-fat diets in body weight control: a meta-analysis of ad libitum dietary intervention studies. International Journal of Obesity 24, 1545–1552.

Augustsson, K., D.S. Michaud, E.B. Rimm, M.F. Leitzmann, M.J. Stampfer, W.C. Willett, & E. Giovannucci. (2003) A prospective study of intake of fish and marine fatty acids and prostate cancer. Cancer Epidemiologyogy, Biomarkers & Prevention 12, 64–67.

Berr, C., F. Portet, I. Carriere, T.N. Akbaraly, C. Feart, V. Gourlet, N. Combe, P. Barberger-Gateau, & K. Ritchie. (2009) Olive oil and cognition: results from the three-city study. Dementia and Geriatric Cognitive Disorders 28, 357–364.

Berry, E.M., S. Eisenberg, Y. Friedlander, D. Harats, N.A. Kaufmann, Y. Norman, & Y. Stein. (1995) Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins – the Jerusalem Nutrition Study: monousaturated vs saturated fatty acids. Nutrition, Metabolism & Cardiovascular Diseases 5, 55–62.

Berry, E.M., S. Eisenberg, D. Haratz, Y. Friedlander, Y. Norman, N.A. Kaufmann, & Y. Stein. (1991) Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins – the Jerusalem Nutrition Study: high MUFAs vs high PUFAs. American Journal of Clinical Nutrition 53, 899–907.

Bes-Rastrollo, M., R.M. van Dam, M.A. Martinez-Gonzalez, T.Y. Li, L.L. Sampson, & F.B. Hu. (2008) Prospective study of dietary energy density and weight gain in women. American Journal of Clinical Nutrition 88, 769–777.

Black, H.S., J.A. Herd, L.H. Goldberg, J.E.J. Wolf, J.I. Thornby, T. Rosen, S. Bruce, J.A. Tschen, J.P. Foreyt, L.W. Scott, S. Jaax, & K. Andrews. (1994) Effect of a low-fat diet on the incidence of actinic keratosis. New England Journal of Medicine 330, 1272–1275.

Boissonneault, G.A., C.E. Elson, & M.W. Pariza. (1986) Net energy effects of dietary fat on chemically induced mammary carcinogenesis in F344 rats. Journal of the National Cancer Institute 76, 335–338.

Borges, T. H., C. Cabrera-Vique, & I. Seiquer. (2015) Antioxidant properties of chemical extracts and bioaccessible fractions obtained from six Spanish monovarietal extra virgin olive oils: assays in Caco-2 cells. Food & Function 6, 2375–2383.

Bosetti, C., C. Pelucchi, & C. La Vecchia. (2009) Diet and cancer in Mediterranean countries: carbohydrates and fats. Public Health Nutrition 12, 1595–1600.

Boyd, N.F., M. Cousins, M. Beaton, V. Kriukov, G. Lockwood, & D. Tritchler. (1990) Quantitative changes in dietary fat intake and serum cholesterol in women: Results from a randomized controlled trial. American Journal of Clinical Nutrition 52, 470–476.

Braga, C., C. La Vecchia, S. Franceschi, E. Negri, M. Parpinel, A. Decarli, A. Giacosa, & D. Trichopoulos. (1998) Olive oil, other seasoning fats, and the risk of colorectal carcinoma. Cancer 82, 448–453.

Bray, F., J. Lortet-Tieulent, J. Ferlay, D. Forman, & A. Auvinen. (2010) Prostate cancer incidence and mortality trends in 37 European countries: an overview. European Journal of Cancer 46, 3040–3052.

Bray, G.A., & B.M. Popkin. (1998) Dietary fat intake does affect obesity! American Journal of Clinical Nutrition 68, 1157–1173.

Brouwer, I.A., A.J. Wanders, & M.B. Katan. (2010) Effect of animal and industrial trans fatty acids on HDL and LDL cholesterol levels in humans – a quantitative review. PLoS One 5, e9434.

Brown, H.B., & I.H. Page. (1958) Lowering blood lipid levels by changing food patterns. Journal of the American Medical Association 168, 1989–1995.

Buckland, G., A. Bach, & L. Serra-Majem. (2008) Obesity and the Mediterranean diet: a systematic review of observational and intervention studies. Obesity Reviews 9, 582–593.

Buckland, G., N. Travier, A. Agudo, A. Fonseca-Nunes, C. Navarro, P. Lagiou, C. Demetriou, P. Amiano, M. Dorronsoro, M.D. Chirlaque, J.M. Huerta, E. Molina, M.J. Perez, E. Ardanaz, C. Moreno-Iribas, J.R. Quiros, A. Naska, D. Trichopoulos, M.C. Giurdanella, R. Tumino, C. Agnoli, S. Grioni, S. Panico, A. Mattiello, G. Masala, C. Sacerdote, S. Polidoro, D. Palli, A. Trichopoulou, & C.A. Gonzalez. (2012) Olive oil intake and breast cancer risk in the Mediterranean countries of the European Prospective Investigation into Cancer and Nutrition study. International Journal of Cancer 131, 2465–2469.

Cade, J.E., E.F. Taylor, V.J. Burley, & D.C. Greenwood. (2011) Does the Mediterranean dietary pattern or the Healthy Diet Index influence the risk of breast cancer in a large British cohort of women? European Journal of Clinical Nutrition 65, 920–928.

Calle, E.E., C. Rodriguez, K. Walker-Thurmond, & M.J. Thun. (2003) Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. New England Journal of Medicine 348, 1625–1638.

Carnevale, R., P. Pignatelli, C. Nocella, L. Loffredo, D. Pastori, T. Vicario, A. Petruccioli, S. Bartimoccia, & F. Violi. (2014) Extra virgin olive oil blunt post-prandial oxidative stress via NOX2 down-regulation. Atherosclerosis 235, 649–658.

Castelli, W.P., R.D. Abbott, & P.M. McNamara. (1983) Summary estimates of cholesterol used to predict coronary heart disease. Circulation 67, 730–734.

Charnock, J.S., P.L. McLennan, & M.Y. Abeywardena. (1992) Dietary modulation of lipid metabolism and mechanical performance of the heart. Molecular and Cellular Biochemistry 116, 19–25.

Chen, J., T.C. Campbell, L. Junyao, & R. Peto. (1990) Diet, life-style, and mortality in China: a study of the characteristics of 65 Chinese counties. Oxford University Press, Oxford.

Chiuve, S.E., T.T. Fung, K.M. Rexrode, D. Spiegelman, J.E. Manson, M.J. Stampfer, & C.M. Albert. (2011) Adherence to a low-risk, healthy lifestyle and risk of sudden cardiac death among women. Journal of the American Medical Association 306, 62–69.

Cho, E., W.Y. Chen, D.J. Hunter, M.J. Stampfer, G.A. Colditz, S.E. Hankinson, & W.C. Willett. (2006) Red meat intake and risk of breast cancer among premenopausal women. Archives of Internal Medicine 166, 2253–2259.

Cho, E., D. Spiegelman, D.J. Hunter, W.Y. Chen, M.J. Stampfer, G.A. Colditz, & W.C. Willett. (2003) Premenopausal fat intake and risk of breast cancer. Journal of the National Cancer Institute 95, 1079–1085.

Cohen, L.A., K. Choi, E.L. Weisburger, & D.P. Rose. (1986a) Effect of varying proportions of dietary fat on the development of N-nitrosomethylurea-induced rat mammary tumors. Anticancer Research 6, 215–218.

Cohen, L.A., D.O. Thompson, K. Choi, R.A. Karmali, & D.P. Rose. (1986b) Dietary fat and mammary cancer. II. Modulation of serum and tumor lipid composition and tumor prostaglandins by different dietary fats: Association with tumor incidence patterns. Journal of the National Cancer Institute 77, 43–51.

Colditz, G.A., W.C. Willett, M.J. Stampfer, S.J. London, M.R. Segal, & F.E. Speizer. (1990) Patterns of weight change and their relation to diet in a cohort of healthy women. American Journal of Clinical Nutrition 51, 1100–1105.

Cottet, V., M. Touvier, A. Fournier, M.S. Touillaud, L. Lafay, F. Clavel-Chapelon, & M.C. Boutron-Ruault. (2009) Postmenopausal breast cancer risk and dietary patterns in the E3N-EPIC prospective cohort study. American Journal of Epidemiology 170, 1257–1267.

Couto, E., S. Sandin, M. Lof, G. Ursin, H.O. Adami, & E. Weiderpass. (2013) Mediterranean dietary pattern and risk of breast cancer. PLoS One 8, e55374.

Cowen, S., S.L. McLaughlin, G. Hobbs, J. Coad, K.H. Martin, I.M. Olfert, & L. Vona-Davis. (2015) High-fat, high-calorie diet enhances mammary carcinogenesis and local inflammation in MMTV-PyMT mouse model of breast cancer. Cancers (Basel) 7, 1125–1142.

Dahm, C.C., R.H. Keogh, M.A. Lentjes, E.A. Spencer, T.J. Key, D.C. Greenwood, J.E. Cade, V.J. Burley, M.J. Shipley, E.J. Brunner, A.M. Stephen, G. Mishra, D. Kuh, I.S. Fentiman, I.R. White, R. Luben, K. T. Khaw, & S.A. Rodwell Bingham. (2010) Intake of dietary fats and colorectal cancer risk: prospective findings from the UK Dietary Cohort Consortium. Cancer Epidemiology 34, 562–567.

Dayton, S., M.L. Pearce, S. Hashimoto, W.J. Dixon, & U. Tomiyasu. (1969) A controlled clinical trial of a diet high in unsaturated fat in preventing complications of atherosclerosis. Circulation 40(suppl. II), 1–63.

de Lorgeril, M., S. Renaud, N. Mamelle, P. Salen, J.L. Martin, I. Monjaud, J. Guidollet, P. Touboul, & J. Delaye. (1994) Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease [Erratum in: *Lancet* 1995, 345:738]. Lancet 343, 1454–1459.

de Lorgeril, M., P. Salen, J.-L. Martin, I. Monjaud, J. Delaye, & N. Mamelle. (1999) Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: Final report of the Lyon Diet Heart Study. Circulation 99, 779–785.

de Roos, B., X. Zhang, G. Rodriguez Gutierrez, S. Wood, G.J. Rucklidge, M.D. Reid, G.J. Duncan, L.L. Cantlay, G.G. Duthie, & N. O'Kennedy. (2011) Anti-platelet effects of olive oil extract: in vitro functional and proteomic studies. European Journal of Nutrition 50, 553–562.
Del Gobbo, L.C., S. Khatibzadeh, F. Imamura, R. Micha, P. Shi, M. Smith, S.S. Myers, & D. Mozaffarian. (2015) Assessing global dietary habits: a comparison of national estimates from the FAO and the Global Dietary Database. American Journal of Clinical Nutrition 101, 1038–1046.
Dimitriadis, E., M. Griffin, P. Collins, A. Johnson, D. Owens, & G.H. Tomkin. (1996) Lipoprotein composition in NIDDM: effects of dietary oleic acid on the composition, oxidisability and function of low and high density lipoproteins. Diabetologia 39, 667–676.
Donini, L.M., L. Serra-Majem, M. Bullo, A. Gil, & J. Salas-Salvado. (2015) The Mediterranean diet: culture, health and science. British Journal of Nutrition 113(suppl. 2), S1–S3.
Esposito, K., C.M. Kastorini, D.B. Panagiotakos, & D. Giugliano. (2011) Mediterranean diet and weight loss: meta-analysis of randomized controlled trials. Metabolic Syndrome and Related Disorders 9, 1–12.
Esterbauer, H., M. Dieber-Rotheneder, G. Striegl, & G. Waeg. (1991) Role of vitamin E in preventing the oxidation of low-density lipoprotein. American Journal of Clinical Nutrition 53(suppl.), 314S–321S.
Estruch, R. (2010) Anti-inflammatory effects of the Mediterranean diet: the experience of the PREDIMED study. Proceedings of the Nutrition Society 69, 333–340.
Estruch, R., M.A. Martinez-Gonzalez, D. Corella, J. Salas-Salvado, V. Ruiz-Gutierrez, M.I. Covas, M. Fiol, E. Gomez-Gracia, M.C. Lopez-Sabater, E. Vinyoles, F. Aros, M. Conde, C. Lahoz, J. Lapetra, G. Saez, E. Ros, & P.S. Investigators. (2006) Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. Annals of Internal Medicine 145, 1–11.
Field, A.E., W.C. Willett, L. Lissner, & G.A. Colditz. (2007) Dietary fat and weight gain among women in the Nurses' Health Study. Obesity (Silver Spring) 15, 967–976.
Flatt, J.P. (1985) Energetics of intermediary metabolism. In: Substrate and energy metabolism in man, ed. J.S. Garrow & D. Halliday. John Libbey, London, 58–69.
Fogel, R.W., & L.A. Helmchen. (2002) Economic and technological development and their relationships to body size and productivity. The nutrition transition, diet and disease in the developing world, ed. B. Caballero & B.M. Popkin. Academic Press, London, 9–24.
Food and Agriculture Organization (FAO). (2015) FAOSTAT, Statistics Division. FAO, Rome.
Food and Agriculture Organization and World Health Organization (FAO & WHO). (2003) Diet, nutrition and the prevention of chronic diseases. WHO Technical Report Series, report of a joint WHO/FAO expert consultation. Geneva, WHO.
Ford, N.A., N.P. Nunez, V.B. Holcomb, & S.D. Hursting. (2013) IGF1 dependence of dietary energy balance effects on murine Met1 mammary tumor progression, epithelial-to-mesenchymal transition, and chemokine expression. Endocrine-Related Cancer 20, 39–51.
Foreign Agricultural Service (FAS). (2012) Greece: retail foods 2012. USDA, Washington, DC.
Foreign Agricultural Service (FAS). (2014) Greece: olive oil annual 2014. USDA, Washington, DC.
Franceschi, S., A. Favero, A. Decarli, E. Negri, C. La Vecchia, M. Ferraroni, A. Russo, S. Salvini, D. Amadori, E. Conti, M. Montella, & A. Giacosa. (1996) Intake of macronutrients and risk of breast cancer. Lancet 347, 1351–1356.
Franco, M.N., T. Galeano-Diaz, O. Lopez, J. G. Fernandez-Bolanos, J. Sanchez, C. De Miguel, M.V. Gil, & D. Martin-Vertedor. (2014) Phenolic compounds and antioxidant capacity of virgin olive oil. Food Chemistry 163, 289–298.
Freedman, L.S., C. Clifford, & M. Messina. (1990) Analysis of dietary fat, calories, body weight, and the development of mammary tumors in rats and mice: a review. Cancer Research 50, 5710–5719.
Fung, T.T., F.B. Hu, M.D. Holmes, B.A. Rosner, D.J. Hunter, G.A. Colditz, & W.C. Willett. (2005) Dietary patterns and the risk of postmenopausal breast cancer. International Journal of Cancer 116, 116–121.
Fung, T.T., K.M. Rexrode, C.S. Mantzoros, J.E. Manson, W.C. Willett, & F.B. Hu. (2009) Mediterranean diet and incidence of and mortality from coronary heart disease and stroke in women. Circulation 119, 1093–1100.
Gaard, M., S. Tretli, & E.B. Loken. (1995) Dietary fat and the risk of breast cancer: a prospective study of 25,892 Norwegian women. International Journal of Cancer 63, 13–17.
Galeone, C., R. Talamini, F. Levi, C. Pelucchi, E. Negri, A. Giacosa, M. Montella, S. Franceschi, & C. La Vecchia. (2007) Fried foods, olive oil and colorectal cancer. Annals of Oncology 18, 36–39.
Garcia-Closas, R., A. Berenguer, & C.A. Gonzalez. (2006) Changes in food supply in Mediterranean countries from 1961 to 2001. Public Health Nutrition 9, 53–60.
Gerbens-Leenes, P.W., S. Nonhebel, & M.S. Krol. (2010) Food consumption patterns and economic growth. Increasing affluence and the use of natural resources. Appetite 55, 597–608.

Giovannucci, E., E.B. Rimm, G.A. Colditz, M.J. Stampfer, A. Ascherio, C.C. Chute, & W.C. Willett. (1993) A prospective study of dietary fat and risk of prostate cancer. Journal of the National Cancer Institute 85, 1571–1579.

Giovannucci, E., E.B. Rimm, M.J. Stampfer, G.A. Colditz, A. Ascherio, & W.C. Willett. (1994) Intake of fat, meat, and fiber in relation to risk of colon cancer in men. Cancer Research 54, 2390–2397.

Gordon, T. (1988) The diet-heart idea: outline of a history. American Journal of Epidemiology 127, 220–225.

Grant, W.B. (2014) A multicountry ecological study of cancer incidence rates in 2008 with respect to various risk-modifying factors. Nutrients 6, 163–189.

Grundy, S.M. (1993) Oxidized LDL and atherogenesis: relation to risk factors for coronary heart disease. Clinical Cardiology 16(suppl.), 13–15.

Hackshaw-McGeagh, L.E., R.E. Perry, V.A. Leach, S. Qandil, M. Jeffreys, R.M. Martin, & J.A. Lane. (2015) A systematic review of dietary, nutritional, and physical activity interventions for the prevention of prostate cancer progression and mortality. Cancer Causes Control 26, 1521–1550.

Hebert, J.R., & A. Rosen. (1996) Nutritional, socioeconomic, and reproductive factors in relation to female breast cancer mortality: findings from a cross-national study. Cancer Detection and Prevention 20, 234–244.

Hegsted, D.M. (1986) Serum-cholesterol response to dietary cholesterol: a re-evaluation. American Journal of Clinical Nutrition 44, 299–305.

Hegsted, D.M., L.M. Ausman, J.A. Johnson, & G.E. Dallal. (1993) Dietary fat and serum lipids: an evaluation of the experimental data. American Journal of Clinical Nutrition 57, 875–883.

Hertog, M.G.L., E.J.M. Feskens, P.C.H. Hollman, M.B. Katan, & D. Kromhout. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 342, 1007–1011.

Howard, B.V., L. Van Horn, J. Hsia, J.E. Manson, M.L. Stefanick, S. Wassertheil-Smoller, L.H. Kuller, A.Z. LaCroix, R.D. Langer, N.L. Lasser, C.E. Lewis, M.C. Limacher, K.L. Margolis, W.J. Mysiw, J.K. Ockene, L.M. Parker, M.G. Perri, L. Phillips, R.L. Prentice, J. Robbins, J.E. Rossouw, G.E. Sarto, I.J. Schatz, L.G. Snetselaar, V.J. Stevens, L.F. Tinker, M. Trevisan, M.Z. Vitolins, G.L. Anderson, A.R. Assaf, T. Bassford, S.A. Beresford, H.R. Black, R.L. Brunner, R.G. Brzyski, B. Caan, R.T. Chlebowski, M. Gass, I. Granek, P. Greenland, J. Hays, D. Heber, G. Heiss, S.L. Hendrix, F.A. Hubbell, K.C. Johnson, & J.M. Kotchen. (2006) Low-fat dietary pattern and risk of cardiovascular disease: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. Journal of the American Medical Association 295, 655–666.

Howe, G.R., K.J. Aronson, E. Benito, R. Castelleto, J. Cornee, S. Duffy, R.P. Gallagher, J.M. Iscovich, J. Dengao, R. Kaaks, G.A. Kune, S. Kune, H.P. Lee, M. Lee, A.B. Miller, R.K. Peters, J.D. Potter, E. Riboli, M.L. Slattery, D. Trichopoulos, A. Tuyns, A. Tzonou, L.E. Watson, A.S. Whittemore, & A.H. Wu Williams. (1997) The relationship between dietary fat intake and risk of colorectal cancer – evidence from the combined analysis of 13 case-control studies. Cancer Causes Control 8, 215–228.

Howe, G.R., C.M. Friedenreich, M. Jain and A.B. Miller. (1991) A cohort study of fat intake and risk of breast cancer. Journal of the National Cancer Institute 83, 336–340.

Hu, F.B., J.E. Manson, & W.C. Willett. (2001) Types of dietary fat and risk of coronary heart disease: a critical review. Journal of the American College of Nutrition 20, 5–19.

Hu, F.B., & W.C. Willett. (2002) Optimal diets for prevention of coronary heart disease. Journal of the American Medical Association 288, 2569–2578.

Hunter, D.J., D. Spiegelman, H.O. Adami, L. Beeson, P.A. van den Brandt, A.R. Folsom, G.E. Fraser, R.A. Goldbohm, S. Graham, G.R. Howe, L.H. Kushi, J.R. Marshall, A. McDermott, A.B. Miller, F.E. Speizer, A. Wolk, S.-S. Yaun, & W.C. Willett. (1996) Cohort studies of fat intake and the risk of breast cancer: a pooled analysis. New England Journal of Medicine 334, 356–361.

IARC GLOBOCAN. (2012) Estimated cancer incidence, mortality and prevalence Available from http://globocan.iarc.fr/Pages/online.aspx.

Institute for Health Metrics and Evaluation. (2013) Global burden of disease statistics. Available from http://www.healthdata.org/gbd

Institute of Medicine. (2005) Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients). Report of the Panel on Macronutrients, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. National Academy of Sciences, Washington, DC. Available from http://www.nap.edu/catalog/10490.html

Ip, C. (1990) Quantitative assessment of fat and calorie as risk factors in mammary carcinogenesis in an experimental model. Recent Progress in Research on Nutrition and Cancer: proceedings of a workshop sponsored by the International Union Against Cancer, held in Nagoya, Japan, November 1–3, 1989. C.J. Mettlin and K. Aoki. New York, NY, Wiley-Liss, Inc., pp. 107–117.

Jacobs, D., G. Blackburn, M. Higgins, D. Reed, H. Iso, G. McMillan, J. Neaton, J. Nelson, J. Potter, B. Rifkind, J. Rossouw, R. Shekeller, S. Usuf, & DPHIL. (1992) Report of the Conference on Low Blood Cholesterol: mortality associations. Circulation 86, 1046–1060.

Jain, M., A.B. Miller, & T. To. (1994) Premorbid diet and the prognosis of women with breast cancer. Journal of the National Cancer Institute 86, 1390–1397.

Jakobsen, M.U., E.J. O'Reilly, B.L. Heitmann, M.A. Pereira, K. Balter, G.E. Fraser, U. Goldbourt, G. Hallmans, P. Knekt, S. Liu, P. Pietinen, D. Spiegelman, J. Stevens, J. Virtamo, W.C. Willett, & A. Ascherio. (2009) Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. American Journal of Clinical Nutrition 89, 1425–1432.

Jeffery, R.W., W.L. Hellerstedt, S.A. French, & J.E. Baxter. (1995) A randomized trial of counseling for fat restriction versus calorie restriction in the treatment of obesity. International Journal of Obesity 19, 132–137.

Kasim, S.E., S. Martino, P.N. Kim, S. Khilnani, A. Boomer, J. Depper, B.A. Reading, & L.K. Heilbrun. (1993) Dietary and anthropometric determinants of plasma lipoproteins during a long-term low-fat diet in healthy women. American Journal of Clinical Nutrition 57, 146–153.

Katan, M.B. (2009) Omega-6 polyunsaturated fatty acids and coronary heart disease. American Journal of Clinical Nutrition 89, 1283–1284.

Kenfield, S.A., N. DuPre, E.L. Richman, M.J. Stampfer, J.M. Chan, & E.L. Giovannucci. (2014) Mediterranean diet and prostate cancer risk and mortality in the Health Professionals Follow-up Study. European Urology 65, 887–894.

Keys, A. (1970) Coronary heart disease in seven countries. American Heart Association Monograph Number 29. Circulation 41, 42 (suppl. I), 1–211.

Keys, A. (1980) Seven Countries: a multivariate analysis of death and coronary heart disease. Harvard University Press, Cambridge, MA.

Keys, A. (1984) Serum-cholesterol response to dietary cholesterol. American Journal of Clinical Nutrition 40, 351–359.

Kim, D.J., R.P. Gallagher, T.G. Hislop, E.J. Holowaty, G.R. Howe, M. Jain, J.R. McLaughlin, C.Z. Teh, & T.E. Rohan. (2000) Premorbid diet in relation to survival from prostate cancer (Canada). Cancer Causes Control 11(1), 65–77.

Kim, E.H., W.C. Willett, G.A. Colditz, S.E. Hankinson, M.J. Stampfer, D.J. Hunter, B. Rosner, & M.D. Holmes. (2006) Dietary fat and risk of postmenopausal breast cancer in a 20-year follow-up. American Journal of Epidemiology 164, 990–997.

Kiritsakis, A. (1991) Olive oil. American Oil Chemists Society, Champaign, IL.

Kromhout, D., A. Keys, C. Aravanis, R. Buzina, F. Fidanza, S. Giampaoli, A. Jansen, A. Menotti, S. Nedeljkovic, & M. Pekkarinen. (1989) Food consumption patterns in the 1960s in seven countries. American Journal of Clinical Nutrition 49, 889–894.

Kushi, L.H., T.A. Sellers, J.D. Potter, C.L. Nelson, R.G. Munger, S.A. Kaye, & A.R. Folsom. (1992) Dietary fat and postmenopausal breast cancer. Journal of the National Cancer Institute 84, 1092–1099.

Landa, M.C., N. Frago, & A. Tres. (1994) Diet and the risk of breast cancer in Spain. European Journal of Cancer Prevention 3, 313–320.

La Vecchia, C., E. Negri, S. Franceschi, A. Decarli, A. Giacosa, & L. Lipworth. (1995) Olive oil, other dietary fats, and the risk of breast cancer (Italy). Cancer Causes Control 6, 545–550.

Law, M.R., N.J. Wald, & S.G. Thompson. (1994) By how much and how quickly does reduction in serum cholesterol concentration lower risk of ischaemic heart disease? British Medical Journal 308, 367–372.

Leaf, A. (2007) Prevention of sudden cardiac death by n-3 polyunsaturated fatty acids. Journal of Cardiovascular Medicine (Hagerstown) 8 Suppl 1, S27–29.

Leaf, A., & P.C. Weber. (1988) Cardiovascular effects of n-3 fatty acids. New England Journal of Medicine 318, 549–557.

Leitzmann, M.F., M.J. Stampfer, D.S. Michaud, K. Augustsson, G.C. Colditz, W.C. Willett, & E.L. Giovannucci. (2004) Dietary intake of n-3 and n-6 fatty acids and the risk of prostate cancer. American Journal of Clinical Nutrition 80, 204–216.

Levi, F., C. Pasche, F. Lucchini, & C. La Vecchia. (2002) Macronutrients and colorectal cancer: a Swiss case-control study. Annals of Oncology 13, 369–373.

Lin, J., S.M. Zhang, N.R. Cook, I.M. Lee, & J.E. Buring. (2004) Dietary fat and fatty acids and risk of colorectal cancer in women. American Journal of Epidemiology 160, 1011–1022.

Liu, L., W. Zhuang, R.Q. Wang, R. Mukherjee, S.M. Xiao, Z. Chen, X.T. Wu, Y. Zhou, & H.Y. Zhang. (2011) Is dietary fat associated with the risk of colorectal cancer? A meta-analysis of 13 prospective cohort studies. European Journal of Nutrition 50, 173–184.

Lof, M., S. Sandin, P. Lagiou, L. Hilakivi-Clarke, D. Trichopoulos, H.O. Adami, & E. Weiderpass. (2007) Dietary fat and breast cancer risk in the Swedish women's lifestyle and health cohort. British Journal of Cancer 97, 1570–1576.

Lopez-Miranda, J., F. Perez-Jimenez, E. Ros, R. De Caterina, L. Badimon, M.I. Covas, E. Escrich, J.M. Ordovas, F. Soriguer, R. Abia, C.A. de la Lastra, M. Battino, D. Corella, J. Chamorro-Quiros, J. Delgado-Lista, D. Giugliano, K. Esposito, R. Estruch, J.M. Fernandez-Real, J.J. Gaforio, C. La Vecchia, D. Lairon, F. Lopez-Segura, P. Mata, J.A. Menendez, F.J. Muriana, J. Osada, D.B. Panagiotakos, J.A. Paniagua, P. Perez-Martinez, J. Perona, M.A. Peinado, M. Pineda-Priego, H. E. Poulsen, J.L. Quiles, M.C. Ramirez-Tortosa, J. Ruano, L. Serra-Majem, R. Sola, M. Solanas, V. Solfrizzi, R. de la Torre-Fornell, A. Trichopoulou, M. Uceda, J.M. Villalba-Montoro, J.R. Villar-Ortiz, F. Visioli, & N. Yiannakouris. (2010) Olive oil and health: summary of the II international conference on olive oil and health consensus report, Jaen and Cordoba (Spain) 2008. Nutrition, Metabolism & Cardiovascular Diseases 20, 284–294.

Martin-Moreno, J.M., W.C. Willett, L. Gorgojo, J.R. Banegas, F. Rodriguez-Artalejo, J.C. Fernandez-Rodriguez, P. Maisonneuve, & P. Boyle. (1994) Dietary fat, olive oil intake and breast cancer risk. International Journal of Cancer 58, 774–780.

Martinez-Gonzalez, M.A., J. Salas-Salvado, R. Estruch, D. Corella, M. Fito, E. Ros, & I. Predimed. (2015) Benefits of the Mediterranean diet: insights from the PREDIMED Study. Progress in Cardiovascular Diseases 58, 50–60.

McManus, K., L. Antinoro, & F. Sacks. (2001) A randomized controlled trial of a moderate-fat, low-energy diet compared with a low fat, low-energy diet for weight loss in overweight adults. International Journal of Obesity and Related Metabolic Disorders 25, 1503–1511.

Menotti, A., A. Alberti-Fidanza, F. Fidanza, M. Lanti, & D. Fruttini. (2012) Factor analysis in the identification of dietary patterns and their predictive role in morbid and fatal events. Public Health Nutrition 15, 1232–1239.

Menotti, A., & P.E. Puddu. (2015) How the Seven Countries Study contributed to the definition and development of the Mediterranean diet concept: a 50-year journey. Nutrition, Metabolism & Cardiovascular Diseases 25, 245–252.

Mensink, R.P., & M.B. Katan. (1987) Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoprotein in healthy men and women. Lancet 1, 122–125.

Mensink, R.P., & M.B. Katan. (1992) Effect of dietary fatty acids on serum lipids and lipoproteins: a meta-analysis of 27 trials. Arteriosclerosis and Thrombosis 12, 911–919.

Mensink, R.P., P.L. Zock, A.D. Kester, & M.B. Katan. (2003) Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. American Journal of Clinical Nutrition 77, 1146–1155.

Meyer, F., I. Bairati, R. Shadmani, Y. Fradet, & L. Moore. (1999) Dietary fat and prostate cancer survival. Cancer Causes Control 10, 245–251.

Meza-Miranda, E.R., A. Camargo, O. A. Rangel-Zuniga, J. Delgado-Lista, A. Garcia-Rios, P. Perez-Martinez, I. Tasset-Cuevas, I. Tunez, F.J. Tinahones, F. Perez-Jimenez, & J. Lopez-Miranda. (2014) Postprandial oxidative stress is modulated by dietary fat in adipose tissue from elderly people. Age (Dordr.) 36, 507–517.

Michaud, D.S., K. Augustsson, E.B. Rimm, M.J. Stampfer, W.C. Willet, & E. Giovannucci. (2001) A prospective study on intake of animal products and risk of prostate cancer. Cancer Causes Control 12, 557–567.

Mitrou, P.N., V. Kipnis, A.C. Thiebaut, J. Reedy, A.F. Subar, E. Wirfalt, A. Flood, T. Mouw, A.R. Hollenbeck, M.F. Leitzmann, & A. Schatzkin. (2007) Mediterranean dietary pattern and prediction of all-cause mortality in a US population: results from the NIH-AARP Diet and Health Study. Archives of Internal Medicine 167, 2461–2468.

Mizuno, N.K., O.P. Rogozina, C.M. Seppanen, D.J. Liao, M.P. Cleary, & M.E. Grossmann. (2013) Combination of intermittent calorie restriction and eicosapentaenoic acid for inhibition of mammary tumors. Cancer Prevention Research (Phila) 6, 540–547.

Morgan, J.W., G.E. Fraser, R.L. Phillips, & M.H. Andress. (1988) Dietary factors and colon cancer incidence among Seventh-Day Adventists. American Journal of Epidemiology 128, 918 (abstract).

Nakamura, T., H. Ishikawa, I. Takeyama, A. Kawano, S. Ishiguro, T. Otani, T. Okuda, Y. Murakami, T. Sakai, & N. Matsuura. (2010) Excessive fat restriction might promote the recurrence of colorectal tumors. Nutrition and Cancer 62, 154–163.

National Diet Heart Study Research Group. (1968) National Diet Heart Study: final report. Circulation 37(suppl. 1), 1–428.

National Research Council, Committee on Diet and Health. (1989) Diet and health: implications for reducing chronic disease risk. National Academy Press, Washington, DC.

Nogueira, L.M., S.M. Dunlap, N.A. Ford, & S.D. Hursting. (2012) Calorie restriction and rapamycin inhibit MMTV-Wnt-1 mammary tumor growth in a mouse model of postmenopausal obesity. Endocrine-Related Cancer 19, 57–68.

Norrish, A.E., R.T. Jackson, S.J. Sharpe, & C.M. Skeaff. (2000) Men who consume vegetable oils rich in monounsaturated fat: their dietary patterns and risk of prostate cancer (New Zealand). Cancer Causes Control 11, 609–615.

Nowak, Z. (2011) Selling the Mediterranean diet: marketing a myth. New Voices in Italian Studies, Rome.

Ooi, E.M., G.F. Watts, T.W. Ng, & P.H. Barrett. (2015) Effect of dietary Fatty acids on human lipoprotein metabolism: a comprehensive update. Nutrients 7, 4416–4425.

Park, S.K., K.L. Tucker, M.S. O'Neill, D. Sparrow, P.S. Vokonas, H. Hu, & J. Schwartz. (2009) Fruit, vegetable, and fish consumption and heart rate variability: the Veterans Administration Normative Aging Study. American Journal of Clinical Nutrition 89, 778–786.

Parthasarathy, S., D. Steinberg, & J.L. Witztum. (1992) The role of oxidized low-density lipoproteins in the pathogenesis of atherosclerosis. Annual Review of Medicine 43, 219–225.

Perez-Jimenez, F., G. Alvarez de Cienfuegos, L. Badimon, G. Barja, M. Battino, A. Blanco, A. Bonanome, R. Colomer, D. Corella-Piquer, I. Covas, J. Chamorro-Quiros, E. Escrich, J.J. Gaforio, P. P. Garcia Luna, L. Hidalgo, A. Kafatos, P.M. Kris-Etherton, D. Lairon, R. Lamuela-Raventos, J. Lopez-Miranda, F. Lopez-Segura, M.A. Martinez-Gonzalez, P. Mata, J. Mataix, J. Ordovas, J. Osada, R. Pacheco-Reyes, M. Perucho, M. Pineda-Priego, J.L. Quiles, M.C. Ramirez-Tortosa, V. Ruiz-Gutierrez, P. Sanchez-Rovira, V. Solfrizzi, F. Soriguer-Escofet, R. de la Torre-Fornell, A. Trichopoulos, J.M. Villalba-Montoro, J.R. Villar-Ortiz, & F. Visioli. (2005) International conference on the healthy effect of virgin olive oil. European Journal of Clinical Investigation 35, 421–424.

Petroni, A., M. Blasevich, M. Salami, N. Papini, G.F. Montedoro, & C. Galli. (1995) Inhibition of platelet aggregation and eicosanoid production by phenolic components of olive oil. Thrombosis Research 78, 151–160.

Psaltopoulou, T., R.I. Kosti, D. Haidopoulos, M. Dimopoulos, & D.B. Panagiotakos. (2011) Olive oil intake is inversely related to cancer prevalence: a systematic review and a meta-analysis of 13,800 patients and 23,340 controls in 19 observational studies. Lipids in Health and Disease 10, 127–143.

Rao, G.N. (1996) Influence of diet on tumors of hormonal tissues. Progress in Clinical Biological Research 394, 41–56.

Reaven, P. (1995) Dietary and pharmacologic regimens to reduce lipid peroxidation in non-insulin-dependent diabetes mellitus. American Journal of Clinical Nutrition 62(6 suppl.), 1483S–1489S.

Richardson, S., M. Gerber, & S. Cenee. (1991) The role of fat animal protein and vitamin consumption in breast cancer: a case-control study in Southern France. International Journal of Cancer 48, 1–9.

Richman, E.L., S.A. Kenfield, J.E. Chavarro, M.J. Stampfer, E.L. Giovannucci, W.C. Willett, & J.M. Chan. (2013) Fat intake after diagnosis and risk of lethal prostate cancer and all-cause mortality. JAMA Internal Medicine 173, 1318–1326.

Riserus, U., W.C. Willett, & F.B. Hu. (2009) Dietary fats and prevention of type 2 diabetes. Progress in Lipid Research 48, 44–51.

Rose, D.P., & J.M. Connolly. (1991) Effects of fatty acids and eicosanoid synthesis inhibitors on the growth of two human prostate cancer cell lines. Prostate 18, 243–254.

Rubio-Senent, F., B. de Roos, G. Duthie, J. Fernandez-Bolanos, & G. Rodriguez-Gutierrez. (2015) Inhibitory and synergistic effects of natural olive phenols on human platelet aggregation and lipid peroxidation of microsomes from vitamin E-deficient rats. European Journal of Nutrition 54, 1287–1295.

Sacks, F.M., G.A. Bray, V.J. Carey, S.R. Smith, D.H. Ryan, S.D. Anton, K. McManus, C.M. Champagne, L.M. Bishop, N. Laranjo, M.S. Leboff, J.C. Rood, L. de Jonge, F.L. Greenway, C.M. Loria, E. Obarzanek, & D.A. Williamson. (2009) Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. New England Journal of Medicine 360, 859–873.

Shai, I., D. Schwarzfuchs, Y. Henkin, D.R. Shahar, S. Witkow, I. Greenberg, R. Golan, D. Fraser, A. Bolotin, H. Vardi, O. Tangi-Rozental, R. Zuk-Ramot, B. Sarusi, D. Brickner, Z. Schwartz, E. Sheiner, R. Marko, E. Katorza, J. Thiery, G.M. Fiedler, M. Bluher, M. Stumvoll, & M.J. Stampfer. (2008) Dietary Intervention Randomized Controlled Trial. Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. New England Journal of Medicine 359, 229–241.

Sheppard, L., A.R. Kristal, & L.H. Kushi. (1991) Weight loss in women participating in a randomized trial of low-fat diets. American Journal of Clinical Nutrition 54, 821–828.

Silva, S., E. Combet, M.E. Figueira, T. Koeck, W. Mullen, & M.R. Bronze. (2015) New perspectives on bioactivity of olive oil: evidence from animal models, human interventions and the use of urinary proteomic biomarkers. Proceedings of the Nutrition Society 74, 268–281.

Simopoulos, A.P. (2001) The Mediterranean diets: what is so special about the diet of Greece? The scientific evidence. Journal of Nutrition 131(11 suppl.), 3065S–3073S.

Simopoulos, A.P. (2004) The traditional diet of Greece and cancer. European Journal of Cancer Prevention 13, 219–230.

Stampfer, M.J., F.M. Sacks, S. Salvini, W.C. Willett, & C.H. Hennekens. (1991) A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. New England Journal of Medicine 325, 373–381.

Steinberg, D., & J.L. Witztum. (1990) Lipoproteins and atherogenesis: current concepts. Journal of the American Medical Association 264, 3047–3052.

Stemmermann, G.N., A. Normura, L.K. Heilbrun, E.S. Pollack, & A. Kagan. (1981) Serum cholesterol and colon cancer incidence in Hawaiian Japanese men. Journal of the National Cancer Institute 67, 1179–1182.

Tobias, D. K., M. Chen, J.E. Manson, D.S. Ludwig, W.C. Willett, & F.B. Hu. (2015) Effect of low-fat diet interventions versus other diet interventions on long-term weight change in adults: a systematic review and meta-analysis. The Lancet Diabetes & Endocrinology 3, 968–979.

Toledo, E., J. Salas-Salvado, C. Donat-Vargas, P. Buil-Cosiales, R. Estruch, E. Ros, D. Corella, M. Fito, F.B. Hu, F. Aros, E. Gomez-Gracia, D. Romaguera, M. Ortega-Calvo, L. Serra-Majem, X. Pinto, H. Schroder, J. Basora, J.V. Sorli, M. Bullo, M. Serra-Mir, & M.A. Martinez-Gonzalez. (2015) Mediterranean diet and invasive breast cancer risk among women at high cardiovascular risk in the PREDIMED trial: a randomized clinical trial. JAMA Internal Medicine 175, 1752–1760.

Toniolo, P., E. Riboli, F. Protta, M. Charrel, & A.P. Cappa. (1989) Calorie-providing nutrients and risk of breast cancer. Journal of the National Cancer Institute 81, 278–286.

Tresserra-Rimbau, A., Medina-Remón, A., Pérez-Jiménez, J., Martínez-González, M.A. Covas, M. Salas-Salvadó, J., Gómez-Gracia, E., Lapetra, J., Arós, F., Fiol, M., Ros, E., Serra Majem, L., Pintó, X., Muñoz, M.A., Saez, G. T., Ruiz-Gutiérrez, V., & Lamuela-Raventós, R.M. (2013). Dietary intake and major food sources of polyphenols in a Spanish population at high cardiovascular risk: the PREDIMED study. Nutrition, Metabolism, and Cardiovascular Diseases 23, 953–959.

Trichopoulou, A., C. Bamia, P. Lagiou, & D. Trichopoulos. (2010) Conformity to traditional Mediterranean diet and breast cancer risk in the Greek EPIC (European Prospective Investigation into Cancer and Nutrition) cohort. American Journal of Clinical Nutrition 92, 620–625.

Trichopoulou, A., T. Costacou, C. Bamia, & D. Trichopoulos. (2003) Adherence to a Mediterranean diet and survival in a Greek population. New England Journal of Medicine 348, 2599–2608.

Trichopoulou, A., K. Katsouyanni, S. Stuver, L. Tzala, C. Gnardellis, E. Rimm, & D. Trichopoulos. (1995) Consumption of olive oil and specific food groups in relation to breast cancer risk in Greece. Journal of the National Cancer Institute 87, 110–116.

Tsakiraki, M., M.G. Grammatikopoulou, C. Stylianou, & M. Tsigga. (2011) Nutrition transition and health status of Cretan women: evidence from two generations. Public Health Nutrition 14, 793–800.

Turpeinen, O., M.J. Karvonen, M. Pekkarinen, M. Miettinen, R. Elosuo, & E. Paavilainen. (1979) Dietary prevention of coronary heart disease: the Finnish Mental Hospital Study. International Journal of Epidemiology 8, 99–118.

Uauy, R., A. Aro, R. Clarke, Ghafoorunissa, M.R. L'Abbe, D. Mozaffarian, C.M. Skeaff, S. Stender, & M. Tavella. (2009) WHO Scientific Update on trans fatty acids: summary and conclusions. European Journal of Clinical Nutrition 63(S2), S68–S75.

Ulbricht, T.L.V., & D.A.T. Southgate. (1991) Coronary heart disease: seven dietary factors. Lancet 338, 985–992.

Valls-Pedret, C., A. Sala-Vila, M. Serra-Mir, D. Corella, R. de la Torre, M.A. Martinez-Gonzalez, E.H. Martinez-Lapiscina, M. Fito, A. Perez-Heras, J. Salas-Salvado, R. Estruch, & E. Ros. (2015) Mediterranean diet and age-related cognitive decline: a randomized clinical trial. JAMA Internal Medicine 175, 1094–1103.

Van den Brandt, P.A., P. Van't Veer, R.A. Goldbohm, E., Dorant, A., Volovics, R.J., Hermus, & E. Sturmans. (1993) A prospective cohort study on dietary fat and the risk of postmenopausal breast cancer. Cancer Research 53, 75–82.

Visioli, F., G. Bellomo, G. Montedoro, & C. Galli. (1995) Low density lipoprotein oxidation is inhibited in vitro by olive oil constituents. Atherosclerosis 117, 25–32.

Visioli, G., & C. Galli. (1995) Natural antioxidants and prevention of coronary heart disease: the potential role of olive oil and its minor constituents. Nutrition, Metabolism & Cardiovascular Diseases 5, 306–314.

Vissers, M.N., P.L. Zock, & M.B. Katan. (2004) Bioavailability and antioxidant effects of olive oil phenols in humans: a review. European Journal of Clinical Nutrition 58, 955–965.

Wakai, K., M. Naito, C. Date, H. Iso, & A. Tamakoshi. (2014) Dietary intakes of fat and total mortality among Japanese populations with a low fat intake: the Japan Collaborative Cohort (JACC) Study. Nutrition & Metabolism 11, 1–13.

Weisburger, J.H., & E.L. Wynder. (1991) Dietary fat intake and cancer. Hematology/Oncology Clinics of North America 5, 7–23.

Welsch, C.W. (1992) Relationship between dietary fat and experimental mammary tumorigenesis: a review and critique. Cancer Research 52(suppl. 7), 2040S–2048S.

Willett, W.C. (1994) Diet and health: what should we eat? Science 264, 532–537.

Willett, W.C. (2002) Dietary fat plays a major role in obesity: no. Obesity Reviews 3, 59–68.

Willett, W.C. (2007) The role of dietary n-6 fatty acids in the prevention of cardiovascular disease. Journal of Cardiovascular Medicine (Hagerstown) 8(suppl. 1), S42–S45.

Willett, W.C. (2010) The WHI joins MRFIT: a revealing look beneath the covers. American Journal of Clinical Nutrition 91, 829–830.

Willett, W.C. (2012) Dietary fats and coronary heart disease. Journal of Internal Medicine 272, 13–24.

Willett, W.C., D.J. Hunter, M.J. Stampfer, G.A. Colditz, J.E. Manson, D. Spiegelman, B. Rosner, C.H. Hennekens, & F.E. Speizer. (1992) Dietary fat and fiber in relation to risk of breast cancer: an 8-year follow-up. Journal of the American Medical Association 268, 2037–2044.

Willett, W.C., & M.J. Stampfer. (1986) Total energy intake: implications for epidemiologic analyses. American Journal of Epidemiology 124, 17–27.

Willett, W.C., & M.J. Stampfer. (1990) Dietary fat and cancer: another view. Cancer Causes Control 1, 103–109.

Willett, W.C., M.J. Stampfer, G.A. Colditz, B.A. Rosner, & F.E. Speizer. (1990) Relation of meat, fat, and fiber intake to the risk of colon cancer in a prospective study among women. New England Journal of Medicine 323, 1664–1672.

Wiseman, S.A., J.N. Mathot, N.J. de Fouw, & L.B. Tijburg. (1996) Dietary non-tocopherol antioxidants present in extra virgin olive oil increase the resistance of low density lipoproteins to oxidation in rabbits. Atherosclerosis 120, 15–23.

Wissler, R.W., & D. Vesselinovitch. (1975) The effects of feeding various dietary fats on the development and regression of hypercholesterolemia and atherosclerosis. Advances in Experimental Medicine and Biology 60, 65–76.

World Cancer Research Fund. (2011) Continuous update project: colorectal cancer. World Cancer Research Fund, London.

World Cancer Research Fund. (2012) Cancer facts and figures: data on specific cancers. WCRF International, London.

World Cancer Research Fund. (2014) Continuous update project. World Cancer Research Fund, London.

Wynder, E.L. (1976) Nutrition and cancer. Federation Proceedings 35, 1309–1315.

Yanai, H., H. Katsuyama, H. Hamasaki, S. Abe, N. Tada, & A. Sako. (2015) Effects of dietary fat intake on HDL metabolism. Journal of Clinical Medicine Research 7, 145–149.

Yannakoulia, M., M. Kontogianni, & N. Scarmeas. (2015) Cognitive health and Mediterranean diet: just diet or lifestyle pattern? Ageing Research Reviews 20, 74–78.

Young, S.G., & S. Parthasarathy. (1994) Why are low-density lipoproteins atherogenic? WesternJournal of Medicine 160, 153–164.

Zhu, Z., W. Jiang, J.H. Zacher, E.S. Neil, J.N. McGinley, & H.J. Thompson. (2012) Effects of energy restriction and wheel running on mammary carcinogenesis and host systemic factors in a rat model. Cancer Prevention Research (Phila) 5, 414–422.

27 Lipidomics and health: an added value to olive oil

Carla Ferreri and Chryssostomos Chatgilialoglu

27.1 Introduction

Olive oil is an important resource as it contains many different compounds and lipids, mainly in the form of triacylglycerol. Since ancient times, olive oil has been referred to as the "secret" of the Mediterranean strength and longevity, and it was included in their dietary habits much before the availability of in-depth analyses and studies. Lipidomics examines the dynamic pathways of food lipids from the uptake to the transformations occurring in the body. It is much interested in olive oil as a source of monounsaturated fatty acids (MUFAs), going from their importance as tissue and cell membrane constituents to the crucial roles played as fine regulators of cell metabolism and signaling. This chapter provides an overview of the MUFAs of olive oil, highlighting some aspects such as the position and geometry of the unsaturation for chemical, biochemical, and analytical implications. Then, it focuses on major and minor MUFA components and summarizes the most recent studies on health implications as evidenced by considering obesity and cancer lipidomics.

27.2 Lipidomics: an added value to olive oil

The importance of nutrition and its relation to health has been recognized since ancient times, and nowadays the paradigm of health is receiving much attention due to the criticisms of the industrialized "Western-type" model and the need of sustainability for health and food of the future (Battino & Ferreiro, 2004; Konstantinidou *et al.*, 2014). Evidence has also been gathered on the need for personalized health approaches, which can ensure both correct nutritional choices and cost–benefit optimization. In this scenario, research on molecular diagnostics makes a major contribution to personalized nutrition with the "-omics" approach, examining the body composition and metabolic transformations in a dynamic and functional manner. Among them, lipidomics is most relevant to edible oils that contain mainly triacylglycerols as the main lipid source for the human body (Hyötyläinen *et al.*, 2013). Oils have typical patterns of fatty acids, and for olive oil there is a high content of MUFAs, namely 9*cis*-octadecenoic acid or oleic acid (9*cis*-18:1), as discussed in other chapters of this book.

Lipidomics of olive oil addresses several aspects, such as determination of the origin and metabolism of the olive plant, offering a modern tool for quality control (Dais & Hatzakis, 2013; Shen *et al.*, 2013). This application will not be discussed in this chapter. For application in health, lipidomics examines crucial steps occurring in the body when olive oil is used as food, from consumption and absorption to biodistribution, metabolism, and bioavailability of lipid components of olive oil. The scope is the full evaluation and understanding of behavior and effects at the molecular level, evidencing key interactions and cascades, influenced especially by the monounsaturated fats and correlated with health effects.

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Oleic acid plays a very important biological role as the most represented MUFA in cell membranes of all tissues, whereas minor components such as 9*cis*-hexadecenoic acid (i.e., palmitoleic acid [9*cis*-16:1]) have only recently been highlighted as a signaling molecule, attracting a lot of attention in research for disease onset such as obesity. The structures of MUFAs, with different carbon atom chain lengths (oleic acid with 18 and palmitoleic acid with 16 carbon atoms) as well as different numbers and positions of double bonds along the chain, are important factors contributing to the diversity of biological effects. The success of lipidomics is assisted by the tremendous advancements of technology that allow researchers to build platforms for successful, highly efficient processing of large numbers of samples when analyzing complex fatty acid mixtures as found in biological samples (Ferreri & Chatgilialoglu, 2012; Köfeler *et al.*, 2012). Nowadays, the dream to individualize lipidomic profiles or phenotypes connected to health conditions is becoming attainable, and this may foster the use of foods and natural oils, targeted to personalized needs, in helping translation of individual molecular outcomes into the nutrition and lifestyle adapted to the profile.

Lipidomic research is now among modern approaches to follow up in detail the so-called lipidome, in plants as well as in living organisms. A definition of "lipidome" is the lipid inventory of each organism, containing the variety of molecules and their metabolites present and formed during life. It must be taken into account that lipidome composition changes according to living conditions, and includes the effects of environmental and nutritional factors. Lipidomics furnishes a comprehensive scenario, thus explaining the found lipidome, tracking the metabolic pathways, but also molecular processes of lipid chemical reactivity, distribution, and competition that occur in the intra- and intercellular environment.

In health, lipidomics is one of the most promising interdisciplinary fields of research, and its scientific achievements are most directly connected with outcomes applicable to the market, for consumer benefits. Lipidomic research has recently been developing strongly based on olive oil characteristics and effects, because olive oil is a dietary component containing bioactive molecules with nutraceutical and curative properties. It has already been shown that there are specific profiles, such as metabolic diseases as well as aging, where a successful personalized nutrition approach with olive oil can be applied.

27.3 Membrane lipidomics and nutrilipidomics: natural oils for a healthy balance

When olive oil is used, cells acquire oleic acid as the most concentrated bioactive component (55–83%) in the form of triacylglycerol (triolein), which after the digestion and absorption processes provides this MUFA to the whole body. The most relevant biological activity carried out by oleic acid is found in the formation of the phospholipid structure, being present as the hydrophobic moiety of the phospholipids as shown in Figure 27.1, profoundly influencing fluidity, permeability, and packing properties of cell membranes. The bent structure of this MUFA creates a larger occupancy in the membrane packing, which contributes to fluidity (Ladha, 1998). It is estimated that an approximately 30° bent of MUFA structures, and the corresponding membrane double-layer packing, forms a vesicle with a larger diameter compared to saturated and trans fatty acid packing (Ferreri *et al.*, 2006a).

The melting points of fatty acids, reflected in the transition temperatures of the phospholipids, also indicate lower melting temperatures for MUFAs compared to corresponding saturated and trans fats, which means that at physiological temperatures MUFAs can be in a fluid state, whereas saturated and trans fats are characterized by higher melting temperatures that result in a more solid and rigid environment (Cevc, 1991). It is also known that membranes with MUFAs can contribute to a better working environment for membrane proteins. Indeed, cell membranes are a "mosaic" of fats embedding proteins that act as pores, channels, and signals, so that properties of the membrane lipidome are very crucial for the optimal occurrence of the whole functioning (Engelman, 2005; Goñi, 2014; Nicolson, 2014).

Based on this information, available due to decades of research, lipidomic research started to focus on understanding how cell membranes work as epigenetic sites, receiving and forming substantial signals that regulate and design the cell fate. It is impressive that there is such a big amount of experimental research, reviews, and books that has been published in the last decade or so, highlighting several aspects such as the membrane lipid organization, the diversity of lipids able to produce a homeostatic control of membrane properties, the role of lipids and lipid rafts in regulating the response of membrane proteins, receptors, and

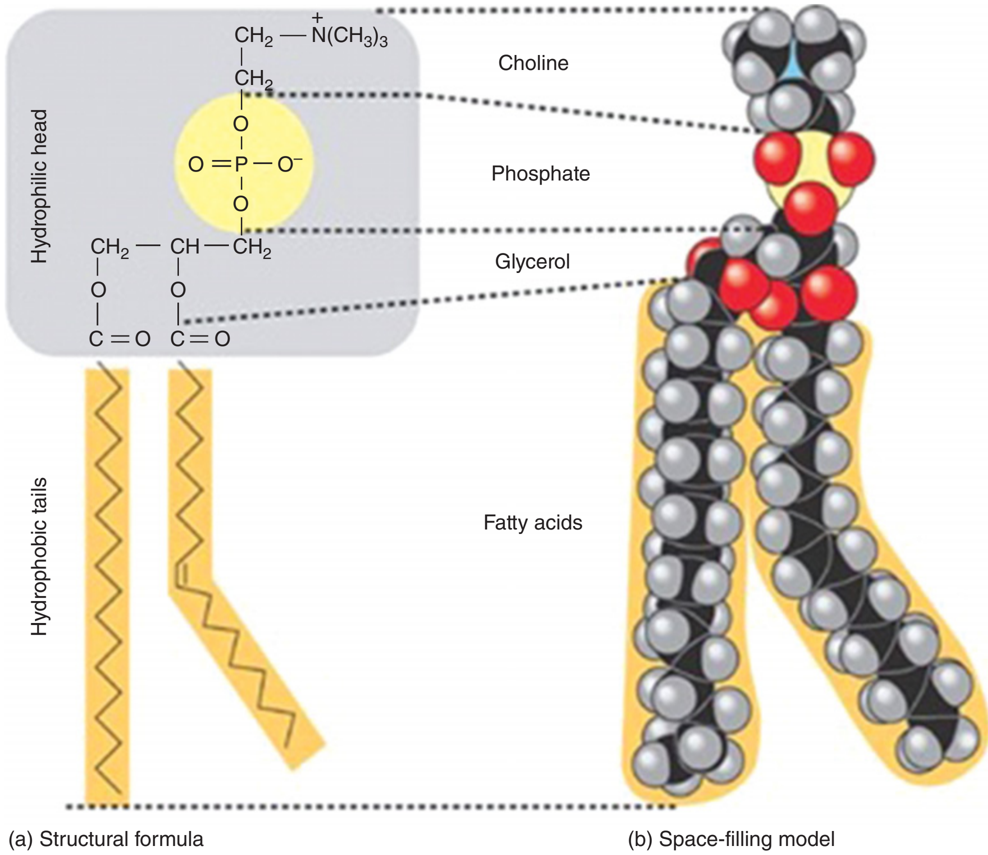

Figure 27.1 The structure of a phospholipid having, in position sn-2, the moiety of oleic acid.

signaling pathways (Simons & Toomre, 2000; Pike, 2003; Escribà, 2014). In research on free radical stress and biological consequences, we found that chemical processes involving membrane and its unsaturated fatty acid components are crucial to explain some aspects of the reactivity and response of cells to environmental and metabolic processes. The occurrence of the change of the natural cis geometry of membrane phospholipids into the corresponding unnatural trans geometry due to free radicals was first evidenced by Ferreri *et al.* (1999). This isomerization process occurs in the same environment where the well-known lipid peroxidation takes place, and affects fluidity and permeability as well as the working environment of membrane proteins (Ferreri *et al.*, 2006b; Chatgilialoglu *et al.*, 2014). The oleic acid moiety, which is the most representative unsaturated fatty acid present in membranes, is affected by isomerization with the formation of 9*trans*-18:1 (i.e., elaidic acid). This isomer can be taken as a biomarker of free radical stress in humans.

From the research carried out so far, it is evident that cell membranes and their phospholipid constituents cannot be “spectators” in the cellular metabolism, and they were defined as “metabolic pacemakers” (Else & Hulbert, 2003). In fact, membranes change influence and regulate the whole cell response. Permeability, fluidity, and thickness of membranes are involved in signal transduction in all biological processes, definitely affecting the important phenomenon of homeostasis, which is the crucial element of cell response and adaptation.

Using the immense knowledge on membrane lipids accumulated by researchers during these years, we postulated the relationship between the membrane homeostasis (i.e., membrane balance), represented mainly by the correct fatty acid composition, and the cell wellness. This concept is also expressed by other approaches, such as “membrane lipid therapy” or “lipid replacement therapy,” which can address the reconstitution of the lipids in tissues and organelles by using a natural medicine approach made of lipids and other

important cofactors for oxidative and enzymatic processes (Nicolson & Ash, 2014). The optimal status of the membrane lipidome corresponds to the recovery of quality of life and is effective in disease prevention, translating the molecular wellness into health of the organism. In this context, we addressed the personalization of the therapy by using the lipidomic analysis of cell membranes and drawing the corresponding lipidome profile of the individual (Ferreri & Chatgilialoglu, 2015). The approach was called "nutrilipidomics," where the choices of foods, dietary intake, and nutraceutical intervention can be carried out on the basis of the individual lipidome condition, to achieve the best result for restoring and maintaining the naturally occurring homeostatic control, with benefits at cellular, metabolic, and physiological levels (Chatgilialoglu & Ferreri, 2012). Nutrilipidomics addresses specifically cell membranes, as relevant sites reporting the results of several processes occurring in the body, using foods and its molecular content in a personalized manner. Since olive oil is an oil mainly composed of oleic acid, which is also the most important MUFA in membranes, the nutrilipidomics approach can be very useful to individualize the exact target and dosage of this food for becoming more and more targeted to an individual's needs. It is worth noting that, since olive oil is mainly made of the omega-9 oleic acid, it has to be combined with oils or foods that contain essential polyunsaturated fatty acids (PUFAs), omega-6 and omega-3, for the optimal balance.

The main international health agencies, the European Food Safety Association (EFSA), World Health Organization (WHO), and Food and Agricultural Organization (FAO), suggest the biodiversity of the fat intake (Burlingame & Dernini, 2010; EFSA Panel, 2010) to ensure the optimal contribution for a healthy life. The nutrilipidomics approach can assign this fat intake in a personalized manner, tailoring the proportion among the fatty acid families to the individual condition.

27.3.1 Effects of fatty acids on membrane properties and biological roles of oleic acid

An overview of the fatty acids and their molecular effect on membrane properties is provided here, and readers may deepen their knowledge on topics of interest by consulting reviews and books published on these subjects (Katsaras & Gutberlet, 2001; Mouritsen, 2005; Ibarguren *et al.*, 2014). The molecular shapes of fatty acids, maintained in the corresponding phospholipids, provide membranes with peculiar organization and characteristics, with notable differences between the saturated and unsaturated families. With the presence of oleic acid as a fatty acid in phospholipids, membranes are less packed than with saturated fats. The oleic acid structure due to the cis configuration of its double bond reduces the order within the lipid layer, favoring the hydration level and increasing membrane fluidity. This can be appreciated by the transition temperature (more or less "melting" temperatures for phospholipids) for dioleoyl-phosphatidylcholine (DOPC) of −20 °C, whereas with two palmitoyl (C16:0) chains (DPPC, dipalmitoyl-phosphatidylcholine) it is 41 °C. Changing the polar heads from choline to ethanolamine, the effect is also reflected in the transition temperatures of DOPE (dioleoyl-phosphatidylethanolamine) and DPPE (dipalmitoyl-phosphatidylethanolamine) of −16 and 41 °C, respectively (Cevc, 1993; Shaikh & Edidin, 2006). Lower melting temperatures correspond, in a simplistic view, to more fluid membranes, and, in a functional way, the ratio of liquid-ordered to liquid-disordered structures in membranes affects the functioning of the lipid double layer, especially that of the lipid rafts (i.e., parts of membrane assembly that accompany channels, receptors, and other protein functions). In addition, in comparison with other fatty acids, such as PUFAs, oleic acid was found to have the greatest disordering effect, suggesting that the position of the double bond along the C18 carbon atom chain is crucial in determining a precise reorganizing effect, as detected in model membranes (Shaikh *et al.*, 2004).

The numerous molecular studies on the use of oleic acid evidenced the importance of its incorporation in relevant regulation sites at the cellular level; therefore, it emerges that the crucial point of its activity is the membrane compartment. Indeed, in this compartment, fatty acids unsaturation can profoundly influence the cell fate by playing important roles in the preservation of the homeoviscous state. As mentioned in this chapter, the quality and organization of membrane lipids can vary the working environment of the proteins embedded in the double layer, and there are several studies evaluating the effects of oleic acid as hydrophobic residue of phospholipids. Examples are taken from an interesting review on the relationship between membrane composition and activity of food constituents (Lopez *et al.*, 2014). Protein interactions with specific regions of the membranes (i.e., those having a liquid-ordered phase or nonlamellar phase) were

evaluated. The hydrolytic activity of Na/K-ATPase was influenced by oleic acid in reconstituted membranes, which are able to affect the packing and create an appropriate topological setting for protein optimal function in the membrane urothelium. Another example of the influence on the membrane packing is given by the membrane docking and resulting activity of the G-protein coupled receptors (GPCRs), which are the largest family of membrane-spanning proteins with properties of detection of several extracellular signals or ligands, such as photons, ions, odors, pheromones, hormones, and neurotransmitters. Furthermore, the regulation via G proteins is also involved in the signaling pathway of adrenergic receptors and adrenoreceptors, key elements in the central and peripheral control of blood pressure. In fact, these receptors are affected by the increase of a different organization of membrane lipids, called the hexagonal phase, and this increase is facilitated by the administration of monounsaturated components, such as oleic acid. Therefore, the vasodilatory pathway was explained as a cascade of signaling derived from oleic acid, directly from its place in membrane organization; this is translated into a nutraceutical use of olive oil as a natural product for hypertension. The contribution given by oleic acid to the membrane organization has been recognized in the regulation of protein channels, such as GLUT4 for glucose uptake, which is governed by the ratio of saturated fatty acids (SFAs) and MUFAs in model membranes. Consequently, this molecular effect can be translated into an accurate use of dietary olive oil in diabetes and metabolic diseases.

Intracellular docking of fatty acids to specific cytoplasmic receptors is another important pathway for intracellular signals arriving to nuclear DNA. Fatty acid types have been found to play different roles, according to the complex that they form. In this case, the free form of fatty acids is involved, as that obtained by the action of lipase on the triacylglycerol depots. The signaling departing from free fatty acids involves several soluble receptors (Papackova & Cahova, 2015). For example, with peroxisome-proliferator-activated receptors (PPARs), oleic acid is important for the transcription of key genes of neuronal differentiation and growth (Rodriguez-Rodriguez *et al.*, 2004; Bento-Abren *et al.*, 2007), and also exerts molecular-protective activity against neurodegenerative diseases (Yang *et al.*, 2010). In macrophages, the PPARs and PPAR-RXR (retinoid X receptor) transcriptional complex are involved in sensing the different proportions of palmitic and oleic acids with influence on cell signaling for postprandial triacylglycerol management (Varela *et al.*, 2013). Such molecular effects suggest a relevant place for olive oil in the dietary indication of metabolic diseases. However, in this case it is recommended to address the previously mentioned fatty acid diversity, with the use of other oil sources providing omega-6 and omega-3 in the right balance, together with olive oil.

Other studies have also focused on the metabolic conversion of oleic acid. An example is the formation of 2-hydroxyoleic acid, which has been proposed as a natural metabolite with hypotensive effects (Alemany *et al.*, 2004; Terés *et al.*, 2008). It is worth adding that research on the biological roles of oleic acid as a component of olive oil evidenced that positive molecular effects can derive from the fact that it substitutes (a) SFAs in the membrane assembly, bringing positive effects for fluidity and permeability, as explained in this chapter; and (b) PUFAs, specifically omega-6, in the process of membrane remodeling, thus reducing the consequences of cellular processes of oxidation and transformations. Moreover, other bioactive components of olive oil, such as antioxidants, can synergize with the effects of oleic acid, reinforcing its molecular effects (Lopez *et al.*, 2014).

27.3.2 Lipidomics of oleic acid in health and disease

Lipidomics addressed oleic acid as a necessary element for the cell membranes, since its biosynthesis is correlated with the insertion of this fatty acid in the double layer, as a phospholipid with effects on membrane properties and signaling pathways. However, there are several pieces of information obtained by lipidomics that indicate the level of oleic acid being involved in several health conditions. Its control by lipidomics can be an efficient tool for both prevention and evaluation of the onset of diseases. This can also indicate the strategy of a personalized prevention plan, based on the monitoring of the membrane status throughout the life of individuals, using targeted intervention mostly based on nutritional elements (Ferreri & Chatgilialoglu, 2015). Research results concerning the role of oleic acid in cell membranes in health and disease conditions are as follows.

The first example is the tumor growth. Researchers have recently been focused on the fact that cancer cell viability depends on the ability to produce phospholipids for supporting the fast cell division and growth. In particular, studies addressed the *de novo* lipid biosynthesis, represented by the activity of the enzymatic

FAS

Palmitic acid (16:0)

Elongase

Stearic acid (18:0)

delta-9 desaturase

Oleic acid (9*cis*-18:1)

Figure 27.2 The saturated–monounsaturated fatty acid pathway with the formation of oleic acid.

complex fatty acid synthase (FAS), which has palmitic acid as a final product, and the subsequent formation of stearic acid by elongase enzyme followed by the stearoyl desaturase enzyme (SCD-1) forming oleic acid. This biosynthetic cascade is shown in Figure 27.2.

The expression of SCD-1 in tumoral cell lines was found to be very high (Roongta *et al.*, 2011; Kamphorst *et al.*, 2013), thus pointing out the role of MUFAs such as oleic acid in sustaining tumor cell life. Other researchers had previously pointed out that the SCD-1 activity produces MUFAs able to increase membrane fluidity and to influence the Akt-induced signaling cascade associated with increased tumorigenesis pathways (Igal, 2010). However, it is debated that the role of oleic acid is not to favor the growth signaling, and hence tumoral growth, but instead that oleic acid is the product of the endogenous stimulation of desaturase enzymatic activity, and therefore palmitic acid production is the most involved pathway in tumoral growth. It is also interesting to note that FAS stimulation and increased palmitic acid production, as well as stimulation of desaturase activity, associate with other metabolic conditions such as overweight and obesity and are connected to excess dietary intake of palmitic acid or carbohydrate, among others. All these conditions are also known to increase cancer risks, and it is remarkable to notice that they all point attention to the saturated–monounsaturated fatty acid pathway. The role of dietary fats in tumoral cell growth has yet to be fully understood, and it would be important to carry out these studies also in connection with the evaluation of new antitumoral strategies. In our hands, the simple dietary conditions in tumoral cell culture experiments demonstrated that palmitic acid is unfavorable for neuroblastoma cell growth, inducing apoptosis, whereas this fate is successfully prevented by oleic acid, which evidently helps several processes, such as the membrane homeoviscous adaptation (Bolognesi *et al.*, 2013).

Overall, this research shows that dietary habits cannot be ignored when considering tumoral growth, thus suggesting to couple nutritional intervention in all cancer therapeutical treatments, clarifying also the role of olive oil in this context. The molecular mechanisms connecting palmitic and oleic acids under overweight and obesity conditions have also been attributed to stimulation of the delta-9 desaturase activity (SCD-1). However, in this metabolic condition, other desaturases are involved, such as delta-6 and delta-5 desaturases (Warensjö *et al.*, 2006; Steffen *et al.*, 2008). It is worth adding that the beneficial effects of MUFAs and oleic acid could also be derived from their replacement of SFAs, which mediate toxic effects in cell metabolism of obese subjects.

As a matter of fact, adipose tissue lipid composition shows a strong presence of oleic acid as a component of triacylglycerol depots, also favoring adipocyte membrane assets that allow the activity of various receptors and the corresponding signaling for lipolysis to occur (Lauritzen *et al.*, 2001; Langin, 2006). It is logical that the normal function of adipocytes is obtained when the natural fat content of depots and membranes is respected. Conversely, the imbalance of fatty acid composition in adipose tissue can favor altered metabolic conditions, bringing interferences in signaling for the natural lipolytic activity, thus changing into an increased accumulation. The most relevant change at this level has been individualized in the higher content of saturated versus monounsaturated fats, and several studies confirm the different signaling derived from these two types of fatty acids. For example, in muscle cells, oleic acid attenuates the palmitate-induced messenger RNA (mRNA) for the dihydroceramide desaturase (DES1), with effects on insulin response and resistance (Hu *et al.*, 2011).

Several studies have shown a net differentiation between membrane signaling effects of SFAs and MUFAs directly produced by endogenous biosynthesis and those derived by activation of the lipolysis of fat depots accumulated from the diet. The reason for such difference is not yet understood. However, it can be hypothesized that a different fate for fatty acids, which is not strictly connected with the triacylglycerols of the fat depots and lipolysis, is represented by the membrane turnover, which occurs with fatty acid–containing molecules, such as lysophospholipids and fatty acyl coenzyme A, as elements of Lands' cycle (described further in this section).

On the other hand, it seems that the interaction of free fatty acids with their cytoplasmic receptors, such as the previously mentioned PPARs, is linked to the lipolysis process liberating other fatty acids from depots, although the mechanisms involved are not yet clear. The latter interaction brings fatty acids to be gene regulatory elements, as previously explained, by their docking with other molecules in order to form complexes with specific interactions on the DNA sequence (Georgiadi & Kersten, 2012).

At this point, it is important to highlight that the influence on cell behavior by fat supplementation occurs also via the so-called "membrane remodeling" process, known since 1958 as Lands' cycle (Lands, 1958).

As shown in Figure 27.3, any stimulus arriving from the exterior of a cell induces a membrane response and results in the activation of the enzyme called phospholipase A_2 (PLA_2), which in turn causes the liberation of the fatty acid moiety in the *sn*-2 position of the membrane phospholipids. The PLA_2 activity produces a free fatty acid and a lysophospholipid, both liberated in the cytoplasm and available for subsequent transformation into lipid mediators (endocannabinoids, prostaglandins, leukotrienes, etc.). The cycle continues with the restoration of the phospholipid loss from the membrane, and this can be rapidly obtained by recruiting a lysophospholipid molecule and a fatty acyl coenzyme A moiety (FA-coA) connected together by the acyl transferase enzymes, thus reconstituting the "new" phospholipid molecule to restore the integrity of the membrane assembly. Stimuli arriving to a cell, an event naturally occurring millions of times in a day of a

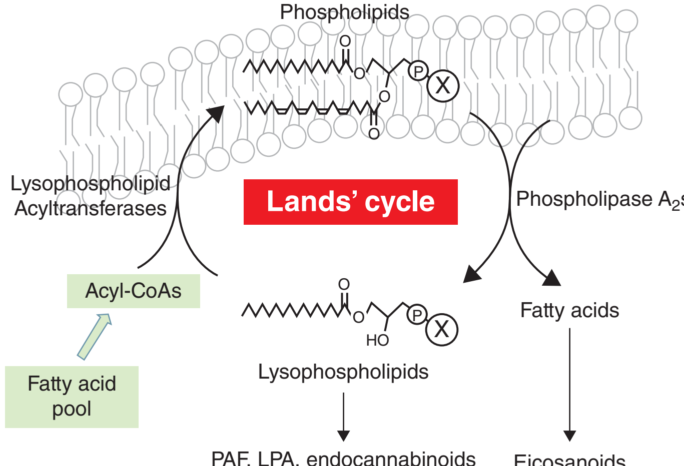

Figure 27.3 Land's cycle for the membrane remodeling after stimulus.

living organism, need an important connection to take place between the control of membrane homeostasis and the status of the individual lipid pool. The correctness of the phospholipid incorporation and functioning in membranes depends on the correctness of the fatty acid availability in the subject. In fact, the lipid pool must be equilibrated in quality and quantity of the fatty acids, in order to give the highest chance during the remodeling pathways to form the most effective membrane assembly. The availability of monounsaturated components, such as oleic acid, in the right quantity in the lipid pool, coming from the equilibrium between metabolism and dietary habits, is crucial for insertion in phospholipid molecules during the remodeling. In particular, oleic acid should be in the correct ratio with respect to the SFA moieties, but also with respect to the PUFA ones, especially omega-6. In fact, the balance of oleic acid versus arachidonic acid can be crucial for the balance of molecular signaling departing from membranes, whereas an imbalance can create a premise for higher cellular reactivity, represented by a membrane rich in omega-6 fatty acid moieties. In fact, upon stimuli, the liberation of omega-6 fatty acids, such as arachidonic acid, can create a high concentration in the cell, compared to a balanced composition, thus inducing cell reactive and inflammatory responses that are hard to put under control by the resolving lipid mediators (Serhan, 2014).

The molecular mechanisms brought about by oleic acid in the membrane lipidome status are very important and nowadays also recognized at the level of favorable clinical effects. For example, it has been reported that skin photoaging is less in subjects who use regularly olive oil in their diet (Latreille *et al.*, 2012), and this phenomenon can be correlated with the molecular effects of ameliorated fluidity with respect to saturated fats and of less oxidizability compared to PUFAs, brought about by the presence of oleic acid in membranes. Analogously, the presence of MUFAs is associated with increased lifespan in all kinds of living organisms, compared with PUFAs that provide higher oxidative stress and affect tissue resistance (Hulbert, 2010).

As mentioned in this chapter, by evaluating the membrane lipidome that is characteristic of each subject, the use of nutritional elements, such as those contained in olive oil, is purposely used in a personalized strategy pointing to the correct balance of cellular compartments and signaling. This molecular tool can also be useful to envisage dietary contributions of oils helping pharmacological treatments, in order to add molecular interactions favorable to the therapy, using the influence of lipids for the sensitive compartment of the membrane.

27.4 Membrane as relevant site for lipidomic analysis

The healthcare approach has improved in the last decade due to the advancement of molecular medicine, with the understanding of signaling and response pathways at the cellular level. It is clear that nutrients from the diet play a crucial role in the regulation of main cellular pathways, the most intriguing one being the recent developments in DNA methylation and histone acetylation as the two epigenetic processes that regulate gene expression and, consequently, metabolic pathways of importance to human health (Bender, 2004; Korzus, 2010). In this respect, another important site for signaling and responses is the cell membrane, and it is also known that DNA methylation regulates genes that code for lipid raft–associated components as well as angiogenesis processes, associated with cell transformation and cancer progression (Patra & Bettuzzi, 2007).

The membrane is also *per se* a relevant site for receiving and emitting signals, inducing an adaptive response and therefore exerting an epigenetic function with their lipid constituents. Indeed, membranes are necessary to define and form a living cell, and as a crucial site for regulating exchange of nutrients, oxygen, as well as information and stimuli that come from the same cell or from cells of other tissues. The knowledge of membrane composition and its regulation by the appropriate mix of the fatty acid residues of membrane phospholipids belong to biochemistry books and academic courses, according to the immense studies done in the past by lipidologists. However, in the last decade or so, the discipline of lipidomics revisited the knowledge of lipid classes and metabolism in a more dynamic and functional way. The lipid pools present in the body compartments, denominated "lipidomes," are considered with a different perspective, not only for their structure–activity relationships but also for their connection to the status of the organism. Lipidome monitoring can also highlight changes that occur under various life conditions, whether healthy or pathological ones. This approach is very promising for two main achievements; (a) in the field of biomarker discovery, for understanding the increase or decrease of a lipid level and its combination with the onset or development of a disease; and (b) in functional lipidomics, to evaluate the lipidome in a holistic sense, that

is, the combination of different lipids that is able to provide the optimal function or, if unbalanced, to create a cell discomfort that affects life quality at first, but can also bring the cell to a stress condition and then to health loss.

27.4.1 Oleic acid as biomarker in lipidomics

The use of oleic acid as a biomarker starts from considering its biosynthetic pathway, described earlier in this chapter and depicted in Figure 27.2. It is clear that to have access to oleic acid, there is only one endogenous pathway represented by the activity of the enzyme delta-9 desaturase (stearoyl desaturase) working with stearic acid. Stearic acid, in turn, is formed by elongation from palmitic acid, which is the first product of the *de novo* fatty acid biosynthesis, carried out by the enzymatic complex of FAS starting from simple two- or three-carbon-atom molecules. As mentioned in the previous section, considering molecular mechanisms, SFA and MUFA components of the tissues are strictly connected to each other in order to provide biological outcomes. Therefore, the increased biosynthesis is required for providing the fatty acid building blocks of phospholipids when the cell division takes place. For this reason, lipidomic research of the last decade or so has focused on the biosynthetic cascade of palmitic–stearic–oleic acids, followed up in organisms under several health conditions, and molecular signaling of cancer and obesity was discovered, as previously explained. In both diseases, the delta-9 desaturase activity is increased, due to the higher demand of lipids for expanding and dividing cells, and the shift to MUFAs provides cells with favorable membrane properties of fluidity and permeability. Evaluating the metabolic responses in human subjects, the increase of delta-9 desaturase activity was associated with dietary habits, such as increased intake of saturated fat, cholesterol, and carbohydrate, whereas it is disfavored by polyunsaturated fat (Ntambi, 1999; Flowers & Ntambi, 2009).

The translation of molecular mechanisms into biomarkers points attention to the increase of oleic acid that cannot be considered as a positive sign when present in the membrane lipidome of an adult subject, except in pregnant women. The use of oleic acid as a biomarker of cell proliferation was proposed, and its evaluation is better performed in membranes than in plasma, in the latter being strongly influenced by daily intake. In postmenopausal women, oleic acid was indeed associated with cancer risk, as evidenced by a meta-analysis of cohort studies (Saadatian-Elahi *et al.*, 2004). This biomarker is not yet used in clinical practice, where it would be important to establish an integrated panel, combining different indicators of proliferation and inflammation that are all favorable for tumor growth, in order to organize an active prevention plan in subjects with high levels of these indicators.

In obesity, an interesting differentiation with the previous condition of cancer growth emerges from the studies in human subjects, pointing attention to palmitoleic acid (9*cis*-16:1) as a relevant biomarker of MUFA biosynthesis (Pietiläinen *et al.*, 2007; Cao *et al.*, 2008). It is worth noting that palmitoleic acid is much less connected to dietary intake than oleic acid, and therefore its level can give important information about endogenous functioning of delta-9 desaturase. Recently, the MUFA components, precisely those belonging to the hexadecenoic fatty acid family, were enriched by a novel fatty acid biomarker, namely sapienic acid (6*cis*-16:1), first assigned only to triacylglycerols. Accurate analytical protocols for gas chromatographic separation of MUFA evidenced the presence of sapienic acid in different lipid classes of human blood, including triacylglycerols and cholesteryl esters, as well as lipoproteins and erythrocyte membrane phospholipids (Sansone *et al.*, 2013). In Figure 27.4, two pathways from palmitic acid are shown, forming palmitoleic acid by delta-9 desaturase and sapienic acid derived by delta-6 desaturase enzyme.

It is worth noting that the latter is an enzyme regularly active for PUFAs and not for SFAs, and this aspect is a matter of current research interest under different health conditions. The finding of positional isomerism in the MUFA family has recently been highlighted, raising attention to the analytical methodologies followed for examination of biological samples. Due to the possibility to confuse fatty acid structures having the same molecular masses, such as in the case of palmitoleic and sapienic acids, protocols of high efficiency in separation and identification are needed. It is desirable that such protocols should be extended to the analysis of olive oils, to compare also different productions, as mentioned in the first section of this chapter. It is worth noting that the content of palmitoleic acid is known, but that of sapienic acid is not.

Another case of the use of oleic acid as a biomarker is the evaluation of free radical stress of the cell membrane components. With oleic acid being less prone to oxidative reactions than PUFAs, MUFAs are considered practically stable under the oxidative metabolic conditions. However, the presence of the double bond is important for cis-trans isomerization, as depicted in Figure 27.5 for oleic acid.

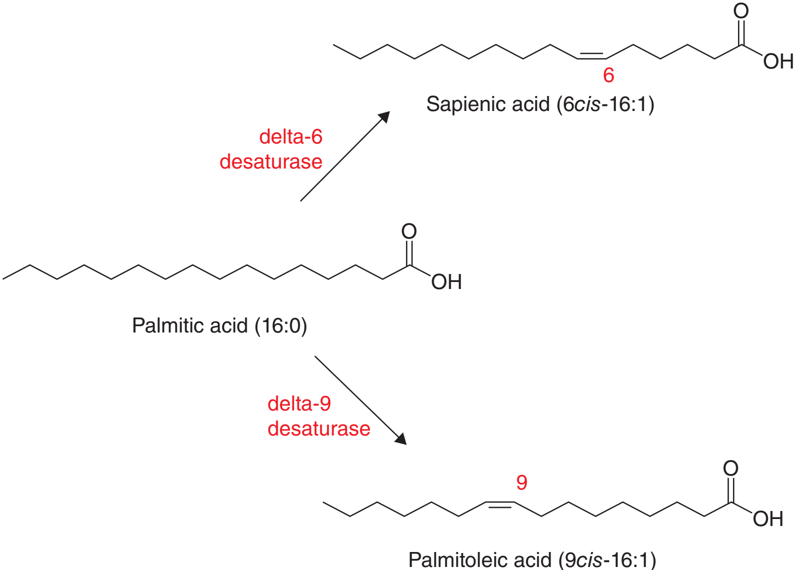

Figure 27.4 The formation of palmitoleic acid and sapienic acid from palmitic acid by the action of delta-9 and delta-6 desaturase enzymes, respectively.

This reaction corresponds to loss of the natural cis geometry, which comprises favorable molecular characteristics of natural unsaturated fatty acids, but this was not evidenced since the first experiments proposed in 1999 using membrane phospholipids (Ferreri *et al.*, 1999). The formation of geometrical trans isomers of natural fatty acids can occur under different conditions of cellular stress, via the mechanism of addition and elimination of free radical species, which is a catalytic cycle (i.e., one radical able to transform several hundreds of molecules before going to the termination step). The cis double bond, which is the natural geometry present in all biologically occurring MUFAs and PUFAs, changes to the unnatural trans isomer without a shift of the original double bond's position (Chatgilialoglu *et al.*, 2014). Before this study, it was not conceived that trans double bonds could be formed endogenously, during the cellular metabolism, whereas it was evidenced that the trans fatty acids, mostly positional isomers, could derive from processed foods containing chemically manipulated (deodorized or partially hydrogenated) vegetable and animal oils. In this

9
O
Oleic acid (9*cis*-18:1)
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Elaidic acid (9*trans*-18:1)

Figure 27.5 The isomerization of oleic acid, with the double bond in position 9, which is transformed from the natural cis geometry to the trans isomer.

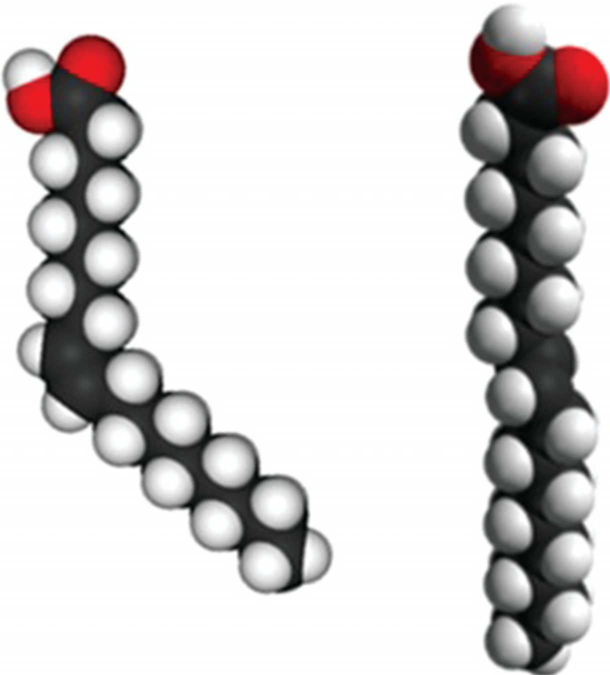

Figure 27.6 The comparison of oleic and elaidic acids' structures to evidence the loss of the bent cis geometry.

respect, the content of natural oils, such as olive oil, acquires more relevance for providing fatty acids in the correct form of the cis isomers, and the presence of trans isomers is considered a sign of adulteration or manipulation using low-quality oils (Vlahov, 1999).

The evidence of the formation of trans isomers of oleic acid (9*trans*-18:1, elaidic acid) and other trans-unsaturated fatty acids during metabolism was conclusively found in cell cultures and animals, both under trans-free dietary conditions (Ferreri *et al.*, 2005; Zambonin *et al.*, 2006). It can be seen in Figure 27.6 that the oleic–elaidic acid transformation produces a dramatic change of the molecular shape from a bent to a linear structure.

A series of model studies were carried out in liposomes, as membrane models, in order to clarify the biological consequences of such transformation. In fact, it is well known that oleic acid is beneficial for the membrane assembly and its properties, as already explained; therefore, the isomer formation corresponds to the loss of these benefits. Trans geometry influences several membrane and cell properties, as reflected in the diameter, which shrinks, and in reduction of fluidity, permeability, and other "sensor" activities (Ferreri *et al.*, 2006a, 2006b). It can also be hypothesized that a minimum local formation of trans isomers in membranes could induce protective responses, which have yet to be unveiled. An estimation of the trans fatty acid content in the erythrocyte membranes of healthy human subjects comes from population surveys, in Italy as well as in other European and non-European countries, focusing on elaidic and mono-trans arachidonic acids (Ferreri & Chatgilialoglu, 2015). The sum of the two isomers can reach 0.1–0.4% (relative percentage) in erythrocyte membranes, considering the cohort of 12 fatty acids taken for reference of the red blood cell membrane profile. For values higher than this interval, trans fatty acids indicate an exceeding membrane exposure to radical stress, which is therefore harmful for membrane lipidome organization. In these subjects, a strategy to diminish such damages has to be adopted.

In the last decade, free radical stress was one of the topics of integrated multidisciplinary research directed toward health and aging, with the participation of biophysicists, biologists, biochemists, pharmacologists, and medical doctors. These studies led to the individualization of the importance of membranes in stress

management, and nowadays membrane stress profiling can be indicated as one of the most promising areas of life science, useful for the development of tools for personalized medicine.

27.4.2 The birth of nutrilipidomics and the role of olive oil

The role of food as a provider of the correct fatty acid pool present in each individual, together with the role of oils and their metabolic transformation, was already explained. The quality of oils and the processes occurring in adsorption and metabolism provide the fatty acids, as crucial elements for the molecular assembly of cell membranes of each tissue in the body and for the membrane phospholipid remodeling during the stimuli, in order to arrive at the optimal functioning. When lipidomics addresses processes and dynamics to form membranes, it cannot be mixed with all other information on metabolomics, since in the metabolome lipids are included as circulating and deposit molecules, thus involved in other functions. The membrane lipidome is crucially different from the lipidome of other cellular compartments, since it represents the priority for cell life, to the same extent as DNA for the hereditary characters. Membrane lipidome is more likely assimilated to a "lipid code" that is different for each cell type, expressing a superior concept of homeostasis through the combination of lipid molecules. For this reason, some years ago scientists started to evaluate membrane lipidome profiles in direct relationship with health, also focusing on the relation between nutrition and the formation of a correct membrane lipidome at the individual level. This approach, called "nutrilipidomics," affects the control of type and quantity of nutritional elements for the final goal to obtain a good control at the molecular level of the lipidome formation (Chatgilialoglu & Ferreri, 2012). Fats are not the only ones indicated as nutritional elements, since the absorption and distribution of fatty acids include many other factors, such as the presence of antioxidants to protect the unsaturated fats from oxidative or chemically aggressive conditions found in the gastric tract and in tissues, emulsifiers or adjuvants that help adsorption by creating a "coating" around the lipid droplets, or the transport of hydrophobic molecules through the bloodstream, among others. In this respect, olive oil has an interesting composition with antioxidant polyphenols, and adjuvants such as sterols and terpenic elements, that certainly render more benefit to the oil. Many of these molecular components are also acquiring additional value *per se*, and the workup of olive trees and oil is becoming interesting from the point of view of utilizing all parts, which in the past constituted a problem as waste.

To render the membrane lipidome profile as a flexible and economical tool in molecular medicine, it was necessary to perform careful evaluation of the representative membrane, to be able to indicate the homeostatic control present in all body tissues. In the literature, stable nutritional and metabolic evaluations, using red blood cells, are available, together with data confirming that these cells can also represent tissues, like muscle, brain, and liver, that need more invasive approaches (Baur *et al.*, 2010; Ferreri & Chatgilialoglu, 2012). The relationship between red blood cells and tissues can only suffer from the stage of body development. In fact, in babies and children, this relationship could be weakened by their fast growth. The red blood cell has a lifetime of four months; therefore, to have a more constant and precise sampling, the selection of mature red blood cells (>3 months old) is necessary to also provide the best "reporter" of metabolic and nutritional contributions in the body. In these membranes, oleic acid concentration was 9–18% (relative percentages) (Ferreri & Chatgilialoglu, 2015), which is in agreement with other reported data (Hodson *et al.*, 2008). The membrane lipidome of mature red blood cells is well described by a selection of ten cis fatty acids and two trans fatty acid isomers (Ferreri & Chatgilialoglu, 2015), as shown in Table 27.1.

After having analyzed the membrane lipidome of mature red blood cells, the evaluation of the individual lipidome profile takes place, evidencing the deviation of different fatty acids from the optimal balance in the individual. The profile brings formulation and dietary choices to the nutraceutical, which are the two indications of the nutrilipidomics approach, an innovative tool for personalized medicine and disease risk reduction. Consolidated dietary habits and lifestyles in different individuals differentiate the corresponding profiles in a specific manner, as found for the increase of SFAs in the membrane profile for dietary habits rich in carbohydrate intake, due to insulin-activated fatty acid biosynthesis, as explained in earlier sections of this chapter.

Membrane lipidomics is an important application of functional lipidomics, which is nowadays directed toward the definition of lipidomic phenotypes. The expansion of the clinical use of membrane lipidomics will allow deepening of knowledge about the profiles that provide optimal body function or, if unbalanced,

Table 27.1 The optimal interval values of the representative cohort of fatty acids forming the lipidomic profile of mature erythrocyte membranes.

FA residues values	Acronym	Normal values
C16:0		17–27
C16:1-Δ9		0.2–0.5
C18:0		13–20
C18:1-Δ9		9–18
C18:1-Δ11		0.7–1.3
C18:2-Δ9, 12	LA	C9–16
C20:3-Δ8, 11, 14	DGLA	1.9–2.4
C20:4-Δ5, 8, 11, 14	AA	13–17
C20:5-Δ5, 8, 11, 14, 17	EPA	0.5–0.9
C22:6-Δ4, 7, 10, 13, 16, 19	DHA	5–7
Total saturated FAs	SFA	30–45
Total MUFAs	MUFA	13–23
Total PUFAs	PUFA	28–39
SFA/MUFAs		1.7–2
Omega-6/omega-3		3.5–5.5
Sum of mono-trans	Trans	≤0.4

Note: AA, arachidonic acid; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; LA, linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

represent conditions of cellular stress, creating a cell discomfort that affects life quality at first, but also has health consequences.

27.5 Conclusion and perspectives

The benefits of olive oil have been described as a function of the presence of an important monounsaturated fatty acid, the oleic acid. The overview given herein on lipidomics and the oleic acid constituent of olive oil can serve as a premise to new developments in the field of healthcare and nutra-based strategies. Understanding the molecular basis of the activity of oleic acid may foster applications for the use of olive oil in natural products, and the nutrilipidomic approach offers a practical tool for personalization that addresses the imbalance of metabolism and provides medical therapies for chronic diseases.

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References

Alemany, R., Terés, S., Baamonde, C., Benet, M., Vogler, O., & Escribá, P.V. (2004) 2-hydroxyoleic acid: a new hypotensive molecule. Hypertension 43, 249–254.

Battino, M., & Ferreiro, M.S. (2004) Ageing and the Mediterranean diet: a review of the role of dietary fats. Public Health Nutrition 7, 953–958.

Baur, L.A., O’Connor, J., Pan, D.A., Wu, B.J., O’Connor, M.J., & Storlien, L.H. (2010) Relationship between the fatty acid composition of muscle and erythrocyte membrane phospholipids in young children and the effect of type of infant feeding. Lipids 35, 77–82.

Bender, J. (2004) DNA methylation and epigenetics. Annual Review of Plant Biology 55, 41–68.
Bento-Abreu, A., Tabernero, A., & Medina, J.M. (2007) Peroxisome proliferator-activated receptor-alpha is required for the neurotrophic effect of oleic acid in neurons. Journal of Neurochemistry 103, 871–881.
Bolognesi, A., Chatgilialoglu, A., Polito, L., & Ferreri C. (2013) Membrane lipidome reorganization correlates with the fate of neuroblastoma cells supplemented with fatty acids, PLoS ONE 8, e55537.
Burlingame, B., & Dernini, S. (Eds.) (2010) Sustainable diets and biodiversity. FAO, Rome.
Cao, H., Gerhold, K., Mayers, J.R., Wiest, M.M., Watkins, S.M., & Hotamisligil, G.S. (2008) Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. Cell 134, 933–944.
Cevc, G. (1991) How membrane chain-melting phase-transition temperature is affected by the lipid chain asymmetry and degree of saturation: an effective chain length model. Biochemistry 30, 7186–7193.
Cevc, G. (1993) Phospholipids handbook. Marcel Dekker, New York.
Chatgilialoglu, C., Ferreri, C., Melchiorre, M., Sansone, A., & Torreggiani. A. (2014) Lipid geometrical isomerism: from chemistry to biology and diagnostics. Chemical Reviews 114, 255–284.
Chatgilialoglu, C., & Ferreri, C. (2012) Nutrilipidomics: a tool for personalized health. Journal of Glycomics & Lipidomics 2, e109.
Dais, P., & Hatzakis, E. (2013) Quality assessment and authentication of virgin oil. Analytica Chimica Acta 765, 1–27.
EFSA Panel. (2010) Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA Journal 8, 1461. doi:10.2903/j.efsa.2010.1461
Else, P.L., & Hulbert, A.J. (2003) Membranes as metabolic pacemakers. Clinical and Experimental Pharmacology and Physiology 30, 559–564.
Engelman, D.M. (2005) Membranes are more mosaic than fluid. Nature 438, 578–580.
Escribà, P.V. (Ed.) (2014) Membrane structure and function: relevance in the cell's physiology, pathology and therapy. Biochimica et Biophysica Acta 1838, 1449–1692.
Ferreri, C., & Chatgilialoglu, C. (2012) The role of fatty acid-based functional lipidomics in the development of molecular diagnostic tools. Expert Review of Molecular Diagnostics 12, 767–780.
Ferreri, C., & Chatgilialoglu, C. (2015) Membrane lipidomics for personalized health. John Wiley & Sons, New York.
Ferreri, C., Costantino, C., Landi, L., Mulazzani, Q.G., & Chatgilialoglu, C. (1999) The thiyl radical mediated isomerization of cis-monounsaturated fatty acid residues in phospholipids: a novel path of membrane damage? Chemical Communications 407–408.
Ferreri, C., Kratzsch, S., Brede, O., Marciniak, B., & Chatgilialoglu, C. (2005) Trans lipids formation induced by thiols in human monocytic leukemia cells. Free Radical Biology and Medicine 38, 1180–1187.
Ferreri, C., Pierotti, S., Barbieri, A., Zambonin, L., Landi, L., Rasi, S., Luisi, P.L., Barigelletti, F., & Chatgilialoglu, C. (2006a) Comparison of phosphatidylcholine vesicle properties related to geometrical isomerism. Photochemistry and Photobiology 82, 274–280.
Ferreri, C., Pierotti, S., Chatgilialoglu, C., Barbieri, A., & Barigelletti, F. (2006b) Probing the influence of cis-trans isomers on model lipid membrane fluidity using cis-parinaric acid and stop-flow technique. Chemical Communications 529–531.
Flowers, M.T., & Ntambi, J.M. (2009) Stearoyl-CoA desaturase and its relation to high-carbohydrate diets and obesity. Biochimica et Biophysica Acta 1791, 85–91.
Georgiadi, A., & Kersten, S. (2012) Mechanisms of gene regulation by fatty acids. Advances in Nutrition 3, 127–134.
Goñi, F.M. (2014) The basic structure and dynamics of cell membranes: an update of the Singer-Nicolson model. Biochimica et Biophysica Acta 1838, 1467–1476.
Hodson, L., Skeaff, C.M., & Fielding, B.A. (2008) Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Progress in Lipid Research 47, 348–380.
Hulbert, A.J. (2010) Membrane and longevity: is there a role for membrane fatty acids? Integrative and Comparative Biology 50, 808–817.
Hu, W., Ross, J., Geng, T., Brice, S.E., & Cowart, L.A. (2011) Differential regulation of dihydroceramide desaturase by palmitate versus monounsaturated fatty acids. Journal of Biological Chemistry 286, 16596–16605.
Hyötyläinen, T., Bondia-Pons, I., & Orešič, M. (2013) Lipidomics in nutrition and food research. Molecular Nutrition & Food Research 57, 1306–1318.
Ibarguren, M., López, D.J., & Escribá, P.V. (2014) The effect of natural and synthetic fatty acids on membrane structure, microdomain organization, cellular functions and human health. Biochimica et Biophysica Acta 1838, 1518–1528
Igal, R.A. (2010) Stearoyl-CoA desaturase-1: a novel key player in the mechanisms of cell proliferation, programmed cell death and transformation to cancer. Carcinogenesis 31, 1509–1515.

Kamphorst, J.J., Cross, J.R., Fan, J., de Stanchina, E., Mathew, R., White, E.P., Thompson, C.B., & Rabinowitz, J.D. (2013) Hypoxic and Ras-transformed cells support growth by scavenging unsaturated fatty acids from lysophospholipids, PNAS 110, 8882–8887.

Katsaras, J., & Gutberlet, T. (Eds.) (2001) Lipid bilayers: structure and interactions. Springer-Verlag, Berlin.

Köfeler, H.C., Fauland, A., Rechberger, G.N., & Trötzmüller, M. (2012) Mass spectrometry based lipidomics: an overview of technological platforms. Metabolites 2, 19–38.

Konstantinidou, V., Daimiel Ruiz, A.A., & Ordovás, J.M. (2014) Personalized nutrition and cardiovascular disease prevention: from Framingham to PREDIMED. Advances in Nutrition 5, 368S–371S.

Korzus, E. (2010) Manipulating brain with epigenetics. Nature Neuroscience 13, 405–406.

Ladha, S. (1998) Lipid heterogeneity and membrane fluidity in a highly polarized cell, the mammalian spermatozoon. Journal of Membrane Biology 165, 1–10.

Lands, W.E.M. (1958) Metabolism of glycerolipids: a comparison of lecithin and triglyceride synthesis. Journal of Biological Chemistry 231, 883–888.

Langin, D. (2006) Adipose tissue lipolysis as a metabolic pathway to define pharmacological strategies against obesity and the metabolic syndrome. Pharmacological Research 53, 482–491.

Latreille, J., Kesse-Guyot, E., Malvy, D., Andreeva, V., Galan, P., Tschachler, E., Hercberg, S., Guinot, C., & Ezzedine, K. (2012) Dietary monounsaturated fatty acids intake and risk of skin photoaging. PLoS ONE 7, e44490.

Lauritzen, L., Hansen, H.S., Jorgensen, M.H., & Michaelsen, K.F. (2001) The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Progress in Lipid Research 40, 1–94.

Lopez, S., Bermudez, B., Montserrat-de la Paz, S., Jaramillo, S., Varela, L.M., Ortega-Gomez, A., Abia, R., & Muriana, F.J.G. (2014) Membrane composition and dynamics: a target of bioactive virgin oil constituents. Biochimica et Biophysica Acta 1838, 1638–1656.

Mouritsen, O.G. (2005) Life: as a matter of fats. Springer-Verlag, Berlin.

Nicolson, G.L. (2014) The fluid-mosaic model of membrane structure: still relevant to understanding the structure, function and dynamics of biological molecules after 40 years. Biochimica et Biophysica Acta 1838, 1451–1466.

Nicolson, G.L., & Ash, M.E. (2014) Lipid replacement therapy: a natural medicine approach to replacing damaged lipids in cellular membranes and organelles and restoring function. Biochimica et Biophysica Acta 1838, 1657–1679.

Ntambi, J.M. (1999) Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. Journal of Lipid Research 40, 1549–1558.

Pike, L.J. (2003) Lipid rafts: bringing order to chaos. Journal of Lipid Research 44, 655–667.

Papackova, Z., & Cahova, M. (2015) Fatty acid signaling: the new function of intracellular lipase. International Journal of Molecular Sciences 16, 3831–3855.

Patra, S.K., & Bettuzzi, S. (2007) Epigenetics DNA-methylation regulation of genes coding for lipid raft-associated components: a role for raft proteins in cell transformation and cancer progression. Oncology Reports 17, 1279–1290.

Pietiläinen, K.H., Sysi-Aho, M., Rissanen, A., Seppänen-Laakso, T., Yki-Järvinen, H., Kaprio, J., & Orešič, M. (2007) Acquired obesity is associated with changes in the serum lipidomic profile independent of genetic effects – a monozygotic twin study. PLoS ONE 2, e218.

Rodriguez-Rodriguez, R.A., Tabernero, A., Velasco, A., Lavado, E.M., & Madina, J.M. (2004) The neurotrophic effect of oleic acid includes dendritic differentiation and the expression of the neuronal basic helix-loop-helix transcription factor NeuroD2. Journal of Neurochemistry 88, 1041–1051.

Roongta, U.V., Pabalan, J.G., Wang, X., Ryseck, R.-P., Fargnoli, J., Henley, B.J., Yang, W.-P., Zhu, J., Madireddi, M.T., Lawrence, M., Wong, T.W., & Rupnow, B.A. (2011) Cancer cell dependence on unsaturated fatty acids implicates stearoyl-coa desaturase as a target for cancer therapy. Molecular Cancer Research 9, 1551–1561.

Saadatian-Elahi, M., Norat, T., Goudable, J., & Riboli, E. (2004) Biomarkers of dietary fatty acid intake and the risk of breast cancer: a meta-analysis. International Journal of Cancer 111, 584–591.

Sansone, A., Melchiorre, M., Chatgilialoglu, C., & Ferreri C. (2013) Hexadecenoic fatty acid isomers: a chemical biology approach for human plasma biomarker development. Chemical Research in Toxicology 26, 1703–1709.

Serhan, C.N. (2014) Pro-resolving lipid mediators are leads for resolution physiology. Nature 510, 92–101.

Shaikh, S.R., Dumaual, A.C., Castillo, A., LoCascio, D., Siddiqui, R.A., Sillwell, W., & Wassall, S.R. (2004) Oleic and docosahexaenoic acid differentially phase separate from lipid raft molecules: a comparative NMR, DSC, AFM, and detergent extraction study. Biophysical Journal 87, 1752–1766.

Shaikh, S.R., & Edidin, M. (2006) Polyunsaturated fatty acids, membrane organization, T cells, and antigen presentation. American Journal of Clinical Nutrition 84, 1277–1289.

Shen, Q., Dong, W., Yang, M., Baibado, J.T., Wang, Y., Alqouqa, I., & Cheung, H.-Y. (2013) Lipidomic study of olive fruit and oil using TiO2 nanoparticle based matrix solid-phase dispersion and MALDI-TOF/MS. Food Research International 54, 2054–2061.

Simons, K., & Toomre, D. (2000) Lipid rafts and signal transduction. Nature Reviews Molecular Cell Biology 1, 31–39.

Steffen, L.M., Vessby, B., Jacobs, D.R., Steinberger, J.J., Moran, A., Hong, C.-P., & Sinaiko, A.R. (2008) Serum phospholipid and cholesteryl ester fatty acids and estimated desaturase activities are related to overweight and cardiovascular risk factors in adolescents. International Journal of Obesity 32, 1297–1304.

Terés, S., Barceló-Coblijn, G., Benet, M., Álvarez, R., Bressani, R., Halver, J.E., & Escribá, P.V. (2008) Oleic acid content is responsible for the reduction in blood pressure induced by olive oil. PNAS 105, 13811–13816.

Varela, L.M., Ortega-Gomez, A., Lopez, S., Abia, R., Muriana, F.J., & Bermudez, B. (2013) The effects of dietary fatty acids on the postprandial triglyceride-rich lipoprotein/apoB48 receptor axis in human monocyte/macrophage cells. Journal of Nutritional Biochemistry 24, 2031–2039.

Vlahov, G. (1999) Application of NMR to the study of olive oil. Progress in Nuclear Magnetic Resonance Spectroscopy 35, 341–357.

Warensjö, E., Ohrvall, M., & Vessby, B. (2006) Fatty acid composition and estimated desaturase activities are associated with obesity and lifestyle variables in men and women. Nutrition, Metabolism & Cardiovascular Diseases 16, 128–136.

Yang, X., Askarova, S., & Lee, J.C. (2010) Membrane biophysics and mechanics in Alzheimer's disease. Molecular Neurobiology 41, 138–148.

Zambonin, L., Ferreri, C., Cabrini, L., Prata, C., Chatgilialoglu, C., & Landi, L. (2006) Occurrence of trans fatty acids in rats fed a trans-free diet: a free radical-mediated formation? Free Radical Biology and Medicine 40, 1549–1556.

28 Analysis of olive oil quality

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28.1 Introduction

Olive oil is a rich source of monounsaturated fatty acids, mainly oleic and natural antioxidants such as tocopherols, sterols, carotenoids, and phenolic compounds. The consumption of extra virgin olive oil (25–50 ml/day) as a part of a Mediterranean diet has been shown to reduce the risk of chronic diseases (i.e. cardiovascular disease (CVD), atherosclerosis, some types of cancers, and Alzheimer's disease), as well as increase life expectancy (Sofi *et al.*, 2010). The nutritional value of olive oil originates primarily from its high level of oleic acid and minor components, including phenolic compounds. The major phenolic compound in olive fruit is oleuropein, which may account for up to 14% of total phenolics in dried fruit, while hydroxytyrosol is the major phenolic in olive oil (Amiot *et al.*, 1986). Two main flavonoids of olive fruits are rutin and luteolin 7-glucoside, and the most abundant anthocyanins include cyanidin 3-*O*-glucoside and cyanidin 3-*O*-rutinoside (Romani *et al.*, 1999). González-Santiago *et al.* (2010) reported that hydroxytyrosol is one of the major components of virgin olive oil and olive oil mill waste with a strong antioxidant potential. Several other researchers also reported that secoiridoids, oleuropein, demethyloleuropein, ligstroside, and verbascoside (caffeoylrhamnosylglucoside of hydroxytyrosol) are the main phenolic compounds of olive fruits (Angerosa *et al.*, 1995; Borzillo *et al.*, 2000; Czerwińska *et al.*, 2003; Shahidi & Ambigaipalan, 2015). Other phenolic compounds included hydroxytyrosol, 3,4-dihydroxyphenylacetic, 4-hydroxyphenylacetic, and 4-hydroxybenzoic acids as well as protocatechuic, vanillic, syringic, and *p*-coumaric acids (Cabrini *et al.*, 2001). Tyrosol is present at the highest concentration; however, this compound contributes very little, if any, to the stability of olive oil (Cabrini *et al.*, 2001). Moreover, two new phenolic compounds were identified in olive oil, namely pinoresinol and 1-acetoxypinoresinol (Brenes *et al.*, 2000). In addition, by-products and residues derived from the olive-processing industry are also good sources of natural antioxidants. Erbay and Icier (2010) have shown the enrichment of oils with olive leaves, olive leaf extract, as well as the main secoiridoid compound (oleuropein). Hayes *et al.* (2011) identified hydroxytyrosol, tyrosol, luteolin-7-O-glucoside, verbascoside, apigenin-7-O-glucoside, and oleuropein in olive leaf extracts. Olive oil mill waste water is also considered as a source of phenolics (Aggelis *et al.*, 2003).

Another class of phenolic compounds of extra virgin olive oil, hydroxyisochromans (l-phenyl-6,7-dihydroxyisochroman, and 1-[3′-methoxy-4′-hydroxy] phenyl-6,7-dihydroxyisochroman), was found in different samples of extra virgin olive oil (Bianco *et al.*, 2002). Phenolic compounds in virgin olive oil are a complex mixture of components that include α- and γ-tocopherols, hydroxytyrosol, tyrosol, phenolic acids (caffeic acid, vanillic acid, syringic acid), lignans (pinoresinol, 1-acetoxypinoresinol), and secoiridoids (oleuropein aglycone, oleuropein, demethyloleuropein, ligstroside) (Cioffi *et al.*, 2010). It has also been reported that one of the well-known phenolic compounds present in newly pressed extra virgin olive oil, the dialdehydic form of deacetoxy-ligstroside aglycone, called oleocanthal, is one of the main substances responsible for the bitter taste of olive oil; furthermore, oleocanthal possesses ibuprofen-like cyclooxygenases (COX-1 and COX-2) inhibitory activity and is responsible for its anti-inflammatory effect (Beuchamp *et al.*, 2005). Oleacein is a more potent scavenger of hypochlorous acid than oleuropein as well as a

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stronger inhibitor of reactive oxygen species (ROS) production and myeloperoxidase release in neutrophils (Czerwińska *et al*., 2012).

The nutritional value and pleasant flavor arising from volatile compounds (Kiritsakis & Min, 1989; Kiritsakis, 1993, 1998a) have contributed to an increase in consumption of olive oil (Patumi *et al.*, 2002). The International Olive Council (IOC, 2001) and the European Union (EU) Commission Regulation (1991) have created the quality standards for olive oil. The quality of olive oil is defined by parameters that include free fatty acid (FFA) content, peroxide value (PV), UV-specific extinction coefficients (K_{232} and K_{270}), and a sensory (organoleptic) evaluation score (Kalua *et al.*, 2007). The commercial grades of olive oils have been classified based on the quantity of FFAs (percentage of oleic acid). Depending on the maximum limits of FFAs as oleic acid percentage, olive oil has been classified as ordinary virgin olive oil (3.3%), lampante virgin olive oil (3.3%), virgin olive oil (2.0%), olive oil (1.0%), olive pomace oil (1.0%), extra virgin olive oil (0.8%), refined olive oil (0.3%), and refined olive pomace oil (0.3%) (Kalua *et al.*, 2007). Olive oil obtained only by mechanical or physical press of olive fruit is named virgin, whereas other oils contain refined oil (Kiritsakis, 1998b). In addition, Kalua *et al.* (2007) suggested that some parameters that are not included in the IOC (2001) and EU (1991) standards, such as phenolic content, are also known to have a significant effect on the stability and sensory (organoleptic) characteristics of olive oil.

This chapter describes a number of basic methods for olive oil quality analysis. The role of these methods is to ensure that olive oils are consistent with the categories declared in terms of olive oil quality and to investigate olive oil adulteration or identity. The degree of triacylglycerol (TAG) hydrolysis, degree of oxidation, and compositional analysis are the three main categories of the parameters used for this assessment. The methods described are not necessarily suggested by the IOC or by the Commission of the European Union, whereas sensory analysis and examination of residues and contaminants are not considered in this chapter.

The characteristics of olive oil and the relevant methods of analysis are described by the legislation of the EU Commission Regulation (2013) (Commission Implementing Regulation No. 299/2013 of 26 March 2013 and No. 1348/2013 of 16 December 2013 amending Regulation No. 2568/91). Moreover, the IOC suggests a variety of validated methods on the basis of the opinions of chemical experts. A number of validated methods are verified by the Association of Official Analytical Chemists (AOAC) and American Chemical Society (ACS), while the Codex Alimentarius and individual researcher suggestions of novel methods for olive oil analysis are reviewed.

28.2 Fatty acid composition and analysis

Fatty acids (FAs) are the most important components of olive oils, and total TAGs constitute about 95–98% of olive oil. Table 28.1 shows the list of allowable levels of FAs for an olive oil to be accepted as extra virgin olive oil, as established by the IOC.

Oxidative susceptibility of oils mainly depends on the composition of their FAs, especially their degree of unsaturation or their methylene bridge index (MBI, the mean number of bisallylic methylene positions) (Shahidi & Zhong, 2010). Hamam and Shahidi (2006) reported the oxidation rate of the FA series stearic,

Table 28.1 Allowable limits of fatty acids for extra virgin olive oil.

Fatty acid	Allowable limit (%)
Oleic acid (C18:1)	55–83
Palmitic acid (C16:0)	7.5–20
Linoleic acid (C18:2)	3.5–21
Stearic acid (C18:1)	0.5–5
Palmitoleic acid (C16:1)	0.3–3.5
Linolenic acid (C18:3)	<1
Arachidic acid (C20:0)	<0.6
Gadoleic acid (C20:1)	<0.4

Adapted from Bell and Gillatt (1994), Mailer (2006), and IOC (2013).

oleic, linoleic, and linolenic acids, for instance, to be in the ratio of 1:100:1200:2500. Olive oils contain a high proportion of oleic acid in the range of 60–80% of the total FAs and approximately 90% of the monounsaturated fatty acids (MUFAs) (Kiritsakis & Markakis, 1987; Maggio *et al.*, 2009). Polyunsaturated fatty acids (PUFAs), such as linoleic and linolenic acids, account for approximately 5–8% of total FAs (Maggio *et al.*, 2009). Generally, MUFAs and PUFAs are determined by capillary gas chromatography with flame ionization detection (GC-FID). FFAs are formed upon hydrolytic rancidity of oils. FFAs of oils are recorded as an acid value, where a sample is dissolved in a mixture of solvents and the FFAs present are titrated using an ethanolic solution of potassium hydroxide. The acid value of oil affords the percentage of the FFAs as oleic acid in the oil being tested, which could subsequently reflect the stability of oil and its susceptibility to rancidity. Maggio *et al.* (2009) reported that the Fourier transformed infrared (FTIR) attenuated total reflectance (ATR) spectroscopic method could be used to determine the FA profile and PV of virgin olive oil. In addition, smoke point (temperature at which an oil sample starts to smoke continuously) is also used to detect the hydrolytic rancidity (AOCS, 1999). According to the European Union Commission (1991), the maximum acidity for extra virgin olive oil is 0.8%.

28.2.1 Acidity

Acidity is a measure of the free fatty acids (FFAs) present in olive oil. Acidity determination in olive oils is of much interest, mainly because it is related to the quality and authenticity of the oils, while increased values increase the risk for oil oxidation. Storage time, temperature (heating, frying, etc.), moisture, bacteria, and enzymes induce the hydrolysis of fat (TAGs) into free fatty acids and diacylglycerols, thereby increasing the value of acidity. Thus, acidity reflects the quality of olives used for olive oil production and the conditions of processing and preserving the oil. Thus, acidity is used for olive oil categorization and as a marker of olive oil quality.

FFA determination methods include both classical (titration) and instrumental methods (colorimetry, electrochemistry, spectroscopy, etc.). Official methods are usually based on alkaline titration. In these, the sample is dissolved in a neutralized solvent, and then it is titrated against a standard base solution (KOH or NaOH) in the presence of phenolphthalein as an indicator. A solvent is chosen in order to extract the acids from the oily medium or to develop an emulsion so that the base solution reacts with the FFAs. This method is simple but extremely laborious, and moreover it needs large amounts of expensive chemicals associated with environmental issues and requires a trained analyst. Thereby, many kits are available as an alternative for bulk FFA determination, and various instrumental methods have been developed in recent years. These methods include colorimetric, electrochemical, and spectroscopic techniques of detection, alone or coupled with techniques for FFA fraction or individual FFA separation. Automated methods have also been developed over the years, while a fully robotic method for the determination of acid values in olive oil without titration was developed by Velasco-Arjona and De Luque Castro (1998). Table 28.2 shows some of the methods mentioned that were tested for olive oil.

Diacylglycerols determination may be used as an alternative method for determining the degree of the hydrolysis of olive oil. This method is more sensitive than the method of determining acidity. There is a linear correlation between diacylglycerols and acidity. Ismail *et al.* (1993) proposed a rapid quantitative determination of FFAs of oils by FTIR spectroscopy. The determination is based on both transmission and attenuated total reflectance approaches, covering an analytical range of 0.2–0.8% FFAs. Calibration curves are prepared by adding oleic acid to the oil chosen for analysis and measuring the C=O band at 1711 cm^{-1} after rationing the sample spectrum against that of the same oil, free of FAs. In a recent study, the use of ^{31}P-NMR was employed for the diacylglycerol determination (Petrakis *et al.*, 2008). For determining the acidity of olive oil, the methods proposed by the IOC (1968) or EU (1991) are usually applied.

28.3 Measurement of oxidation

Autoxidation is the main deteriorative process that occurs in lipids and adversely affects their quality, particularly in relation to the off-flavor and rancidity development. Measurement of oxidation of olive oil is

Table 28.2 Methods used for the determination of acidity.

Method	Principle	Reagents-measurements
Classical		
Aricetti & Tubino (2012)	Titrimetry	Distilled water/ethanol, aqueous NaOH solution, phenolphthalein
AOCS Cd 3d-63 (2003)	Titrimetry	Toluene/isopropyl alcohol, KOH in isopropyl alcohol, phenolphthalein
AOCS (1998) Ca 5a-40	Titrimetry	
European Commission Regulation (1991)	Titrimetry (cold method)	Diethyl oxide/ethanol, ethanolic solution of KOH, phenolphthalein
Colorimetric		
Sato *et al.* (2014)	Capillary zone electrophoresis	Indirect detection at 224 nm
Makahleh & Saad (2011)	Flow injection analysis (FIA)	Conductivity detection (CD)
Balesteros *et al.* (2007)	Capillary zone electrophoresis	Indirect detection at 224 nm
Mariotti *et al.* (2001)	FIA titration automated	562 nm (phenolphthalein)
Nouros *et al.* (1997)	Automated flow injection spectrophotometric – titrimetric	562 nm (phenolphthalein)
Zhi *et al.* (1996)	Automated flow injection analysis (FIA) flow-reversal injection liquid–liquid extraction	716 nm (Cu^{2+}–FFA complex)
Electrochemical		
Takamura *et al.* (1999)	Voltametric reduction of quinones	
Velasco-Arjona & De Luque Castro (1998)	pH measurements of emulsion	
Spectroscopic		
Fronimaki *et al.* (2002), Dais *et al.* (2007)	^{31}P-NMR spectroscopy	134.79 ppm
Skiera *et al.* (2014)	^{1}H-NMR spectroscopy	11–12 ppm
Sacchi *et al.* (1997)	^{13}C-NMR spectroscopy	Comparing 176–178 ppm with 171–174 ppm
El-Abassy *et al.* (2009)	FT Raman in combination with partial least-squares (PLS) regression	1600–945 cm^{-1}
Iñón *et al.* (2003)	Fourier transform infrared spectroscopy (FTIR) attenuated total reflectance (ATR)	1710 cm^{-1}
Muik *et al.* (2003)	FT Raman spectrometry in combination with partial least-squares (PLS) regression	Five latent variables in the region 1200–1800 cm^{-1}
Bertran *et al.* (1999)	ATR FTIR using partial least-squares regression (PLSR)	1775–1689 cm^{-1}

essential in providing quality standards for regulatory issues and health claims. High temperature; visible or diffused light (Kiritsakis & Dugan, 1985) and oxygen; contact with metal surfaces, such as copper; damage to fruit; and delays between harvest and processing time could increase the risk of oxidation of olive oils (Kiritsakis, 1998b; Mailer & Beckingham, 2006). Lipid oxidation is conventionally studied by determination of PV, thiobarbituric acid reactive substances (TBARS), conjugated dienes (CDs), anisidine value (AV), total carbonyls, assessing headspace volatile compounds, oil stability index (OSI), and measurement of free radicals. Table 28.3 summarizes the methods commonly used for measuring oxidation. In the determination of oxidative rancidity of olive oil, more than one method should be used. Each method has its own drawbacks and advantages. The use of two or more methods gives a better indication of the oxidative deterioration and quality of olive oil (Kiritsakis, 1991).

Table 28.3 Methods for measuring oxidation of fats and oils.

Methods	Units
Peroxide value (PV)	Milliequivalents of oxygen per kilogram of sample (meq/kg)
Conjugated dienes and trienes	Conjugable oxidation products (COPs)
Thiobarbituric acid reactive substances (TBARS) assay	Milligrams of malonaldehyde (MA) equivalents per kilogram sample or micromoles of MA equivalents per gram of sample (meq/g)
p-Anisidine value (*p*-AnV)	Absorbance of a solution resulting from the reaction of 1 g of fat in isooctane solution (100 ml) with *p*-anisidine
Total carbonyls	Absorbance of a solution resulting from the reaction of the carbonyl compounds with 2,4-dinitrophenylhydrazine (DNPH) followed by reaction of the resulting hydrazones with alkali
Electrical conductivity method	Oil stability index (OSI) value, which is defined as the point of maximal change of the rate of oxidation
Free radical measurement	Detecting the formation of radicals by using electron paramagnetic resonance (EPR) spectroscopy

28.3.1 Peroxide value

The most common method used for evaluating the extent of oxidation in fats, oils, and food lipids is to determine the PV. A number of methods have been developed for the determination of PV, among which iodometric titration, measurement of ferric ion complex by spectrophotometry, and infrared spectroscopy are the most frequently employed (Shahidi & Zhong, 2007, 2015). Peroxides are primary products in the oxidative breakdown of lipids, and their formation is the net result of the production rate and the decomposition rate. As the decomposition rate of peroxides is a function of at least pH and temperature, the production rate is difficult to quantify. The end products of lipid oxidation are the result of several reaction pathways from peroxides; these reactions do not follow one line, but several breakdown lines are possible, giving different end products from the same peroxide molecule (Kiritsakis *et al.*, 1983, 2009; Kiritsakis, 1998b). Meanwhile, an increased interest in antioxidant activity has led to the development of a wide array of indirect methods to measure the antioxidant capacity of different commodities, including olive oil (Huang *et al.*, 2005; Roginsky & Lissi, 2005). According to the official EU method (1995), PV is based on the titration of iodine liberated from potassium iodide by peroxides present in the oil. The titration method has several disadvantages, such as being time-consuming and labor intensive, requiring a large amount of samples, and involving the absorption of iodine across unsaturated bonds and the oxidation of iodide by dissolved oxygen. To overcome these drawbacks, novel methods based on the same reaction such as colorimetric determination at 560 nm, potentiometric end point determination, and electrochemical (platinum electrode at constant potential) and spectrophotometric determination of the I_3^- chromophore at 290 or 360 nm have been proposed (Hicks & Gebicki, 1979; Gebicki & Guille, 1989; Dobarganes & Velasco, 2002; Shahidi & Zhong, 2007, 2015). Oxidation of ferrous ion in an acidic medium and colorimetric detection of ferric ion as ferric thiocyanate, a red-violet complex with strong absorption at 500–510 nm, are more sensitive than the standard method based on iodometry. Oxidation of ferrous ion in an acidic medium containing xylenol orange (FOX) dye is another method that has been reported to have high sensitivity comparable to, or even better than, that of the spectrophotometric iodometric assay. The resulting ferric ions and dye form a blue-purple complex with a maximum of absorbance between 550 and 600 nm (Jiang *et al.*, 1991; Dobarganes & Velasco, 2002). However, many factors such as the amount of oil sample, solvent used, and source of FOX may affect the absorption coefficient. Therefore, knowledge of the nature of hydroperoxides present in the oil sample and careful control of the conditions used are required for accurate measurements (Dobarganes & Velasco, 2002; Shahidi & Zhong, 2007). Terao and Matsushita (1977) proposed another colorimetric method for measuring hydroperoxides in photooxidized oils, where cadmium acetate was used and the absorbance of the color formed was measured at 350 nm.

A number of alternative methods and techniques for the determination of lipid hydroperoxides have been reported in the literature (Dhaouadi *et al.*, 2006). For the simultaneous determination of peroxides, several

HPLC methods have been reported, including UV (Oliveri-Vigh & Hainsworth, 1978; Gaddipati *et al.*, 1983), electrochemical (Iwahashi, 2000), fluorescence (Akasaka *et al.*, 1993; Wang & Glaze, 1998) and chemiluminescence detection (Miyazawa *et al.*, 1994; Yasuda & Narita, 1997; Pegg *et al.*, 2007). Flow-injection analysis (FIA) procedures have also been reported with spectrophotometric (Perez-Ruiz *et al.*, 1993; Nouros *et al.*, 1999; Saad *et al.*, 2006), fluorimetric (Perez-Ruiz *et al.*, 1993), and FTIR spectroscopic detection (Ruız *et al.*, 2001; Dobarganes & Velasco, 2002). Dhaouadi *et al.* (2006) described a flow-injection method for measuring the PV of edible oils. The technique is based on spectrophotometric monitoring at 660 nm of methylene blue (MB), generated from leucomethylene blue (LMB) oxidation with peroxides present in oil samples; this method was found to be rapid (30 samples/h), simple, reproducible, low cost, and flexible. Electrochemical methods using both polarographic and amperometric detectors (Mulchandani & Rudolph, 1995; Evans, 1999) as well as biosensors or enzymatically modified electrodes (Mulchandani *et al.*, 1995; Li *et al.*, 1998) were employed with some success in the detection of lipid hydroperoxides. Most of these techniques, however, suffer from some disadvantages, such as low specificity, possible appearance of interfering substances, critical enzyme preparation, or expensive instrumentation (Dhaouadi *et al.*, 2006). Recently, Talpur *et al.* (2010) developed a simple method of using UV spectrometry for determination of PV of frying oils. The basis of the PV determination was the stoichiometric reaction of triphenylphosphine (TPP) with the hydroperoxides present in the frying oil to produce triphenylphosphine oxide (TPPO), which exhibits a readily measurable absorption band at 240 nm. They found that the UV method has a major advantage over the titration method in terms of reducing solvent and reagent, which not only cuts the cost but also avoids disposal and environmental problems associated with toxic chemicals. Furthermore, it reduces labor requirements in routine quality control analysis of edible fats and oils. The proposed method was correlated with the official American Oil Chemist's Society (AOCS, 1989) titration method, and a good correlation coefficient ($R^2 = 0.99525$) was attained.

Van de Voort *et al.* (1994) proposed the determination of the oxidation of oils by FTIR. FTIR spectroscopy provides a simple and rapid means of following complex changes that take place as a lipid oxidizes. The critical absorption bands associated with common oxidation end products are identified by relating them to those of spectroscopically representative reference compounds. A quantitative approach is proposed in which the standards used are spectroscopically representative of oxidative end products and by which the oxidative state of the oil can be defined in terms of percent hydroperoxides, percent alcohols, and total carbonyl compounds. FTIR analysis provides a rapid means of evaluating the oxidative state of oil or of monitoring changes in the oils undergoing thermal stress (van de Voort *et al.*, 1994). Recently, Pizarro *et al.* (2013) studied the feasibility of implementing Fourier transform mid-infrared (FT-MIR) spectroscopy for the routine analysis of PV in extra virgin olive oil. Mathäus (1996) used a chemiluminescence method to determine the oxidation of oils. This method detects peroxyesters and dialkylperoxides as well, whereas the iodiometric method is not suitable for determining these compounds because of their redox potential. For the chemiluminescence method, a small quantity of oil is dissolved in a mixture of acetone/ethanol (2:1, v/v), and 1 ml of a luminol solution (consisting of luminol, hemin, and sodium carbonate) is added. A photomultiplier below the sample vessel receives the light and converts it to an electric pulse. A good correlation ($r = 0.9865$) existed between the Rancimat and the chemiluminescence methods for determination of the induction period of oils (Mathäus, 1996).

There are several methods (Lea, 1931; Wheeler, 1932; IOC, 1968; AOCS, 1989; EU, 1991) for measuring the total hydroperoxides as the primary products of oxidation of olive oil. The IOC (1995) and the EU (1995) set up a maximum PV for each classification of olive oil (Table 28.4). The IOC standard for an oil sample is ≤20 mEq O_2/kg oil (Mailer & Beckingham, 2006). Van de Voort *et al.* (1994) reported that the official method established by the European Union for PV determination in olive oil (EU, 1991) is laborious and time-consuming, it requires the use of organic solvents, and, even more importantly, its accuracy strongly depends on experimental conditions.

28.3.2 Conjugated dienes and trienes

Polyunsaturated fats and oils are generally methylene-interrupted in nature. Upon oxidation, conjugated dienes and trienes are formed, and these give rise to absorption peaks at 232 and 270 nm in the UV region, respectively (Kiritsakis, 1991, 1998b; Shahidi & Wanasundara, 2002). Conjugated hydroperoxides formed as primary oxidation products of olive oil absorb at 232 nm. The secondary oxidation products (aldehydes

Table 28.4 European Regulation Standard limits of PV and acidity for olive oil.

Oil	PV (meq O_2/kg of oil)	Acidity (% oleic acid)
Lampante olive oil	>20	>3.3
Extra virgin olive oil	≤20	≤1.0
Virgin olive oil	≤20	≤2.0
Ordinary virgin olive oil	≤20	≤3.3
Olive oil	≤15	≤1.5
Olive-pomace oil	≤15	≤1.5
Refined olive oil	≤5	≤0.5
Refined olive-pomace oil	≤5	≤0.5
Crude olive-pomace oil	–	>0.5

and ketones) absorb at higher wavelengths (270 nm). According to the EU (1991) Regulation 2568/91, the purity of olive oil is measured using an UV spectrophotometric technique, known as UV-specific extinction coefficients (K_{232} and K_{270}). In this method, K_{232} and K_{270} refer to respective absorbances at 232 and 270 nm, and are related to conjugated dienes and trienes. A low absorption in this region is indicative of a high-quality olive oil, while adulterated or refined oils show an increased level of absorption in this region (Puente, 2008). The EU (1991) has developed maximum permitted values for K_{232} and K_{270} (Table 28.5).

The refining process favors the formation of conjugated polyenes (mainly when active earth is used for the discoloration). Conjugated dienes and trienes, formed during the refining or bleaching of olive oil, also absorb in the region of 260–280 nm (IOC, 1968; EU, 1995; Kiritsakis, 1998b). Good correlations existed between the CDs and PVs (Shahidi *et al.*, 1994; Wanasundara *et al.*, 1995). This method is simple and fast, and it requires no chemical reagents. It also has better sensitivity than PV measurement (Antolovich *et al.*, 2002; Shahidi & Zhong, 2007, 2015). However, the result may be affected by the presence of compounds such as carotenoids, which absorb in the same region (Shahidi & Wanasundara, 2002). CD measurement often cannot be performed directly on tissues and body fluids because many other interfering substances are present, such as heme proteins, chlorophylls, purines, and pyrimidines that absorb strongly in the UV region. Extraction of lipids into organic solvents before analysis is a common approach to alleviate this problem (Antolovich *et al.*, 2002). Farhoosh *et al.* (2012) observed the changes in polar value (POV), conjugated diene value (CDV), and carbonyl value (CV) of a set of olive oil samples with a wide range of compositional parameters during 16 h of heating at 180 °C. These authors found a significant correlation of CDV and CV of olive oil samples with POV ($R^2 > 0.98$). They reported that heated oils should be discarded when CDs reach 44 mmol/l.

28.3.3 Thiobarbituric acid reactive substances assay

During lipid oxidation, malonaldehyde (MA), a minor component of fatty acids with three or more double bonds, is formed as a result of the degradation of PUFAs. MA reacts with 2-thiobarbituric acid (TBA) to form a pink MA–TBA complex that is measured spectrophotometrically at its absorption maximum at 530–535 nm, usually 532 nm (Kiritsakis & Markakis, 1987; Antolovich *et al.*, 2002; Jardine *et al.*, 2002; Shahidi & Zhong, 2007). MA forms readily when trienoic or tetraenoic acids are present (Dugan, 1976). It may also be formed from dienoic acid (linoleic), during autoxidation and photooxidation of olive oil (Kiritsakis, 1982) or other oils. Photooxidation of dienoic acids in olive oil can lead to β,γ-unsaturated hydroperoxides.

Table 28.5 Maximum permitted values of K_{232} and K_{270} for olive oils.

Oil	K_{232}	K_{270}
Extra virgin olive oil	≤2.4	≤0.20
Virgin olive oil	≤2.5	≤0.25
Olive oil	≤3.3	≤1.0

The β,γ-systems presumably can undergo cyclization and scission to form MA, which can participate in the TBA reaction (Pryor *et al.*, 1976).

Many other substances, including alkenals, alkadienals, proteins, sucrose, and urea, may react with TBA to yield colored species and thus contribute to overestimation of the extent of lipid peroxidation (Jardine *et al.*, 2002). Some specificity is provided by the choice of analytical wavelength, as alka-2,4-dienals and, to a lesser extent, alka-2-enals produce a red pigment absorbing at 530 nm, whereas alkanals in general produce a yellow chromogen absorbing at 450 nm. Further enhancement in specificity has been achieved by HPLC separation of the complex prior to measurement (Bird *et al.*, 1983; Jardine *et al.*, 2002). Other approaches to improving specificity and sensitivity include extraction of the MA prior to the formation of the chromogen and/or derivative spectrophotometry (Botsoglou *et al.*, 1994; Jardine *et al.*, 2002). Shahidi and Zhong (2007) reported that reduction of heating temperature, extraction of the MA prior to the formation of the chromogen, and direct FTIR analysis of TBARS may improve the specificity and sensitivity of TBARS. Antolovich *et al.* (2002) suggested another method for detecting peroxidation in lipids of biological origin that involved the so-called LPO-586 assay (a colorimetric assay of lipid peroxidation). This method apparently responds to both MA and 4-hydroxyalkenals but is not specific to either group. The chromophore(s) formed in the condensation of aldehydes with *N*-methyl-2-phenylindole absorb(s) strongly close to 586 nm, and the method can be used as an alternative to the TBARS method.

The TBA test has been applied by several workers (Casillo, 1968; Kiritsakis, 1982) to determine the oxidation of olive oil. Casillo (1968) reported that the TBA test detects the rancidity of olive oil at a lower level than PV. Nouros *et al.* (2000) have shown that a flow-injection system incorporating multiple incubation coils could be used for the automated determination of TBARS in olive oil samples.

28.3.4 p-Anisidine value (p-AnV)

The *p*-anisidine value is a measure of the content of aldehydes (principally 2-alkenals and 2,4-alkadienals) generated during the decomposition of hydroperoxides. *p*-Methoxyaniline (anisidine) reacts with aldehydes and produces a yellow-colored product that absorbs at 350 nm (Doleschall *et al.*, 2002). The *p*-AV is defined as 100 times the absorbance of a solution resulting from 1 g of fat or oil mixed with 100 mL of isooctane/acetic acid/*p*-anisidine reagent. This test is more sensitive to unsaturated aldehydes than to saturated aldehydes because the colored products from unsaturated aldehydes absorb more strongly at this wavelength (Shahidi & Zhong, 2007). However, it correlates well with the amount of total volatile substances (Doleschall *et al.*, 2002). The *p*-AnV is comparable only within the same type of oil because initial *p*-AnV varies among oil sources. For instance, oils with high levels of PUFAs might have higher *p*-AnV even when fresh (Shahidi & Zhong, 2007). The combined value of *p*-AnV and PV is referred to as the totox number (TN), which indicates the overall oxidative status of oil (AOCS, 1997) and is calculated as:

$$\text{Totox} = 2\text{PV} + p\text{-AnV}$$

Adding two units of PV with one unit of *p*-AnV gives the TN. Labrinea *et al.* (2001) reported that there is no standard or set limit for *p*-AnV in the olive oil codex, but it is considered as a very useful indicator of oil quality that complements the PV test.

28.3.5 Total carbonyls

Since carbonyl compounds are the major cause of rancid and unpleasant flavor, it is essential to measure the CV of heated fats and oils. Total carbonyl content can be measured by a colorimetric procedure using 2,4-dinitrophenylhydrazine that produces 2,4-dinitrophenylhydrazone. However, selected model systems should be used in the absence of any protein, as carbonyls are also produced from protein oxidation, which may then give rise to higher values than those expected from lipid oxidation alone (Shahidi & Zhong, 2007). Farhoosh and Moosavi (2008) reported that for frying oils, if the CV is $\leq$43.5 μmol/g, the oil is still considered safe and acceptable, flavor wise.

28.3.6 Polar value

The most reliable method for the evaluation of oil quality during heating and frying processes is the measurement of the contents of polar compounds, which is known as the POV. The POV is determined by measuring the change in dielectric constant. Paul *et al.* (1997) reported that the regulatory agencies in European countries established a rejection point for heated oils at the level of 24–27% POV.

28.3.7 Electrical conductivity method

During lipid oxidation, volatile organic acids, mainly formic and acetic acids, are produced at high temperatures, simultaneously with hydroperoxides (Shahidi & Zhong, 2007). In addition, other secondary products including alcohols and carbonyl compounds can be further oxidized to carboxylic acids (Kiritsakis *et al.*, 2002). The formation of volatile acids can be measured by monitoring the change in electrical conductivity when the effluent from oxidizing oils is passed through water (Shahidi & Zhong, 2007). This method is not always recommended for measuring antioxidant activity, especially for evaluation of thermally sensitive compounds. Volatile antioxidants may be swept out of the oil by the air flow under test conditions, and also the oils are severely deteriorated when the end point is reached (Shahidi & Zhong, 2015). The solid-phase microextraction (SPME) method coupled to GC-MS and GC-FID combined techniques has been employed in isolation and in the characterization of volatile components from olive oil (Manai *et al.*, 2007; Zarrouk *et al.*, 2007).

The Oxidative Stability Index (OSI) generally correlates more accurately with the shelf life of an oil sample. The continuous measurement of an increase in conductivity due to the ionizable short-chain fatty acids, formic acid and acetic acid resulting from the induced oxidation of the oil sample, determines the degree of rancidity (Pike, 1998; Morales & Przybylski, 2000). A decreasing trend in OSI was observed when virgin olive oil samples were stored in dark, sealed bottles for 12 months (Morelló *et al.*, 2004a, 2004b). Researchers have shown that there is a decreasing trend in hydroxytyrosol, total phenolics, carotenoid content, and tocopherol content with overripening of the olive fruit as well as increase in the storage time (Roca & Minguez-Mosquera, 2001; Morello *et al.*, 2004a, 2004b). Baldioli *et al.* (1996) have demonstrated that the OSI of olive oil measured with a Rancimat instrument did not show any significant correlation with the concentration of total tocopherols in the sample, while OSI positively correlated with the concentration of total phenolic compounds.

28.3.8 Nuclear magnetic resonance (NMR) spectroscopy

High-resolution ^{1}H NMR spectroscopy, in which hydrogen atoms (proton, ^{1}H) with various locations in the TAG molecules are determined, has been used to evaluate oxidative deterioration of fats and oils. Wanasundara *et al.* (1995) found good correlations between PVs and the NMR results, and reported that the NMR methodology provides a rapid, nondestructive, and simple procedure for evaluation of the oxidative changes during storage of edible oils. In addition to ^{1}H NMR, ^{13}C NMR and ^{31}P NMR are also powerful tools to predict oxidative stability of oils (Medina *et al.*, 1998; Hidalgo *et al.*, 2002). ^{13}C NMR enables direct observation of carbon atoms. The selectivity and dispersion of ^{13}C NMR spectra are very high. However, because the abundance of the NMR active ^{13}C nucleus isotope is only 1.12% of ^{12}C, the sensitivity of ^{13}C NMR is usually much lower than that of ^{1}H NMR (Medina *et al.*, 1998; Shahidi & Zhong, 2007). Several authors have used NMR spectroscopy for the characterization of olive oils (Vlahov, 1999; Fauhl *et al.*, 2000; Vigli *et al.*, 2003; Rezzi *et al.*, 2005). Petrakis *et al.* (2008) used ^{1}H and ^{31}P NMR spectroscopy for characterizing fatty acids, phenolics, diacylglycerols, total free sterols, free acidity, and iodine number of Greek virgin olive oil.

28.4 Determination of chlorophylls

The greenish color of virgin olive oils is due to chlorophyll pigments, chlorophylls, pheophytins, and pyropheophytins (Kiritsakis & Markakis, 1987). However, refined olive oil does not contain any natural chlorophyll pigments. Thus, for economical purposes, synthetic stable chlorophyll pigments containing

copper have been used in refined oils in order to cheat the customers. However, the use of copper pheophytin (E 141, a food-coloring agent) in olive oils is illegal (Del Giovine & Fabietti, 2005). In order to prove the authenticity of the product, it is mandatory to detect the copper chlorophyll. In the literature, there are various methods that can detect chlorophyll. Traditionally, the chlorophyll content of olive oil may be determined by measuring its absorbance at 630, 670, and 710 nm, using visible spectrophotometry (AOCS, 1989). Rahmani and Csallany (1985) proposed a rapid and precise HPLC method for determining chlorophylls a and b and pheophytins a and b in olive oil. The oil sample is diluted 20 times (w/v) with the solvent (hexane–isopropanol, 98.5:1.5, v/v) and then is injected into a μ-Porasil column. It has been noted that carbon tetrachloride is a known carcinogen. The AOCS (1989) official method Ch 4-91 specifies either methylene chloride (also a carcinogen) or methyl iso-butylketone (MIBK) as the solvent. Del Giovine and Fabietti (2005) proposed a capillary zone electrophoresis (CZE) method with a laser-induced fluorescence (LIF) detector to separate copper chlorophyll from other chlorophyll pigments in order to determine its possible presence in olive oil samples.

28.5 Determination of phenols

The phenol content of olive oil can be determined using the method proposed by Gutfinger (1981). This is one of the most widely used methods for the routine determination of total phenolic content in olive oil. This colorimetric assay is based on the reaction of Folin-Ciocalteu reagent with the functional hydroxyl groups of the analytes (Gutfinger, 1981). There are several other methods that may also be used to determine total phenols in olive oil. Garcia-Mesa *et al.* (1993) developed a robotic-FIA method for determination of total polyphenols in virgin olive oil. In another study, a simple and rapid capillary electrophoretic method was used for characterization of the polyphenolic fraction of extra virgin olive oil (Carrasco-Pancorbo *et al.*, 2006). In this method, the detection was carried out by UV absorption at 200, 240, 280, and 330 nm in order to facilitate the identification of the compounds. However, the above methods require the separation of analytes in order to avoid the overlap with other compounds, and they are also time-consuming. Recently, the application of chemometric tools such as multivariate calibration has been proposed to overcome the lack of selectivity when using UV-visible spectrophotometric detection for the analysis of olive oil phenolics (Fuentes *et al.*, 2012).

28.6 Cold test

The cold test determines the resistance of olive oil and other oils to crystallization, and is commonly used as an index for evaluating the winterization or similar stearin removing processes (Kiritsakis, 1998b).In the winterization process, containers of the samples are immersed in an ice and/or a water bath or kept in a cold room for about $5\frac{1}{2}$ hours to remove any solids; the sample must be clear, limpid, and brilliant at the end of the test period in order to pass the test.

28.7 Determination of sterol content

The determination of phytosterols is a widely accepted method and one of the most important markers for the detection of adulterated olive oils. In general, the sterols in olive oil were analyzed using GC. Bohacenko and Kopicova (2001) reported that in GC analysis, a wide spectrum of other compounds in addition to sterols, such as higher hydrocarbons, aliphatic alcohols, tocopherols, triterpene-based alcohols, and waxes, could interfere with the measurement. These authors suggested a method using the preparative LC with silica gel packed-column and gradient elution with three mixtures of hexane and diethyl ether in order to separate undesirable interfering compounds in the unsaponifiable fraction before the determination of sterols using GC. The predominant sterols in olive oils are 4-desmethyl sterols and β-sitosterol. Minor compounds include Δ^5-avenasterol, stigmasterol, sitostanol, and cholesterol (Volin, 2001). The official method involves saponification of the oil and extraction of the unsaponifiable fraction with diethyl ether. Then, the extract is fractionated by thin-layer chromatography (TLC) on silica gel plates, and quantification of the silanized

sterol fraction is performed by capillary-column GC (EU, 1991). Martínez-Vidal *et al.* (2007) proposed an analytical procedure based on LC-MS with atmospheric-pressure chemical ionization (APCI), with saponification of sterols but without TLC fractionation, in order to minimize sample preparation. According to the IOC (2013), the total sterol content of virgin olive oil, refined olive oil, and olive oil is ≥1000 mg/kg of oil.

28.8 Differential scanning calorimetry (DSC) of olive oil

DSC has been used to assess adulteration of olive oil with other vegetable or seed oils of lower quality and/or economic value. DSC is a rapid, solvent-free method to characterize oxidation of olive oil. A study on extra virgin olive oil subjected to various accelerated oxidation treatments using modulated DSC showed a very good correlation between thermograph parameters selected for the main crystallization peak and the various off flavors derived mainly from the oleic acid degradation (Kanavouras & Coutelieris, 2004).

28.9 Authentication and authenticity of olive oil

The detection of adulteration in olive oil is essential for the safety of products and protection of consumers (Kiritsakis, 1998b). Various olive oil standards exist in the world. Some of them are national standards, while Codex Alimentarius and the IOC have developed the international standards. Based on the IOC standard, the European Union has put in place a mandatory standard for olive oil and olive-pomace oil (Valentine, 2013). These official and trade standards are classified by a number of physicochemical parameters and sensory characteristics. These include the determination of fatty acid composition, free fatty acids, triacylglycerols, chlorophyll content, pyropheophytin content, sterols composition, phenols, peroxide value, and UV extinction coefficients (K_{232} and K_{270}). Volin (2001) suggested that the unsaponifiable fraction (predominantly sterols) of olive oil may be used for detecting adulteration or checking for authenticity, because this fraction is more characteristic than the fatty acid profile. European Community legislation has introduced the Protected Designation of Origin (PDO) mark, which allows the labeling of virgin olive oils with the names of the areas where they are produced (Yildrim, 2009). This certification is believed to protect producers of high-quality olive oils and ensure consumer awareness of product quality while it increases the value of oil. Recently, Yang *et al.* (2013) identified the adulteration of extra virgin olive oil with corn, peanut, rapeseed, and sunflower oils on the basis of 22 fatty acids and six significant parameters (including 18:2/18:3, 18:1/18:2, total saturated fatty acids [SFAs], monounsaturated fatty acids, and polyunsaturated fatty acids). Detection of adulteration of olive oil is also discussed elsewhere in this book.

References

Aggelis, G., Iconomou, D., Christou, M., Bokas, D., Kotzailias, S., Christou, G., Tsagou, V., & Papanikolaou, S. (2003) Phenolic removal in a model olive oil mill wastewater using *Pleurotus ostreatus* in bioreactor cultures and biological evaluation of the process. Water Research 37, 3897–3904.

Akasaka, K., Ohrui, H., & Meguro, H. (1993) Simultaneous determination of hydroperoxides of phosphatidylcholine, cholesterol esters and triacylglycerols by column-switching high-performance liquid chromatography with a post-column detection system. Journal of Chromatography – Biomedical Applications 622, 153–159.

American Oil Chemists' Society (AOCS). (1999) Acid value: sampling and analysis of commercial fats and oils. In: Official methods and recommended practices (Method Cd 3d-63). AOCS, Champaign, IL.

American Oil Chemists' Society (AOCS). (1989) Official methods and recommended practices of the American Oil Chemists' Society (Method Cd 8b-90), 4th ed. AOCS, Champaign, IL.

American Oil Chemists' Society (AOCS). (1998) Official methods and recommended practices of the American Oil Chemists Society, 5th ed. AOCS, Champaign, IL.

American Oil Chemists' Society (AOCS). (1997) *p*-Anisidine value: sampling and analysis of commercial fats and oils. In: Official methods and recommended practices of the American Oil Chemists' Society (Method Cd 18-90). AOCS, Champaign, IL.

Amiot, M.J., Fleuriet, A., & Macheix, J.J. (1986) Accumulation of oleuropein derivatives during olive maturation. Journal of Agriculture and Food Chemistry 34, 823–826.

Angerosa, F., d'Alessandro, N., Konstantinou, P., & Di Giacinto, L. (1995) GC-MS evaluation of phenolic compounds in virgin olive oil. Journal of Agriculture and Food Chemistry43, 1802–1807.
Antolovich, M., Prenzler, P.D., Patsalides, E., McDonald, S., & Robards, K. (2002) Methods for testing antioxidant activity. Analyst 127, 183–198.
Baldioli, M., Servili, M., Perretti, G., & Montedoro, G.F. (1996) Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. Journal of the American Oils Chemists' Society 73, 1589–1593.
Bell, J.R., & Gillatt, P.N. (1994) Standards to ensure the authenticity of edible oils and fats. Food, Nutrition and Agriculture, Food and Agriculture Organization of the United Nations, Rome.
Beuchamp, G.K., Keast, R.S., Morel, D., & Lin, J. (2005) Enzymes in an inflammation pathway are inhibited by oleocanthal, a component of olive oil. Nature 437, 45–46.
Bianco, A., Coccioli, F., Guiso, M., & Marra, C. (2002) The occurrence in olive oil of a new class of phenolic compounds: hydroxy-isochromans. Food Chemistry 77, 405–411.
Bird, R.P., Hung, S.S.O., Hadley, M., & Draper, H.H. (1983) Determination of malonaldehyde in biological materials by high-pressure liquid chromatography. Analytical Biochemistry 128, 240–244.
Bohacenko, I., & Kopicova, Z.D.E.N.A. (2001) Detection of olive oils authenticity by determination of their sterol content using LC/GC. Czech Journal of Food Sciences 19, 97–104.
Borzillo, A., Iannotta, N., & Uccella, N. (2000) Oinotria table olives: quality evaluation during ripening and processing by biomolecular components. European Food Research and Technology 212, 113–121.
Botsoglou, N.A., Fletouris, D.J., Papageorgiou, G.E., Vassilopoulos, V.N., Mantis, A.J., & Trakatellis, A.G. (1994) Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. Journal of Agriculture and Food Chemistryn 42, 1931–1937.
Brenes, M., Hidalgo, F. J., García, A., Rios, J. J., García, P., Zamora, R., & Garrido, A. (2000) Pinoresinol and 1-acetoxypinoresinol, two new phenolic compounds identified in olive oil. Journal of the American Oils Chemists' Society 77, 715–720.
Cabrini, L., Barzanti, V., Cipollone, M., Fiorentini, D., Grossi, G., Tolomelli, B., Zambonin, L., & Landi, L. (2001) Antioxidants and total peroxyl radical-trapping ability of olive and seed oils. Journal of Agriculture and Food Chemistry 49, 6026–6032.
Carrasco-Pancorbo, A., Gómez-Caravaca, A. M., Cerretani, L., Bendini, A., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2006) A simple and rapid electrophoretic method to characterize simple phenols, lignans, complex phenols, phenolic acids, and flavonoids in extra-virgin olive oil. Journal of Separation Science 29, 2221–2233.
Casillo, R. (1968) Quality evaluation of virgin olive oils: thiobarbituric acid test. Rivista Italiana delle Sostanze Grasse 45, 753–757.
Cioffi, G., Pesca, M.S., De Caprariis, P., Braca, A., Severino, L., & De Tommasi, N. (2010) Phenolic compounds in olive oil and olive pomace from Cilento (Campania, Italy) and their antioxidant activity. Food Chemistry 121, 105–111.
Czerwińska, M., Kiss, A.K., & Naruszewicz, M. (2012) A comparison of antioxidant activities of oleuropein and its dialdehydic derivative from olive oil, oleacein. Food Chemistry 131, 940–947.
Del Giovine, L., & Fabietti, F. (2005) Copper chlorophyll in olive oils: identification and determination by LIF capillary electrophoresis. Food Control 16, 267–272.
Dhaouadi, A., Monser, L., Sadok, S., & Adhoum, N. (2006) Flow-injection methylene blue-based spectrophotometric method for the determination of peroxide values in edible oils. Analytica Chimica Acta 576, 270–274.
Dobarganes, M.C., & Velasco, J. (2002) Analysis of lipid hydroperoxides. European Journal of Lipid Science and Technology 104, 420–428.
Doleschall, F., Kemény, Z., Recseg, K., & Kővári, K. (2002) A new analytical method to monitor lipid peroxidation during bleaching. European Journal of Lipid Science and Technology 104, 14–18.
Dugan, L.R. (1976) Lipids. In: Principles of food science, Part I food chemistry, ed. O. Fennema. Marcel Dekker, New York, 140–203.
Erbay, Z., & Icier, F. (2010) The importance and potential uses of olive leaves. Food Reviews International 26, 319–334.
European Union (EU) Commission Regulation. (2013) Commission Implementing Regulation No 299/2013 of 26 March 2013 and No 1348/2013 of 16 December 2013 amending Regulation No 2568/91, No 294/2013.
European Union (EU) Commission Regulation. (1995) (Modification of No. 2568/91 Regulation) On the characteristics of olive oil and olive: residue oil and the relevant methods of analysis. Official Journal of the European communities. No L 069.
European Union (EU) Commission Regulation. (1991) On the characteristics of olive oil and olive – residue oil and the relevant methods of analysis. Official Journal of the European communities, No L 248.
Evans, O. (1999) On-line deoxygenation in reductive (and oxidative) amperometric detection: Environmental applications in the liquid chromatography of organic peroxides. Analyst 124, 1811–1816.

Farhoosh, R., Khodaparast, M.H.H., Sharif, A., & Rafiee, S.A. (2012) Olive oil oxidation: rejection points in terms of polar, conjugated diene, and carbonyl values. Food Chemistry 131, 1385–1390.

Farhoosh, R., & Moosavi, S.M.R. (2008) Carbonyl value in monitoring of the quality of used frying oils. Analytica Chimica Acta 617, 18–21.

Fauhl, C., Reniero, F., & Guillou, C. (2000) ^{1}H NMR as a tool for the analysis of mixtures of virgin olive oil with oils of different botanical origin. Magnetic Resonance in Chemistry 38, 436–443.

Fronimaki, P., Spyros, A., Christophoridou, S., & Dais, P. (2002) Determination of the diglyceride content in Greek virgin olive oils and some commercial olive oils by employing 31P NMR spectroscopy. Journal of Agricultural and Food Chemistry, 50, 2207–2213.

Fuentes, E., Báez, M.E., Bravo, M., Cid, C., & Labra, F. (2012) Determination of total phenolic content in olive oil samples by UV-visible spectrometry and multivariate calibration. Food Analytical Methods 5, 1311–1319.

Gaddipati, N., Volpe, F., & Anthony, G. (1983) Quantitative determination of benzoyl peroxide by high-performance liquid chromatography and comparison to the iodometric method. Journal of Pharmaceutical Sciences 72, 1398–1400.

Garcia-Mesa, J.A., Luque de Castro, M.D., & Valcarcel, M. (1993) Coupled robot-flow injection analysis system for fully automated determination of total polyphenols in olive oil. Analytical Chemistry 65, 3540 –3542.

Gebicki, J.M., & Guille, J. (1989) Spectrophotometric and high-performance chromatographic assays of hydroperoxides by the iodometric technique. Analytical Biochemistry 176, 360–364.

González-Santiago, M., Fonollá, J., & Lopez-Huertas, E. (2010) Human absorption of a supplement containing purified hydroxytyrosol, a natural antioxidant from olive oil, and evidence for its transient association with low-density lipoproteins. Pharmacological Research 61, 364–370.

Gutfinger, T. (1981) Polyphenols in olive oils. Journal of the American Oils Chemists' Society 58, 966–968.

Hamam, F., & Shahidi, F. (2006) Acidolysis reactions lead to esterification of endogenous tocopherols and compromised oxidative stability of modified oils. Journal of Agriculture and Food Chemistry 54, 7319–7323.

Hayes, J.E., Allen, P., Brunton, N., O'Grady, M.N., & Kerry, J.P. (2011) Phenolic composition and in vitro antioxidant capacity of four commercial phytochemical products: olive leaf extract (*Olea europaea* L.), lutein, sesamol and ellagic acid. Food Chemistry 126, 948–955.

Hicks, M., & Gebicki, J.M. (1979) A spectrophotometric method for the determination of lipid hydroperoxides. Analytical Biochemistry 99, 249–253.

Hidalgo, F.J., Gómez, G., Navarro, J.L., & Zamora, R. (2002) Oil stability prediction by high-resolution ^{13}C nuclear magnetic resonance spectroscopy. Journal of Agriculture and Food Chemistry 50, 5825–5831.

Huang, D., Boxin, O., & Prior, R.L. (2005) The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry 53, 1841–1856.

International Olive Council (IOC). (1968) Common methods for analysis of olive oils. T.14/DOC No4/Corr 1. IOC, Madrid, Spain.

International Olive Council (IOC). (1995) Trade standards applying to olive oil and olive pomace oil. COI/T. 15/NC No.2 Rev. 2. IOC, Madrid, Spain.

International Olive Council (IOC). (2001) Trade standards applying to olive oil and olive pomace oil. In COI/T.15/NC no. 2/Rev. 10. IOC, Madrid, Spain.

International Olive Council (IOC). (2013) Trade standards applying to olive oil and olive pomace oil. COI/T. 15/NC No 3/Rev. 7. IOC, Madrid, Spain.

Ismail, A., van de Voort, F.R., Emo, G., &. Sedman, J. (1993) Rapid quantitative determination of free fatty acids in fats and oils by Fourier transform infrared spectroscopy. Journal of the American Oils Chemists' Society 70, 335–341.

Iwahashi, H. (2000) High-performance liquid chromatographic determination of linoleic acid peroxide-derived radicals using electrochemical detection. Journal of Chromatography A 904, 197–202.

Jardine, D., Antolovich, M., Prenzler, P.D., & Robards, K. (2002) Liquid chromatography−mass spectrometry (LC-MS) investigation of the thiobarbituric acid reactive substances (TBARS) reaction. Journal of Agriculture and Food Chemistry 50, 1720–1724.

Jiang, Z.Y., Woollard, A.C.S., & Wolff, S.P. (1991) Lipid hydroperoxide measurement by oxidation of Fe^{2+} in the presence of xilenol orange. Comparison with the TBA assay and the iodometric method. Lipids 26, 853–856.

Kalua, C.M., Allen, M.S., Bedgood, D.R., Bishop, A.G., Prenzler, P.D., & Robards, K. (2007) Olive oil volatile compounds, flavour development and quality: a critical review. Food Chemistry 100, 273–286.

Kanavouras, A., & Coutelieris, F.A. (2004) Shelf-life predictions for packaged olive oil based on simulations. Food Chemistry 96, 48–55.

Kiritsakis, A. (1982) Quality studies on olive oil. PhD dissertation, Michigan State University, East Lansing, MI.

Kiritsakis, A. (1991) Olive oil. American Oil Chemists' Society, Champaign, IL, USA.

Kiritsakis, A.K. (1993) Flavor chemistry of olive oil. Olivae 45, 28–33.

Kiritsakis, A.K. (1998a) Flavor components of olive oil: a review. Journal of the American Oils Chemists' Society 75, 673–681.

Kiritsakis A.K. (1998b) Olive oil: from the tree to the table, 2nd ed. Food and Nutrition Press, Trumbull, CT.

Kiritsakis, A., & Dugan, L.R. (1985) Studies in photooxidation of olive oil. Journal of the American Oils Chemists' Society 62, 892–896.

Kiritsakis, A.K., Kanavouras A., & Kiritsakis, K.A. (2002) Chemical analysis, quality control and packaging issues of olive oil. European Journal of Lipid Science and Technology 104, 628–638.

Kiritsakis, K., Kontominas, M.G., Kontogiorgis, C., Hadjipavlou-Litina, D., Moustakas, A., & Kiritsakis, A. (2009) Composition and antioxidant activity of olive leaf extracts from Greek olive cultivars. Journal of the American Oil Chemists' Society 87, 369–376.

Kiritsakis, A.K., & Markakis, P. (1987) Olive oil: a review. Advances in Food Research 31, 453–482.

Kiritsakis, A.K., & Min, D. (1989) Flavor chemistry of olive oil. In: Flavor chemistry of lipid foods, ed. D. Min & T. Smouse. Champaign, IL USA.

Kiritsakis, A K., Stine, C.M., & Dugan, L.R. (1983) Effect of selected antioxidants on the stability of virgin olive oil. Journal of the American Oils Chemists' Society 60, 286–290.

Kristinova, V., Mozuraityte, R., Storrø, I., & Rustad, T. (2009) Antioxidant activity of phenolic acids in lipid oxidation catalyzed by different prooxidants. Journal of Agriculture and Food Chemistry 57, 10377–10385.

Labrinea, E.P., Thomaidis, N.S., & Giorgiou, C.A. (2001) Direct olive oil anisidine value determination by flow injection. Analytica Chimica Acta 448, 201–206.

Lea, C.H. (1931) The effect of light on the oxidation of fats. Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character 108, 175–189.

Li, J., Tan, S.N., & Oh, J.T. (1998) Silica sol-gel immobilized amperometric enzyme electrode for peroxide determination in the organic phase. Journal of Electroanalytical Chemistry 448, 69–77.

Maggio, R.M., Kaufman, T.S., Del Carlo, M., Cerretani, L., Bendini, A., Cichelli, A., & Compagnone, D. (2009) Monitoring of fatty acid composition in virgin olive oil by Fourier transformed infrared spectroscopy coupled with partial least squares. Food Chemistry 114, 1549–1554.

Mailer, R. (2006) Chemistry and quality of olive oil. NSW Department of Primary Industries 227, 1–4.

Mailer, R., & Beckingham, C. (2006) Testing olive oil quality: chemical and sensory methods. NSW Department of Primary Industries 231, 1–5.

Makahleh, A., & Saad, B. (2011) Flow injection determination of free fatty acids in vegetable oils using capacitively coupled contactless conductivity detection. Analytica Chimica Acta 694, 90–94.

Manai, H., Haddada, M.F., Daoud, D., Fernandez, X., Lizzani-Cuvelier, L., & Zarrouk, M. (2007) Profiles of volatile compounds from some monovarietal Tunisian virgin olive oils: comparison with French PDO. Food Chemistry 103, 467–476.

Martínez-Vidal, J.L., Garrido-Frenich, A., Escobar-García, M.A., & Romero-González, R. (2007) LC–MS determination of sterols in olive oil. Chromatographia 65, 695–699.

Mathäus, B.W. (1996) Determination of the oxidative stability of vegetable oils by Rancimat and conductivity and chemiluminescence measurements. Journal of the American Oils Chemists' Society 73, 1039–1043.

Medina, I., Sacchi, R., Giudicianni, I., & Aubourg, S. (1998) Oxidation in fish lipids during thermal stress as studied by ^{13}C nuclear magnetic resonance spectroscopy. Journal of the American Oils Chemists' Society 75, 147–154.

Miyazawa, T., Fujimoto, K., Suzuki, T., & Yasuda, K. (1994) Determination of phospholipid hydroperoxides using luminol chemiluminescence-high-performance liquid chromatography. Methods in Enzymology 233, 324–332.

Morales, M.T., & Przybylski, R. (2000) Olive oil oxidation. In J Harwood and R Aparicio, Handbook of olive oil. Aspen, Gaithersburg, MD, 459–490.

Morelló, J.R, Motilva, M.J., Tovar, M.J., & Romero, M.P. (2004a) Changes in commercial virgin olive oil (cv Arbequina) during storage, with special emphasis on the phenolic fraction. Food Chemistry 85, 357–364.

Morelló, J.R., Romero, M.P., & Motilva, M.J. (2004b) Effect of the maturation process of the olive fruit on the phenolic fraction of drupes and oils from Arbequina, Farga, and Morrut cultivars. Journal of Agricultural and Food Chemistry 52, 6002–6009.

Mulchandani, A., & Rudolph, D.C. (1995) Amperometric determination of lipid hydroperoxides. Analytical Biochemistry 225, 277–282.

Mulchandani, A., Wang, C.L., & Weetall, H.H. (1995) Amperometric detection of peroxides with poly (anilinomethylferrocene)-modified enzyme electrodes. Analytical Chemistry 67, 94–100.

Nouros, P.G., Georgiou, C.A., & Polissiou, M.G. (1999) Direct parallel flow injection multichannel spectrophotometric determination of olive oil peroxide value. Analytica Chimica Acta 389, 239–245.

Nouros, P.G., Georgiou, C.A., & Polissiou, M. (2000) Determination of olive oil 2-thiobarbituric acid reactive substances by parallel flow injection. Analytica Chimica Acta 417, 119–124.

Oliveri-Vigh, S., & Hainsworth, F.T. (1978) Benzoyl peroxide assay using high-pressure liquid chromatography. Journal of Pharmaceutical Sciences 67, 1035–1036.

Patumi, M., d'Andria, R., Marsilio, V., Fontanazza, G., Morelli, G., & Lanza, B. (2002) Olive and olive oil quality after intensive monocone olive growing (*Olea europaea* L., cv. Kalamata) in different irrigation regimes. Food Chemistry 77, 27–34.

Paul, S., Mittal, G.S., & Chinnan, M.S. (1997) Regulating the use of degraded oil/fat in deep-fat/oil food frying. Critical Reviews in Food Science and Nutrition 37, 635–662.

Pegg, R.B., Amarowicz, R., Naczk, M., & Shahidi, F. (2007) Photochem for determination of antioxidant capacity of plant extracts. In: Antioxidant measurement and applications, ed. F. Shahidi, & C-T. Ho, ACS Symposium Series 956, American Chemical Society, Washington, DC.

Perez-Ruiz, T., Martinez-Lozano, C., Tomas, V., & Val, O. (1993) Fluorimetric flow-injection determination of hydroperoxides in foodstuffs. Food Chemistry 46, 301–305.

Petrakis, P., Agiomyrgianaki, A., Christophoridou, S., Spyros, A., & Dais, P. (2008) Geographical characterization of Greek virgin olive oils (Cv. Koroneiki) using 1H and 31P NMR fingerprinting with canonical discriminant analysis and classification binary trees. Journal of Agriculture and Food Chemistry 56, 3200–3208.

Pike, O.A. (1998) Fat characterization. In: Food analysis, 2nd ed., ed. S.S. Nielsen. Aspen, Gaithersburg, MD, 217–235.

Pizarro, C., Esteban-Díez, I., Rodríguez-Tecedor, S., & González-Sáiz, J.M. (2013) Determination of the peroxide value in extra virgin olive oils through the application of the stepwise orthogonalisation of predictors to mid-infrared spectra. Food Control 34, 158–167.

Pryor, W.A., Stanley, J.P., & Blair, E. (1976) A suggested mechanism for the formation of TBA-reactive material from prostaglandins-like endoperoxides. Lipids 11, 370–379.

Puente, J. (2008) Determination of olive oil purity and degree of oxidation using the LAMBDA XLS. PerkinElmer, Waltham, MA.

Rahmani, M., & Csallany, A.S. (1985) Mise au point d'une methode de chromatographie liquide à haute performance pour la determination des pigments chlorophylliens dans les huiles vegetables. Revue française des corps gras 32, 257–259.

Rezzi, S., Axelson, D.E., Héberger, K., Reniero, F., Mariani, C., & Guillou, C. (2005) Classification of olive oils using high throughput flow ^{1}H NMR fingerprinting with principal component analysis, linear discriminant analysis and probabilistic neural networks. Analytica Chimica Acta 552, 13–24.

Roca, M., & Mínguez-Mosquera, I. (2001) Change in the natural ratio between chlorophylls and carotenoids in olive fruit during processing for virgin olive oil. Journal of the American Oils Chemists' Society 78, 133–138.

Roginsky, V., & Lissi, E.A. (2005) Review of methods to determine chain-breaking antioxidant activity in food. Food Chemistry 92, 235–254.

Romani, A., Mulinacci, N., Pinelli, P., Vincieri, F.F., & Cimato, A. (1999) Polyphenolic content in five Tuscany cultivars of *Olea europaea* L. Journal of Agriculture and Food Chemistry 47, 964–967.

Rotondi, A., Bendini, A., Cerretani, L., Mari, M., Lercker, G., & Toschi, T.G. (2004) Effect of olive ripening degree on the oxidative stability and organoleptic properties of Cv. Nostrana di Brisighella extra virgin olive oil. Journal of Agriculture and Food Chemistry 52, 3649–3654.

Ruíz, A., Canada, M.J.A., & Lendl, B. (2001) A rapid method for peroxide value determination in edible oils based on flow analysis with Fourier transform infrared spectroscopic detection. Analyst 126, 242–246.

Saad, B., Wai, W.T., Lim, B.P., & Saleh, M.I. (2006) Flow injection determination of peroxide value in edible oils using triiodide detector. Analytica Chimica Acta 565, 261–270.

Shahidi, F., & Ambigaipalan, P. (2015) Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects – a review. Journal of Functional Foods 18, 820–897.

Shahidi, F., & Wanasundara, U. (2002) Methods for measuring oxidative rancidity in fats and oils. Food Science and Technology, 4650–488.

Shahidi, F., Wanasundara, U., & Brunet. N. (1994) Oxidative stability of oil from blubber of harp seal (*Phoca groenlandica*) as assessed by NMR and standard procedures. Food Research International 27, 555–562.

Shahidi, F., & Zhong, Y. (2010) Lipid oxidation and improving the oxidative stability. Chemical Society Reviews 39, 4067–4079.

Shahidi, F., & Zhong, Y. (2007) Measurement of antioxidant activity in food and biological systems. In: Antioxidant measurement and applications, ed. F. Shahidi & C.T. Ho. ACS Symposium series 956. American Chemical Society, Washington, DC, 36–66.

Shahidi, F., & Zhong, Y. (2015) Measurement of antioxidant activity. Journal of Functional Foods 18, 757–759.

Skiera, C., Steliopoulos, P., Kuballa, T., Diehl, B., & Holzgrabe, U. (2014) Determination of free fatty acids in pharmaceutical lipids by 1H NMR and comparison with the classical acid value. Journal of Pharmaceutical and Biomedical Analysis, 93, 43–50.

Sofi, F., Abbate, R., Gensini, G.F., & Casini, A. (2010) Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. The American Journal of Clinical Nutrition 92, 1189–1196.
Talpur, M.Y., Sherazi, S.T.H., Mahesar, S.A., & Bhutto, A.A. (2010) A simplified UV spectrometric method for determination of peroxide value in thermally oxidized canola oil. Talanta 80, 1823–1826.
Terao, J., & Matsushita, S. (1977) Products formed by photosensitized oxidation of unsaturated fatty acid esters. Journal of the American Oils Chemists' Society 54, 234–238.
Valentine, S. (2013) Trade standards for olive oil and olive-pomace oil in the world. Workshop "Authentification of Olive Oil", Madrid, Spain.
van de Voort, F.R., Ismail, A.A., Sedman, J., & Emo, G. (1994) Monitoring the oxidation of edible oils by Fourier transform infrared spectroscopy. Journal of the American Oils Chemists' Society 71, 243–253.
Velasco-Arjona, A., & De Luque Castro, M.D. (1998) A fully robotic method for the determination of acid values in olive oil without titration. Journal of the American Oils Chemists' Society 75, 1849–1853.
Vigli, G., Philippidis, A., Spyros, A., & Dais, P. (2003) Classification of edible oils by employing 31P and 1H NMR spectroscopy in combination with multivariate statistical analysis. A proposal for the detection of seed oil adulteration in virgin olive oils. Journal of Agriculture and Food Chemistry 51, 5715–5722.
Vlahov, G. (1999) Application of NMR to the study of olive oils. Progress in Nuclear Magnetic Resonance Spectroscopy 35, 341–357.
Volin, P. (2001) Analysis of steroidal lipids by gas and liquid chromatography. Journal of Chromatography A 935, 125–140.
Wanasundara, U.N., Shahidi, F., & Jablonski, C.R. (1995) Comparison of standard and NMR methodologies for assessment of oxidative stability of canola and soybean oils. Food Chemistry 52, 249–253.
Wang, K., & Glaze, W.H. (1998) High-performance liquid chromatography with post column derivatization for simultaneous determination of organic peroxides and hydrogen peroxide. Journal of Chromatography A 822, 207–213.
Wheeler, D.H. (1932) Peroxide formation as a measure of autoxidative deterioration. Oil and Soap 9, 89–97.
Yang, Y., Ferro, M.D., Cavaco, I., & Liang, Y. (2013) Detection and identification of extra virgin olive oil adulteration by GC-MS combined with chemometrics. Journal of Agriculture and Food Chemistry 61, 3693–3702.
Yasuda, M., & Narita, S. (1997) Simultaneous determination of phospholipid hydroperoxides and cholesteryl ester hydroperoxides in human plasma by high-performance liquid chromatography with chemiluminescence detection. Journal of Chromatography B: Biomedical Applications 693, 211–217.
Yildrim, G. (2009) Effect of storage time on olive oil quality. Doctoral dissertation, İzmir, Institute of Technology.
Zarrouk, W., Haddadai, F.M., Baccourin, B., Queslatin, I., & Taamallin, W. (2007) Characterization of virgin olive oil from southern Tunisia. European Journal of Lipid Science and Technology 110, 81–88.

29 Detection of extra virgin olive oil adulteration

Hazem Jabeur, Akram Zribi, and Mohamed Bouaziz

29.1 Introduction

Virgin olive oil is a valuable plant oil extracted from fresh and healthy olive fruits (*Olea europeae* L.) by mechanical processes (pressing or centrifuging) and without heat, solvents, or any preliminary refining (García & Yousfi, 2006; Ammar *et al.*, 2014a). Therefore, it is practically the only vegetable oil that can be consumed directly in its raw state. Extra virgin olive oil (EVOO) is considered as the best olive oil for its superior organoleptic characteristics (aroma and taste). It has potential health benefits, and remarkable antioxidant properties and chemical composition (Méndez & Falqué, 2007; Jafari *et al.*, 2009; Ammar *et al.*, 2014b). Since EVOO is a premium food product requiring a relatively high price, it is a target for adulteration with less expensive vegetable oils. The most common adulterants found in EVOO are refined olive oil; seed oils, such as sunflower, corn, soybean, and rapeseed oils (Guimet *et al.*, 2005); as well as nut oils, including peanut and hazelnut oils (Blanch *et al.*, 1998). These are used as ingredients in several foods, cooking oils, salad oils, and fried foods. Given the difference in price between EVOO and other edible oils, adding cheaper oils to virgin olive oil has become a common practice for profit. This practice is, however, harmful since consumers buy olive oil for its health benefits (Kafatos & Comas, 1991) and are badly deceived when they receive oil that does not provide them with what they seek. Authenticity covers many aspects, including adulteration, mislabeling, mischaracterization, and misleading origin (Frankel, 2010). Therefore, the detection of edible oil adulteration is crucial in food quality, safety control, and the vegetable oil product trade. Monitoring the authenticity of EVOO is carried out using instrumental techniques that provide data about their qualitative and quantitative composition.

Most of the current work on edible oil adulteration is based on chromatographic analysis, namely gas chromatography (GC), high-performance liquid chromatography (HPLC), and gas or liquid chromatography coupled with mass spectrometry (GC-MS and LC-MS). However, these separating techniques have been complemented with, or substituted by, many other modern fingerprinting techniques, such as Fourier transform near-infrared (FT-NIR) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, FT-Raman spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, dielectric spectroscopy (DS), differential scanning calorimetry (DSC), and total synchronous fluorescence spectroscopy (TSyFS). Added to that, a common strategy of these methods is to conduct multivariate statistical analyses of the contents of various components, such as fatty acids and triacylglycerols (TAGs) as well as sterols, and classify samples of different botanical origins using chemometric tools.

Other fingerprinting molecular spectroscopy techniques have been developed and used in recent years. The advantages of the spectroscopy techniques are the negligible sample preparation, the small amounts of organic solvents or reagents used, the noninvasive approach, the relatively easy and quick data acquisition, and the possibility to provide information on a wide range of components in a single experiment maintaining the natural ratio of the substances (Vigli *et al.*, 2003; Berrueta *et al.*, 2007).

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It is in this context that the present chapter examines the effectiveness of both single and coupled (combined) techniques with various analytical instruments to detect the adulteration of EVOO. Here, two main parts are considered: in Section 29.2, the evaluation of authenticity of EVOO followed by various chemical parameters of purities using several sophisticated analytical instruments to detect potential adulterants; and, in Section 29.3, other specific methods are discussed that have been used not only to classify directly oil samples according to botanical origin but also to determine the composition of binary mixtures of EVOO with cheaper oils.

29.2 Parameters suitable for authenticity assessment of EVOO

Olive oil is usually more expensive than other edible oils, which makes it a candidate for adulteration with other cheaper oils. To assess the authenticity of EVOO, it is fundamental to know the technologies applied, the fat modification techniques used, and the chemical composition of the authentic olive oil and that of the potential adulterants. Therefore, different methods have been developed to detect falsification perpetrated.

The central problem for the authenticity assessment of EVOO is to define one or more parameters within the lipid fraction, which allows checking for the identity and purity of the specified olive oil. Ideally, such markers are chemical compounds present in the adulterant oil (soybean, corn, or sunflower oil) and absent in the original one (olive oil). However, very often marker substances are not totally absent in olive oil but present only in concentrations different from that in the adulterated oil. Therefore, the profiles of authentic oils must be compared with the olive oil to be tested.

Chemically, there are two parts of compounds (saponifiable and unsaponifiable matter). In the saponifiable matter, the main constituents of vegetable oils are triacyglycerols (TAGs). The TAGs of EVOO contain mixtures of palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic acids and traces of myristic, arachidic, heptadecanoic, and eicosanoic acids. Other constituents include partial acylglycerols (diacylglycerols [DAGs] and monoacylglycerols [MAGs]) and esters of fatty acids with saturated fatty alcohols of linear chain. The unsaponifiable matter, which makes up around 2% of all oils, includes many chemical substances of a very different structure, such as hydrocarbons, tocopherols, pigments, sterols, alcohols, terpenic dialcohols, volatile compounds affecting aroma and flavors, phenolic acid, and flavonoid compounds and proteins.

The detection and determination of the adulteration of EVOO are not simple tasks. Actually, they traditionally require the monitoring of several organic compounds to establish a comparison with typical unadulterated oils so as to identify the change of composition that could be related to adulteration. In this respect, the detection of the characteristics of the chemical components has been proposed as a suitable indication for the presence of other oils in EVOO.

The purity control of EVOO is becoming more stringent, and strict laws are being enforced, especially for avoiding adulteration. The public bodies that are responsible for the prevention of the adulteration of foodstuffs necessitate methods of analysis that could facilitate large-scale *in situ* controls.

29.2.1 Adulteration within fatty acids

The TAGs are composed of three fatty acids attached to a glycerol backbone. Actually, it is the well-balanced fatty acid composition that confers to olive oil its high nutritional values. The composition of the fatty acids of EVOO also depends on several factors, such as soil, climate, processing, harvesting, and changes occurring during storage (Dyer *et al.*, 2008), as well as using peak areas for quantitative analysis without employing an internal standard. The fatty acids of edible oil have always been one of the main issues encountered when dealing with the oil origin and for detection of mixtures, although the wide variation in edible oils from different geographical origins is a limiting factor in the interpretation of data with regard to adulteration (Aparicio & Aparicio-Ruíz, 2000).

The official method for the separation and quantification of cis- and trans-fatty acids from oils involves capillary gas chromatography (GC) analysis, with a high-polar stationary phase (cyanopropyl polysiloxane) and flame ionization detection (FID), after being converted into fatty acid methyl esters (FAMEs) by

alkali/catalyzed transesterification of vegetable oils. FAMEs are quantified according to their area percentage, obtained by the integration of the peaks. The results were expressed as percentages of individual fatty acids in the lipid fraction (International Olive Council [IOC], 2001a, 2001b).

In order to evaluate the possibility of detecting Chemlali EVOO adulteration with low-cost seed oils, Jabeur *et al.* (2014) prepared various blends of EVOO and soybean, corn, or sunflower oil and analyzed their fatty acids. The adulteration percentages ranged from 1 to 10% to determine a threshold of detection. Fatty acids composition as an indicator of purity suggests that linolenic acid content could be used as a parameter for the detection of EVOO fraud with 5% soybean oil. The adulteration could also be detected by the increase of the trans-fatty acid contents with 3% soybean oil, 2% corn oil, and 4% sunflower oil (Jabeur *et al.*, 2014). It is important to note that most studies based on the compositions of fatty acids have only focused on the detection of adulterations without considering the type of vegetable oil involved.

For determination of the percentage of olive oil in a blend with other types of plant oils (peanut, rice, corn, and grapeseed oils), analysis of FAMEs by GC-FID, followed by chemometric tools (principal component analysis [PCA], target factor analysis [TFA], soft independent models of class analogy [SIMCA], and partial least squares [PLS]), is used. This method leads to the construction of models capable of verifying and recognizing the percentage of olive oil in a binary blend. Good classification models are obtained by adding blends containing 45 and 55% olive oil. In this case, the best results are achieved by applying SIMCA to separate the data sets of binary blends rather than to the overall data set (Monfreda *et al.*, 2014).

Nuclear magnetic resonance (NMR) spectroscopy has become the preeminent technique for determining the structure of organic compounds. The basic information given by NMR is a spectral line characterized by its spectral position (given in parts per million [ppm] relative to a reference frequency) and its intensity. The line intensity is measured as the area under the spectral line and reflects accurately the number of equivalent nuclei in the environment (Mannina *et al.*, 2003). In order to evaluate the composition of the oils and to differentiate between them, ^{1}H and ^{13}C NMR resonance groups are integrated and their percentage from the total signal is estimated.

The ^{1}H and ^{13}C NMR analyses are used to determine the saturated fatty acids, oleic acid, linoleic acid, linolenic acid, and iodine value. The ^{1}H, ^{13}C integrals, and fatty acids concentrations of the 0, 0.5, 1, 5, and 100% olive oil in sunflower oil mixtures were used to make calibration curves (percentage of olive oil in function of the ^{1}H signal/^{13}C signal/fatty acid concentration). These calibration curves were tested by using a sample at 1% (w/w). Popescu *et al.* (2015), using the ^{1}H NMR spectrum, reported 2.77 ppm (—CH═CH—C$\underline{H_2}$—CH═CH− of linoleyl and linolenyl) and 2.03 ppm (—C$\underline{H}$2—CH═CH− of unsaturated fatty acids) are obtained; and from the ^{13}C NMR spectrum, 130.22 (C9 of linoleyl and linolenyl) and 29.42 ppm (CH_2) are obtained in their study. Table 29.1 summarizes the assignment of the principal resonances in the ^{1}H and ^{13}C NMR spectrum of vegetable oil.

NMR is a fast method, faster than most of the techniques used in oil analysis, and it requires only a simple sample preparation. It also enables the recording of more or less all constituents of a complex mixture in a single experiment. This feature can be used for fast screening of a large number of samples and the development of a database for authentic products.

Capote *et al.* (2007) investigated different kinds of adulterants (sunflower, corn, peanut, and coconut oils) in olive oil. However, despite its effective identification, only eight different samples of pure olive oil were considered, which could not eliminate the variability between them (Aguilera *et al.*, 2005; Gargouri *et al.*, 2013). In these, the peak areas were used to quantify without the use of any internal standard.

A semiquantitative method using ^{13}C NMR in the olefinic region (127.5–130 ppm) was reported to detect the presence of plant oils (cottonseed, sunflower seed, soybean, and corn oils) in EVOO, which affected the intensities of 12 peaks and the α/β ratios of oleic acid (1.1) and linoleic acid (1.5) (Mavromoustakos *et al.*, 2000).

Gamazo-Vázquez *et al.* (2003) determined FAMEs after saponification and derivatization by capillary GC coupled to mass spectrometry (GC-MS). This method can be used in bottling plants to efficiently discriminate between pure olive oil and olive oil adulterated by other oils at low percentages. The optimal discriminatory parameter is the ratio between the peak areas of the oleic and linoleic derivatives in the chromatograms obtained using full-scan MS between 35 and 350 amu. This ratio would allow the diagnosis of the contamination of olive oil with sunflower oil at least at the 1% level with >95% certainty in bottling plants.

Table 29.1 Assignment of the main resonances in the ^{1}H and ^{13}C NMR spectrum of vegetable oil.

Chemical shift (ppm) (no.)	^{1}H	Compound
0.87 (1)	$-CH_2-CH_2-CH_2-C\underline{H}_3$	All acids except linolenyl
1.02 (2)	$-CH{=}CH-CH_2-C\underline{H}_3$	Linolenyl
1.30 (3)	$(C\underline{H}_2)n$	All acyl chains
1.62 (4)	$-C\underline{H}_2-CH_2-COOH$	All acyl chains
2.03 (5)	$-C\underline{H}_2-CH{=}CH-$	All unsaturated fatty acids
2.32 (6)	$-C\underline{H}_2-COOH$	All acyl chains
2.77 (7)	$-CH{=}CH-C\underline{H}_2-CH{=}CH-$	Linoleyl and linolenyl
4.22 (8)	$-C\underline{H}_2-OCO-R$	Glycerol (triacylglycerols)
5.26 (9)	$C\underline{H}-OCO-R$	Glycerol (triacylglycerols)
5.37 (10)	$-C\underline{H}{=}C\underline{H}-$	All unsaturated fatty acids
Chemical shift (ppm)	**^{13}C**	**Compound**
14.07–14.28	C18 (ω1)	All acyl chains
20.56–22.70	C17 (ω2)	All acyl chains
24.49–24.89	C3	All acyl chains
25.56–25.72	C11	Linoleyl and linolenyl
	C14	Linolenyl
27.22–27.38	C8	Oleyl and linoleyl
	C11	Oleyl
29.42	C4–C7	All acyl chains
	C12–C15	Oleyl
	C8–C15	Stearoil
	C8–C13	Palmitoil
31.55–31.94	C16 (ω3)	Linoleyl
34.06–34.20	C2, sn-2	All acyl chains
62.12	CH_2O-, sn-1,3	Glycerol (triacylglycerols)
	CH_2O-, sn-1	Glycerol (1,2-diacylglycerols)
65.07	CH_2O-, sn-1	Glycerol (monoacylglycerols)
68.93	$CHO-$, sn-2	Glycerol (triacylglycerols)
77.01	CDCl3	Solvent
127.13–127.92	C12	Linoleyl
	C10, C15	Linolenyl
128.10–28.47	C10	Linoleyl
	C12, C13	Linolenyl
129.49–129.70	C9, C10	Oleyl
130.02–130.45	C9	Linoleyl and linolenyl
	C13	Linoleyl
172.81	C1, sn-2	Triacylglycerols
173.23	C1, sn-1,3	Triacylglycerols

Ozen *et al.* (2003) successfully classified adulterated and pure oil and the detection limit for adulteration. FTIR and common chemometric techniques (including discriminant analysis [DA], Mahalanobis distances, and Cooman plots) were used to classify various types of dietary supplement oils (DSOs) and cheaper edible oils. These analyses indicated that the developed FTIR method and chemometric analysis are very useful for quantifying the adulterant oil added to the DSO at the 2–20% (v/v) level.

29.2.2 Triacylglycerols

TAGs generally account for 95–98% (w/w) of vegetable oils and show a characteristic distribution. As a consequence, the addition of other edible vegetable oils to olive oils modifies TAG distribution, and because of that, they are considered to be good fingerprints for adulteration detection purposes (Aparicio & Aparicio-Ruíz, 2000).

The reverse-phase high-performance liquid chromatography (RP-HPLC) quantitative analysis of TAGs is considered as an effective method for the detection of EVOO adulteration (De la Mata-Espinosa *et al.*, 2011). In this case, the stationary phase usually consists of a nonpolar octadecylsilane (C_{18}) bonded phase, while the mobile phase is a polar solvent (acetonitrile/acetone [50:50, v/v]). The refractometer detector is most appropriate for quantitative analysis carried out using thermostated cells and isocratic elution. The advantage of using a TAG profile includes the distribution of fatty acids between the different stereospecific positions on the glycerol molecule. TAGs are separated according to the equivalent carbon number (ECN) and the positions of double bond(s). Until recently, the most prominent methods to detect the adulteration of EVOO with other vegetable oils was the trilinolein (LLL) content and the difference between the theoretical value of TAGs with an equivalent carbon number of 42 ($ECN42_{theoretical}$). An appropriate software is used to compute the $\Delta ECN42$ based on data of the fatty acids composition and analytical triacylglycerol results ($ECN42_{HPLC}$) (IOC, 2010; García-González *et al.*, 2007). Better results were obtained by Jabeur *et al.* (2014) using $\Delta ECN42$, which proved to be effective in the Chemlali EVOO adulteration at levels as low as: 1% sunflower oil, 3% soybean oil, and 3% corn oil. TAG compositions of EVOO and the adulterated EVOO oil mixed with 10% (w/w) are depicted in Figure 29.1.

High-performance liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry (HPLC-APCI-MS) was used to determine the adulteration of EVOO with 10–50% hazelnut oil based on TAG composition and non-TAG components (Parcerisa *et al.*, 2000). Discriminant analysis (DA) showed that hazelnut oil and mixtures with olive oil were clearly separated according to their TAG composition.

Lerma-García *et al.* (2011) developed a new method for the determination of TAGs in vegetable oils from different botanical origins by using HPLC with ultraviolet-visible (UV-Vis) detection. Using a core–shell particle packed column (C_{18}, 2.6 μm), isocratic elution with acetonitrile/*n*-pentanol at 10 °C and within a total analysis time of 15 min was achieved. Using mass spectrometric detection, a total of peaks, which were common to the oils of six different botanical origins (corn, EVOO, grapeseed, hazelnut, peanut, and soybean oils), were identified. These peaks were used to construct linear discriminant analysis (LDA) models

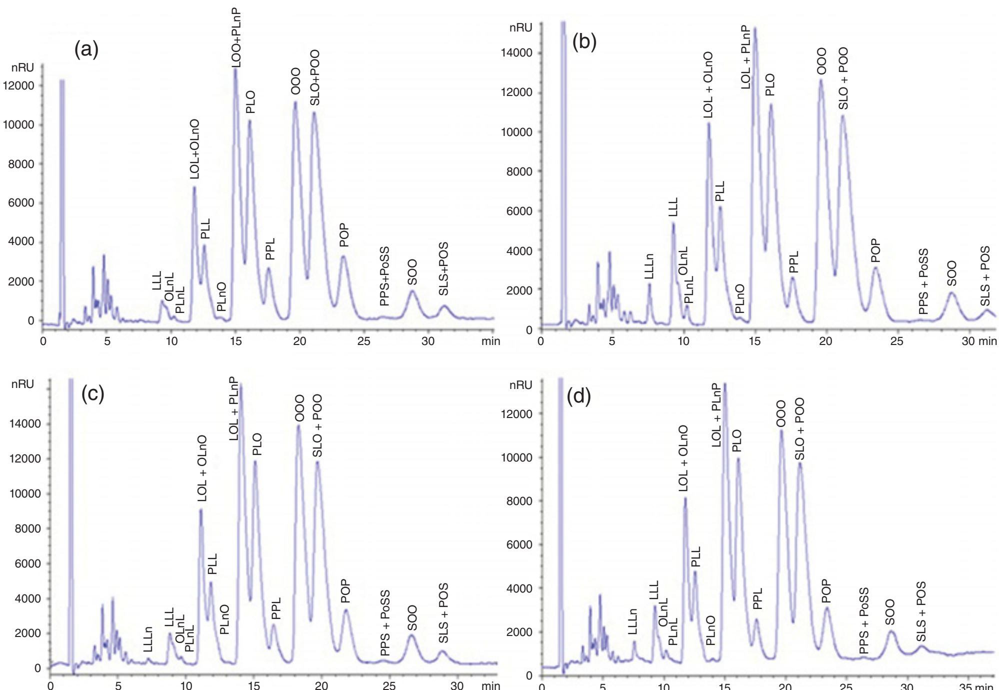

Figure 29.1 Chromatograms showing the (a) TAG profiles of Chemlali EVOO; and binary mixtures containing 90% EVOO and 10% of either (b) soybean, (c) sunflower, or (d) corn oils using HPLC-RID.

for botanical origin prediction. Afterward, and in order to evaluate the possibility of detection of EVOO adulteration with low-cost oils, binary mixtures containing 90% EVOO with 10% of either corn, grapeseed, hazelnut, peanut, or soybean oils were prepared. When EVOO was adulterated with grapeseed oil, the relative areas of OLL (oleic-linoleic-linoleic), LLP (linoleic-linoleic-palmitic), and particularly LLL largely increased. Similarly, the presence of hazelnut oil was evidenced by an increase of the areas of LLL and OLL. The adulteration with corn oil resulted in a large increase in the areas of LLL, OLL, and LLP. When peanut oil was present, the areas of LLL, OLL, LLP, and LLBe increased largely. Finally, the adulteration with soybean oil produced a large increase in the areas of LLLn (linoleic-linoleic-linolenic), LLL, OLLn (oleic-linoleic-linolenic), OLL, and LLP. Therefore, adulteration of EVOO with small percentages of other oils was clearly proven in all cases, although with a moderate sensitivity for hazelnut oil (Lerma-García *et al.*, 2011).

Cunha and Oliveira (2006) used HPLC–evaporative light scattering detection (ELCD) to determine TAG composition in oils. The chromatographic separation was achieved using a Kromasil 100 C_{18} column (at 25 °C) and gradient elution with acetone and acetonitrile. After the methodology implementation and validation, it was applied to the study of the TAG profiles of eight plant oils (sunflower, corn, peanut, soybean, hazelnut, walnut, sesame, and olive oil). A categorical principal component analysis (CATPCA) was performed to simplify the data from TAG profiles of vegetable oils, and to easily distinguish vegetable oils except hazelnut oil. These oils can be differentiated from the others by their high levels of triolein (OOO). Sunflower and walnut oils are discriminated from the other oils, mainly with LLL and palmitodilinolein (PLL) parameters, which also permit the differentiation between both of them. On the other hand, the other plant oils (peanut, corn, sesame, and soybean) have different profiles mainly pertaining to the contents of PLO (palmitic-linoleic-oleic), SPO (stearic-palmitic-oleic), and POP (palmitic-oleic-palmitic). Furthermore, the determination of TAG composition may be an important parameter for detecting the adulteration of such products during purity control.

The optimization and application of methods of triacylglycerol evaluation for characterization of olive oil adulteration by soybean oil with HPLC-APCI-MS/MS (tandem MS) have been developed by Fasciotti and Pereira-Netto (2010). HPLC separation was also carried out using an octadecylsilica LiChrospher column (250 mm × 3 mm; 5 μm) and a gradient composed of acetonitrile and 2-propanol. APCI-MS run in positive mode and an ion trap mass analyzer were applied in the study of olive and soybean oils and their mixtures. Multiple reaction monitoring (MRM) employing the transition of protonated TAG molecules ($[M+H]^+$) to the protonated diacylglycerol fragments ($[M+H-R]^+$) improved the selectivity of TAG detection and was used for quantitative analysis. The quantitative studies were based on the estimates of mixtures of soybean oil and olive oil proportions by comparison of TAG areas found in the mixtures of both oils. Good agreement with expected or labeled values was found for a commercial blend containing 15% (w/w) olive oil in soybean oil and in a 1:1 mixture of both oils, showing the potential of this method in characterizing oil mixtures and estimating oil proportions. Olive oils of different origins were also evaluated by mass spectral data obtained after direct injection of oil solutions and PCA.

A reliable procedure for identification and quantification of adulteration of olive oils in terms of blending with other vegetable oils (sunflower, corn, seeds, sesame, and soybean) was developed by Ruiz-Samblás *et al.* (2012). The high-temperature GC method proposed, chemometric class-modeling techniques such as SIMCA, and quantification techniques PLS and genetic algorithms (GA-PLS) with feature selection appear to be appropriate tools to verify the percentage of olive oil in blends with vegetable oils; and they could become an important instrument to verify the labeling compliance and for quality control in the detection of adulteration. Indeed, reliability of the proposed qualification model is very high as the kind of vegetable oils used for blending was correctly identified for all samples. Moreover, reliable quantification models were built for each of the different blend kinds. Lastly, for the possibility of quantifying the purity of oil samples regardless of the adulterating vegetable oil, promising results were obtained by applying PLS (on the entire chromatogram or with GA variable selection) to the whole data set without preliminary classification of the oils.

29.2.3 Sterols

Sterols are part of the unsaponifiable matter and are found in almost all fats and oils, including EVOO. They are also characteristics of the genuineness of vegetable oils (Gargouri *et al.*, 2015). Indeed, several

phytosterols are present in virgin olive oil, mainly β-sitosterol, which is the dominant member of the total sterol fraction (Kiritsakis, 1998). In general, sterols are useful markers and fingerprinting components for assessing authenticity of oils. Considering that β-sitosterol is the most abundant in a majority of oils, its value has only limited use for the authenticity assessment and differentiation of vegetable oils. However, it has been shown to be useful for tracing vegetable oils in the fats of animal origin, as the latter contains cholesterol as the primary sterol.

While EVOO has elevated levels of β-sitosterol and Δ5-avenasterol, safflower and sunflower oils contain significant levels of Δ7-stigmastenol, whereas soybean and corn oils have high levels of campesterol and stigmasterol, yet brassicasterol is mainly present in rapeseed and mustard seed oils. These apparent differences make them most suitable for determining the botanical origin of oils and, hence, for detecting the adulteration of olive oil with other cheaper or lower quality vegetable oils (Bohačenko *et al.*, 2001; Galeano-Diaz *et al.*, 2005). Thus, the composition of the sterol fraction of olive oil is a very useful parameter for detecting the adulteration or to check authenticity, since it can be considered as a fingerprint.

Chromatographic methods are currently most widely used for qualitative and quantitative analyses of this extensive series of compounds clustered in 4-demethylsterols or simply sterols. Indeed, gas chromatography of unsaponifiable matter is the most prevalent technique.

An official method for the isolation of total sterols from EVOO and EVOO adulteration implies the saponification of the oil, extraction of the unsaponifiable matter with diethyl ether, and washing of the extract with water. The extract is fractionated by thin-layer chromatography (TLC) on silica gel. Injection is usually performed after derivatization with a silating reactant. Capillary columns give the best performance since they can resolve the sterols almost completely (IOC, 2001c).

A recent study by Jabeur *et al.* (2014) reported that the sterols profile is almost decisive in clarifying the adulteration of olive oils with cheaper oils; 1% sunflower oil could be detected by the increase of Δ7-stigmastenol, and 4% corn oil by the increase of campesterol. Moreover, they have confirmed that linear discriminant analysis (LDA) could represent a powerful tool for faster and cheaper evaluation of EVOO adulteration (Figure 29.2).

Al-Ismail *et al.* (2010) were able to detect the adulteration of virgin olive oil with some refined vegetable oils by direct sterol analysis using gas–liquid chromatography (GLC) equipped with a polar column and high thermal stability. This method is based on the determination of the sum of campesterol and stigmasterol percentages. Mixtures of corn, soybean, sunflower, and cottonseed oils in olive oil at levels of 5, 10, and

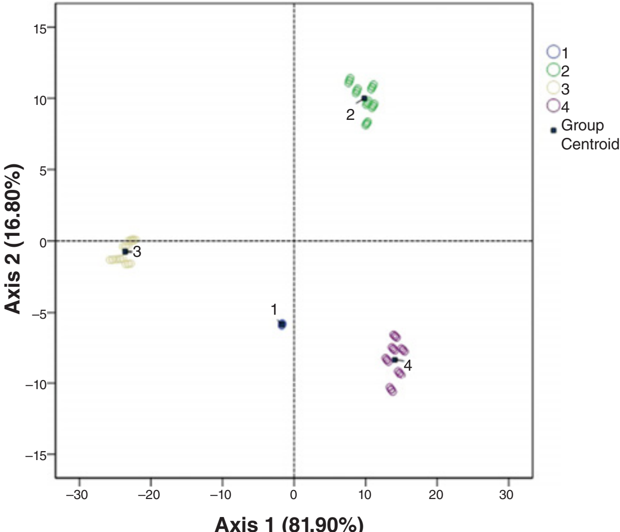

Figure 29.2 LDA score plot of pure EVOO and adulterated EVOO based on all the analyses performed with four determinations. (1) EVOO: extra virgin olive oil; (2) EVOO + (1–10%) of soybean oil; (3) EVOO + (1–10%) of corn oil; and (4) EVOO + (1–10%) of sunflower oil.

20% were studied. An olive oil authenticity factor based on the summation of campesterol and stigmasterol percentages was established as an indicator of olive oil adulteration with vegetable oils. The results indicate the possibility to detect the addition of these vegetable oils in olive oil at less than 5%.

For identification of brassicasterol in olive oil, GC–electron ionization–MS (GC-EI-MS) of their sterols has been used. Brumley *et al.* (1985) detected the presence of brassicasterol in EVOO by the addition of rapeseed or canola oil, taking into account that the content of brassicasterol is $\leq$0.1% for olive oil, while it is $\geq$12 and 5% in rapeseed and canola oils, respectively.

Grob *et al.* (1994a) detected the adulteration of virgin olive oil with plant oils (rapeseed, soybean, sunflower, and grapeseed) by direct analysis of the sterols using on-line coupled LC-GC-FID. The addition of approximately 2% rapeseed oil was detected by determining the content of brassicasterol. The addition of 10% soybean oil was detected by determining the concentration of campesterol and stigmasterol. Besides, the contamination of olive oil by sunflower oil higher than 5% could be detected by the increase of campesterol, stigmasterol, and especially Δ7-stigmastenol. Adulteration with 10% or more of grapeseed oil would increase the concentration of campesterol and stigmasterol.

A strategy to avoid the detection of the adulteration of olive oil with sunflower oil is the elimination of the Δ7-sterol fraction by refining under extreme conditions (oils treated strongly with bleaching earth). However, in the course of this process, the Δ5-sterols are dehydrated to sterenes and Δ7-sterols undergo isomerization to Δ8 (14) and Δ14-sterols. The determination of Δ8 (14) and Δ14-sterols has been proposed for detecting desterolized high-oleic sunflower oil in olive oil, since these sterols arise from the isomerization of Δ7-stigmastenol. After elimination of a majority of β-sitosterol by means of TLC on silica gel plates, these reaction products can be detected by GC equipped with a polar column (70% phenylmethylsilicone) (Mariani & Bellan, 2011).

29.2.4 Stigmasta-3,5-diene

In comparison with EVOO, refined vegetable oils are characterized by stigmasta-3,5-diene; the origin of this hydrocarbon, which has been studied by Cert *et al.* (1994), is the dehydration of β-sitosterol during the refining of edible oils and fats (see the mechanism of sterol dehydration in Figure 29.3). This hydrocarbon is very useful in detecting the presence of refined oil in crude oils. Many vegetable oils are refined by different steps, including bleaching and deodorization, by treatment with acid bleaching earth and steaming at high temperatures. These processes dehydrate the sterols present in the oil to a series of steroidal hydrocarbons or sterenes (Cert *et al.*, 1994). The major plant sterol is β-sitosterol from principally 24-ethylcholesta-3,5-diene

Figure 29.3 Mechanism of sterols dehydration.

(stigmasta-3,5-diene) and lesser quantities of its positional isomers. Other sterols form similar compounds: for example, 24-methylcholesta-3,5-diene (campesta-3,5-diene) is from campesterol, and 24-ethylcholesta-3,5,22-triene (stigmasta-3,5,22-triene) is formed from stigmasterol.

Currently, adulteration with refined vegetable oils is a major issue in the EVOO market. The stigmastadiene test is the most effective means of detecting this type of adulteration.

The official methods for determination of stigmastadiene in virgin olive oil have been adopted by the IOC (2001d) with an application limit between 0.01 and 4 mg kg^{-1}. The isolation of steroidal hydrocarbons in virgin olive oil is usually performed using low-pressure column chromatography on the silica gel of the unsaponifiable matter of 20 g of oil, using *n*-hexane as eluent. For determination of stigmastadiene in virgin olive oil, the first fraction eluted from the column is discarded, and the second one is analyzed by GC on a fused-silica capillary column coated with 5% phenylmethylpolysiloxane and equipped with a FID detector. Stigmastadiene is quantified by the comparison of the combined peak area with that of a single point addition of an internal standard of cholestadiene (IOC, 2001d). Steroidal hydrocarbons in refined oils are found in higher concentrations than in crude oils, and the saponification step can be avoided. The oil dissolved in hexane is directly fractioned on a silica gel column, and the corresponding fraction analyzed (IOC, 2001e).

In this context, Crews *et al.* (2014) described a rapid GC-MS method for the determination of stigmastadiene, which is faster and more sensitive than the current official procedure, based on GC-FID. This method does not require a saponification step for cold-pressed oils, but rather uses a stigmastadiene standard for quantification. It has a low limit of quantification (0.015 mg kg^{-1}) and gives excellent confirmation of peak identity at the current regulatory limit of 0.5 mg kg^{-1}. The availability of selected ion monitoring allows the detection of other sterenes in vegetable oils, which can help in identifying the source of added plant oils. By simple inclusion of additional fragment ions, other sterenes can be monitored. For example, monitoring of the response at m/z 394 and measuring against the available standard allow the quantification of stigmasta-3,5,22-triene, and monitoring of the response at m/z 381 allows the detection of campesta-3,5,22-triene derived from refined rapeseed oil.

The 3,5-steradienes compounds are the main dehydration products of Δ5-sterols, but other isomers are also formed along with the degradation products of Δ7-sterols, methylsterols, and triterpenic alcohols. In order to identify these minor compounds, new isolation procedures have been employed by Grob *et al.* (1994b), using on-line HPLC-GC-MS on silica gel columns to identify 3,5-, 2,4-, and 2,5-steradienes; the 3,5-cyclo-6-enes; and 2,4,6-trienes. The first column separates the sterenes (except squalene) from the triacylglycerols and other polar compounds, and the second one separates different fractions identified with UV detection: stereradienes at 235 nm and steradiene at 309 nm.

Jabeur *et al.* (2015b) recently showed that the profile of sterenes is almost decisive in clarifying the contamination of EVOO with some cheaper refined plant oils. The use of stigmasta-3,5-diene proved to be more effective in detecting even low levels of adulteration of Chemlali EVOO with most of the refined vegetable oils under study. The determination of stigmasta-3,5-diene can be used as a parameter for the detection of EVOO fraud with each studied refined oil: 2% olive (0.081 ppm > 0.05 ppm), 0.4% pomace olive (0.062 ppm > 0.05 ppm), 1% palm (0.063 ppm > 0.05 ppm), 0.2% soybean (0.069 ppm > 0.05 ppm), 0.5% sunflower (0.062 ppm > 0.05 ppm), and 0.1% corn (0.065 ppm > 0.05 ppm) oils.

29.2.5 Fatty acid alkyl esters

Fatty acid alkyl esters (FAAEs), mainly fatty acid ethyl esters (FAEEs) and FAMEs, represent the family of natural neutral lipids present in olive oils and formed by the esterification of free fatty acids (FFAs) with low molecular alcohols, such as methanol and ethanol. They can easily occur in an acid medium and are catalyzed by the presence of certain enzymes.

Blends between EVOO and mild thermal deodorized oil do not produce the easily detectable modifications of the chemical composition. Indeed, the parameters usually checked as quality indicators, such as TAG composition, sterols, and newly formed steroid hydrocarbons, are not appreciably altered by blending (Saba *et al.*, 2005). The most reliable technique seems to be the determination of FAAEs and FAMEs, which are present in the waxy fraction of olive oils (Biedermann *et al.*, 2008). In good-quality EVOOs, FAMEs and FAEEs are present in very small amounts, yet they are present in higher amounts in lampante virgin olive oils (Mariani & Bellan, 2011) and in the second olive-processing oil (the so-called *repaso*) (Cerretani *et al.*, 2011).

Saba *et al.* (2005) identified 9(*E*), 11(*E*) octadecadienoate (C18:2) in the FAMEs by means of GC coupled to acetonitrile CI-MS and CI-MS/MS of some extra virgin and deodorized olive oils. From the obtained results, the conjugated linoleic acid methyl ester formation is related to the heating temperature and time in olive oil samples; therefore, it may represent a good marker for the detection of deodorized oil addition to EVOO at a very low level.

An analytical procedure for quantitative determination of FAAEs together with squalene in vegetable oils has been developed (Pérez-Camino *et al.*, 2002). The fraction containing these compounds was isolated from the oil by solid phase extraction (SPE) on silica gel cartridges and then quantitatively analyzed by GC. This method was applied to extra and lampante virgin olive oil categories as well as to oils obtained from olive pomace by second centrifugation and solvent extraction. EVOO oils contained low amounts of FAMEs and FAEEs, while oils obtained from altered olive or olive pomace showed high concentrations of FAAEs, mainly ethyl esters. The correlation between oil acidity and ethyl esters concentration was poor.

The influence of soft deodorization on the composition of FAMEs and FAEEs in olive oils was investigated by Pérez-Camino *et al.* (2008) using GC. FAAEs can be considered as good markers of low-quality olive oil subjected to soft deodorization (Jabeur *et al.*, 2015a). From all data analyses on the studied oils, it can be confirmed that the virgin olive oil contains less than 70 mg/kg (mean value, 24.3) FAAEs and a FAEE–FAME ratio lower than 2. Other oils that comply with the analytical requirements actually established for EVOOs and having higher FAAEs and FAEE–FAME ratios are suspected of being subjected to soft deodorization. The total amount of methyl and ethyl esters of fatty acids (FAAEs) and the ratio between ethyl and methyl esters (i.e., the ratio of FAEEs to FAMEs) of the adulterated EVOO mixed with 1–50% (w/w) mild deodorized olive oil are summarized in Table 29.2.

The fast Fourier transform mid-infrared (FT-MIR) spectroscopy combined with PLS methodology has been used by Valli *et al.* (2013) for predicting the level of low-quality virgin olive oil adulteration in EVOO. Results were statistically similar to the official procedure (IOC, 2009) in terms of analytical performance for the total amount of FAMEs and FAEEs and the ratio between ethyl and methyl esters in EVOO; there was a good agreement between the predicted and actual values on calibration data sets was 0.98 and 0.83, respectively, and the limit of quantification was low enough (29.3 mg kg^{-1}), the actual limits for Σ (FAMEs + FAEEs).

29.2.6 Adulteration with copper–chlorophyll

Carotenes present in olive oil are responsible for its yellow color, while chlorophylls are responsible for the greenish color. The presence of these pigments in olive oil not only determines its color but also plays an important role in its oxidative stability due to their antioxidant activity in the dark and pro-oxidant activity in the light (Kiritsakis, 1998; Ouesleti *et al.*, 2009).

Some oils marked as virgin olive oils have been found to contain synthetic pigment Cu–chlorophyll (E141) or other green pigments, added illegally. An analytical method for identification and quantification of Cu–chlorophyll adulteration in edible oils has been developed by Fang *et al.* (2015). High-resolution MS with a high-performance liquid-quadripole (HPLC-Q)-Orbitrap system and HPLC coupled with photodiode-array detector analyses are applied to a survey of E141 pigment in commercial plant oils, including olive pomace oil, EVOO, olive oil, grapeseed oil, and blended oils. From all data analyses on the studied oils, it can be confirmed that the presence of Cu–chlorophyll derivatives is indicative of fraudulent adulteration of oils.

29.3 Direct authenticity assessment of EVOO

To prevent oil adulteration, antifraud controls require that specific tests be performed to assess the quality of EVOOs, which usually rely on highly sophisticated and expensive methods. The chemical methods officially employed for the control of authenticity of virgin olive oil, such as GC and HPLC, are expensive and time-consuming and require skilled operators.

New and complementary analytical techniques devoid of such troubles could act as supporting tools for currently used methods. Among them, calorimetric techniques seem to be very promising, and the application of DSC to make evident the adulteration of EVOO has been reported by Chiavaro *et al.* (2008). The

Table 29.2 Content of fatty acid ethyl esters in the mixtures of extra virgin olive oil with deodorized olive oil (DO).

	Grams of DO added to EVOO in 100 g of oil mixture									
	0	**1**	**5**	**10**	**15**	**20**	**30**	**40**	**50**	**100**
FAMEs (ppm)	6.80±0.61	8.20±0.34*	16.20±0.58***	21.00±0.36***	30.30±0.27***	35.20±0.42***	47.80±0.55***	53.70±0.77***	66.40±0.51***	108.30±1.23***
FAEEs (ppm)	5.00±0.21	7.80±0.50***	18.00±0.37***	26.40±0.67***	41.70±1.09***	50.00±0.31***	74.30±0.69***	87.60±0.41***	121.20±1.66***	271.70±0.86***
Total FAAEs (ppm)	11.80±0.82	16.00±0.84**	34.20±0.95***	47.40±1.03***	72.00±1.36***	85.20±0.73***	122.10±1.24***	141.30±1.18***	187.60±2.17***	380.00±2.09***
FAEEs/FAMEs	0.73	0.95	1.11	1.25	1.37	1.42	1.55	1.63	1.82	2.50
Classification	EVOO	EVOO	EVOO	EVOO	EVOO	EVOO	VOO	VOO	VOO	DO

Note: EVOO = extra virgin olive oil; FAEEs = fatty acid ethyl esters; FAMEs = fatty acid methyl esters; VOO = virgin olive oil. Each value represents the mean of three determinations ($n = 3$) ± standard deviation. Significant differences between the EVOO and the mixtures of EVOO with deodorized olive oil (DO) groups.*
$p < 0.05$;** $p < 0.01$; *** $p < 0.001$.

potential application of DSC to verify the adulteration of EVOO with refined hazelnut oil was evaluated. EVOO and hazelnut oil were characterized by significantly different cooling and heating DSC thermal profiles. The addition of hazelnut oil significantly enhanced crystallization enthalpy (at hazelnut oil ≥20%) and shifted the transition toward lower temperatures (at hazelnut oil ≥5%). Finally, both cooling and heating DSC thermograms undergo significant changes as a result of the addition of hazelnut oil to EVOO, and they may be a tool for the detection of adulteration of EVOO with refined hazelnut oil.

Two methods to quantify the adulteration of EVOO based on physical characteristics of adulterated samples have been described by Torrecilla *et al.* (2011). Firstly, the adulterant agent concentration is determined using the density and/or refractive indices (RIs) of the adulterated samples of EVOO with sunflower or corn oils by suitable linear correlations between density and/or RI. Finally, the models based on the combination of DSC equipment and a chaotic parameter (lag-k autocorrelation coefficients [LCCs]) are defined here to quantify the adulterations of EVOO with refined, refined olive pomace, sunflower, or corn oils. In both studied models, the adulterant agent concentrations were less than 14% (w/w). The former is adequate to calculate the concentration of the adulterant with a correlation coefficient (R^2) higher than 0.927 and a mean square error (MSE) less than 8.9%.

Microwave dielectric spectroscopy appears as a promising solution, and it has potential as a tool for adulteration detection and for monitoring vegetable oils. The dielectric spectroscopy has become an effective method for qualitative characterization and other intrinsic physical characteristics of foodstuff materials (Miura *et al.*, 2003; Venkatesh & Raghavan, 2004). To this end, the Cole-Cole dielectric parameters (and, in particular, relaxation frequency) of different vegetable oils were evaluated through an innovative automatable procedure. Successively, typical cases of adulteration conditions were considered by mixing olive oil and sunflower oil in different percentages. In this case, the relaxation frequency appears to be the Cole-Cole parameter that indicates the presence of adulterants (Cataldo *et al.*, 2010).

The correct discrimination of EVOO samples were obtained by HPLC-MS with direct injection and positive APCI detection without chemical derivatization and purification by using stepwise discriminant function analysis (SDFA) to select the variables and LDA (Nagy *et al.*, 2005). The correct classification and 99% prediction rate were obtained with samples from three Italian olive cultivars. The authors claim qualitative detection of adulteration to be above 91 and 88% identification of the type of adulterant (sunflower, corn, peanut, and coconut oils).

Direct infusion ESI-MS was used to differentiate qualitatively unrefined olive oil from vegetable oils, to detect the aging and adulteration of vegetable oils by analyzing the polar components extracted with methanol–water (1:1) from different oils and mixtures (Catharino *et al.*, 2005). PCA was used to distinguish unique major diagnostic ions of olive oil from those of other vegetable oils (soybean, corn, canola, sunflower, and cottonseed). The corresponding ESI-MS fingerprints in the negative mode also differentiate olive oil from the other refined vegetable oils and oxidized soybean oil, showing additional ions than those in fresh oil.

Mid-IR and FTIR spectroscopy combined with chemometrics (PLS and PCA models) were used to detect and quantify the adulteration of EVOO with edible oils (Gurdeniz & Ozen, 2009). The model, based on PLS analysis developed to detect adulteration, was limited to 10%. In another study, FTIR was used to classify oil samples according to botanical origin and determine the composition of the binary mixtures of EVOO with cheaper oils (sunflower, corn, soybean, and hazelnut oils) (Lerma-García *et al.*, 2010). Absorbance peak areas were normalized within the FTIR spectra as predictors of botanical origin by LDA. Multiple linear regression (MLR) models were used to determine binary mixtures as low as 5% of EVOO with other vegetable oils.

High-power gradient NMR diffusion coefficients (D) were determined to detect the adulteration of EVOO for the rapid screening of adulteration of olive oils with cheaper vegetable oils (Šmejkalová & Piccolo, 2010). Changes in D values could be detected with the adulteration of 10% for sunflower and soybean oils and 30% for hazelnut and peanut oils.

Dielectric spectroscopy (DS) was adopted for quantitative determination of the levels of adulterant in olive oil (Toyoda, 2003). As a simple, rapid, and nondestructive measuring technique, DS provides information about the dielectric response of materials to electromagnetic fields. The dielectric spectra of a binary mixture of olive oil spiked with other oils increased linearly with the increase in the concentration of soybean, corn, canola, sesame, and perilla oils from 0 to 100% (w/w). The dielectric properties of the binary mixture of oils were investigated in the frequency range of 101 Hz–1 MHz. A PLS model was developed and used to verify

the concentrations of the adulterant. Furthermore, PCA was used to classify olive oil samples as distinct from other adulterants based on their dielectric spectra (Lizhi *et al.*, 2010).

Maggio *et al.* (2010) developed a multistage strategy combining FTIR with PLS as a multivariate method for monitoring the purity of EVOO and performing qualitative and quantitative determinations of adulterants (canola, hazelnut, pomace, and high-linoleic/high-oleic sunflower) in commercial samples. This general operating procedure represents an improvement toward adulterant assessment in EVOO, using the prediction of adulterant ratio and the spectral residues to determine sample composition. The method developed was suitable for determination of modeled adulterants, but it may also reveal an adulteration.

Downey *et al.* (2002) used visible and near-infrared transflectance spectroscopy to discriminate between authentic EVOOs and the same oils adulterated with the addition of sunflower oil, and to quantify the level of sunflower oil adulterant present. A number of multivariate mathematical approaches were investigated to detect and quantify the sunflower oil adulterant. These include hierarchical cluster analysis, SIMCA, and PLS regression. SIMCA can successfully discriminate between authentic EVOO and the same oils adulterated with sunflower oil at levels as low as 1% (w/w). The greatest classification accuracy was achieved using the first derivative of spectral data in the wavelength range of 1100–2498 nm. Using a confidence level of 1%, a 100% correct classification was achieved in both calibration and prediction sample sets.

Papadopoulos *et al.* (2002) used chemiluminescence (CL) for the detection of the adulteration of the more expensive EVOO with cheaper plant oils (corn and sunflower). The energy is produced by the oxidation of polyunsaturated fatty acid esters, such as linoleic or linolenic acid, and possibly energy transfer to fluorescent species contained in edible oils. A weak CL emission is observed in commercial Greek EVOOs (Knossos, Spitiko, Ananias, Altis, Minerva, and Xenia) and in refined plant oils such as sunflower oils (Marata, Sanola, Sun, Mana, Sol, and Minerva) as well as in corn oils (Flora, Minerva, Marata Sun, and Sol) with potassium superoxide in the aprotic solvent dimethoxyethylene. On measuring the CL of mixtures of EVOOs with the cheaper refined oils, calibrations were produced, which can be used for the determination of the adulteration of olive oils with plant oils down to 3%. Furthermore, depending on the kind of oils, "low"-authenticity CL factors for olive oils (0.8–2.15 μmol L^{-1} gallic acid) and "high"-authenticity CL actors for seed oils (4.5–11.2 μmol L^{-1} gallic acid) were calculated.

Finally, it is necessary to mention that the most important requirement for consumers is to have the highest quality in all purchased goods. This requisite is even more obligatory when the products have health implications. Adulteration of EVOO with other vegetable oils has negative effects on oil quality and nutrition value. In fact, the adulterations are often made with refined oils impacting EVOO fatty acid composition, and contents of antioxidants and vitamins (Darmon *et al.*, 2006). In comparison to seed oils, EVOO has low levels of saturated and high levels of monounsaturated fatty acids, mainly oleic acid. Olive pomace oil is produced through extraction of olive pomace with organic solvents and has significantly lower nutritive value and price in comparison with EVOO (Kiritsakis, 1998).

29.4 Conclusion

The adulteration of olive oil is a very important issue because of its impact on quality, nutritional value, and consumer safety. Due to the inherent high cost of EVOO, the adulteration of this kind of product with cheaper or low-quality oils actually seems to be one of the most common types of fraud. The development of analytical methodologies that allow for the detection of adulterations is warranted since the detection of refined vegetable oil to EVOO at low percentages could be a very challenging task. Briefly, more efforts are needed to exploit new methods that could be assigned as reliable adulteration markers, able to detect with high selectivity, sensitivity, and accuracy blends of EVOO with other plant oils.

To prevent oil adulteration, antifraud controls require that specific tests be performed to assess the quality of oils; such controls usually rely on highly sophisticated and expensive methods (i.e., gas chromatography, liquid chromatography, Fourier transform infrared, and nuclear magnetic resonance) for monitoring the authenticity of EVOO. Moreover, the employment of several multivariate methods such as principal component analysis, canonical analysis, linear discriminant analysis, cluster analysis, partial least squares, and surface response methodology has become a prerequisite for several applications related primarily to food quality control in terms of authentication and adulteration, mainly due to a substantial simplification of the classification and grouping task.

Advances in knowledge and technology have undoubtedly led to greater success in the fight against adulteration over the years. However, it is equally true that the same techniques and knowledge have been used by defrauders in order to invalidate the usefulness of some methods. As a result of the advances in analytical methods, or the new challenges created by fraudsters, official methods and trade standards are periodically revised and upgraded.

Over the last few years, much attention has been given to fraudulent practices associated with EVOO traceability, with special emphasis on the botanical origin due to the recent introduction in the market of high-quality monocultivar olive oil. Aiming to find traceability markers, several studies have been performed allowing the discrimination of compositional and genetical markers by exploring an alternative methodology based on the application of DNA-based detection methods in order to assess the role of DNA as a tool to detect adulteration.

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References

Aguilera, M.P., Beltrán, G., Ortega, D., Fernández, A., Jiménez, A., & Uceda, M. (2005) Characterisation of virgin olive oil of Italian olive cultivars: 'Frantoio' and 'Leccino', grown in Andalusia. Food Chemistry 89, 387–391.

Al-Ismail, K.M., Alsaed, A.K., Ahmad, R., & Al-Dabbas, M. (2010) Detection of olive oil adulteration with some plant oils by GLC analysis of sterols using polar column. Food Chemistry 121, 1255–1259.

Ammar, S., Zribi, A., Mansour, A.B., Ayadi, M., Abdelhedi, R., & Bouaziz, M. (2014a) Effect of processing systems on the quality and stability of Chemlali olive oils. Journal of Oleo Science 63, 311–323.

Ammar, S., Zribi, A., Gargouri, B., Flamini, G., & Bouaziz, M. (2014b) Effect of addition of olive leaves before fruits extraction process to some monovarietal Tunisian extra-virgin olive oils using chemometric analysis. Journal of Agricultural and Food Chemistry 62, 251–263.

Aparicio, R., & Aparicio-Ruíz, R. (2000) Authentication of vegetable oils by chromatographic techniques. Journal of Chromatography A 881, 93–104.

Berrueta, L.A., Alonso-Salces, R.M., & Héberger, K. (2007) Supervised pattern recognition in food analysis. Journal of Chromatography A 1158, 196–214.

Biedermann, M., Bongartz, A., Mariani, C., & Grob, K. (2008) Fatty acid methyl and ethyl esters as well as wax esters for evaluating the quality of olive oils. European Food Research and Technology 228, 65–74.

Blanch, G.B., Del Mar Caja, M., Ruiz del Castillo, M.L., & Herraiz, M. (1998) Comparison of different methods for the evaluation of the authenticity of olive oil and hazelnut oil. Journal of Agricultural and Food Chemistry 46, 3153–3157.

Bohačenko, I., & Kopicová, Z. (2001) Detection of olive oils authenticity by determination of their sterol content using LC/GC. Czech Journal of Food Sciences 19, 97–103.

Brumley, W.C., Sheppard, A.J., Rudolf, T.S., Shen, C.S., Yasaei, P., & Sphon, J.A. (1985) Mass spectrometry and identification of sterols in vegetable oils as butyryl esters and relative quantitation by gas chromatography with flame ionization detection. Journal of the Association of Official Analytical Chemists 68, 701–709.

Capote, F.P., Jiménez, J.R., & de Castro, M.D.L. (2007) Sequential (step-by-step) detection, identification and quantitation of extra virgin olive oil adulteration by chemometric treatment of chromatographic profiles. Analytical and Bioanalytical Chemistry 388, 1859–1865.

Cataldo, A., Piuzzi, E., Cannazza, G., De Benedetto, E., & Tarricone, L. (2010) Quality and anti-adulteration control of vegetable oils through microwave dielectric spectroscopy. Measurement 43, 1031–1039.

Catharino, R.R., Haddad, R., Cabrini, L.G., Cunha, I.B.S., Sawaya, A.C.H.F., & Eberlin, M.N. (2005) Characterization of vegetable oils by electrospray ionization mass spectrometry fingerprinting: classification, quality, adulteration, and aging. Analytical Chemistry 77, 7429–7433.

Cerretani, L., Bendini, A., Valli, E., Chiavaro, E., Morchio, G., & Lercker, G. (2011) Chemical characterization of refined olive oils and second extraction olive oils ("repaso" oils) available on the national and international markets. La Rivista Italiana Delle Sostanze Grasse 88, 82–88.

Cert, A., Lanzón, A., Carelli, A.A., Albi, T., & Amelotti, G. (1994) Formation of stigmasta-3,5-diene in vegetable oils. Food Chemistry 49, 287–293.

Chiavaro, E., Vittadini, E., Rodriguez-Estrada, M.T., Cerretani, L., & Bendini, A. (2008) Differential scanning calorimeter application to the detection of refined hazelnut oil in extra virgin olive oil. Food Chemistry 110, 248–256.

Crews, C., Pye, C., & Macarthur, R. (2014) An improved rapid stigmastadiene test to detect addition of refined oil to extra virgin olive oil. Food Research International 60, 117–122.

Cunha, S.C., & Oliveira, M.B.P.P. (2006) Discrimination of vegetable oils by triacylglycerols evaluation of profile using HPLC/ELSD. Food Chemistry 95, 518–524.

Darmon, N., Darmon, M., & Ferguson, E. (2006) Identification of nutritionally adequate mixtures of vegetable oils by linear programming. Journal of Human Nutrition Dietetics 19, 59–69.

De la Mata-Espinosa, P., Bosque-Sendra, J.M., Bro, R., & Cuadros-Rodríguez, L. (2011) Olive oil quantification of edible vegetable oil blends using triacylglycerols chromatographic fingerprints and chemometric tools. Talanta 85, 177–182.

Downey, G., Mcintyre, P., & Davies, A.N. (2002) Detecting and quantifying sunflower oil adulteration in extra virgin olive oils from the eastern mediterranean by visible and near-infrared spectroscopy. Journal of Agricultural and Food Chemistry 50, 5520–5525.

Dyer, J.M., Stymne, S., Green, A.G., & Carlsson, A.S. (2008) High-value oils from plants. The Plant Journal 54, 640–655.

Fang, M., Tsai, S.F, Yu, G.Y., Tseng, S.H., Cheng, H.F., Kuo, C.H., Hsu, C.L., Kao, Y.M., Chih Shih, D.Y., & Chiang, Y.M. (2015) Identification and quantification of Cu-chlorophyll adulteration of edible oils. Food Additives and Contaminants 8, 157–192.

Fasciotti, M., & Pereira-Netto, A.D. (2010) Optimization and application of methods of triacylglycerol evaluation for characterization of olive oil adulteration by soybean oil with HPLC–APCI-MS–MS. Talanta 81, 1116–1125.

Frankel, E.N. (2010) Chemistry of extra virgin olive oil: adulteration, oxidative stability, and antioxidants. Journal of Agricultural and Food Chemistry 58, 5991–6006.

Galeano-Diaz, T., Durán-Merás, I., Sánchez-Casas, J., & Alexandre-Franco, M.F. (2005) Characterization of virgin olive oils according to its triglycerides and sterols composition by chemometric methods. Food Control 16, 339–347.

Gamazo-Vázquez, J., García-Falcón, M.S., & Simal-Gándara, J. (2003) Control of contamination of olive oil by sunflower seed oil in bottling plants by GC-MS of fatty acid methyl esters. Food Control 14, 463–467.

García, J.M., & Yousfi, K. (2006) The postharvest of mill olives. Grasas y Aceites 57, 16–24.

García-González, D.L., Viera, M., Tena, N., & Aparicio, R. (2007) Evaluation of the methods based on triglycerides and sterols for the detection of hazelnut oil in olive oil. Grasas y Aceites 58, 344–350.

Gargouri, B., Ammar, S., Zribi, A., Mansour, A.B., & Bouaziz, M. (2013) Effect of growing region on quality characteristics and phenolic compounds of chemlali extra-virgin olive oils. Acta Physiologiae Plantarum 35, 2801–2812.

Gargouri, B., Zribi, A., & Bouaziz, M. (2015) Effect of containers on the quality of Chemlali olive oil during storage. Journal of Food Science and Technology 52, 1948–1959.

Grob, K., Biedermann, M., Artho, A., & Schmid, J.P. (1994b) LC, GC, and MS of sterol dehydration products. La Rivista Italiana Delle Sostanze Grasse 71, 533–538.

Grob, K., Giuffré, A.M., Leuzzi, U., & Mincione, B. (1994a) Recognition of adulterated oils by direct analysis of the minor components. European Journal of Lipid Science and Technology 96, 286–290.

Guimet, F., Ferré, J., & Boqué, R. (2005) Rapid detection of olive–pomace oil adulteration in extra virgin olive oils from the protected denomination of origin "Siurana" using excitation–emission fluorescence spectroscopy and three-way methods of analysis. Analytica Chimica Acta 544, 143–152.

Gurdeniz, G., & Ozen, B. (2009) Detection of adulteration of extra-virgin olive oil by chemometric analysis of mid-infrared spectral data. Food Chemistry 116, 519–525.

International Olive Council (IOC). (2001a) Determination of trans unsaturated fatty acids by capillary column gas chromatography. COI/T, 20/Doc. No. 17 Rev. 1. IOC, Madrid, Spain.

International Olive Council (IOC). (2001b) Preparation of the fatty acid methyl esters from olive oil and olive pomace oil. COI/T, 20/Doc. No. 24. IOC, Madrid, Spain.

International Olive Council (IOC). (2001c) Determination of the composition and content of sterols by capillary-column gas chromatography. COI/T, 20/Doc. No. 10 Rev. 1. IOC, Madrid, Spain.

International Olive Council (IOC). (2001d) Determination of stigmastadienes in vegetable oils. COI/T, 20/Doc. No. 11 Rev. 2. IOC, Madrid, Spain.

International Olive Council (IOC). (2001e) Determination of sterenes in refined vegetable oils. COI/T, 20/Doc. No. 16 Rev. 1. IOC, Madrid, Spain.

International Olive Council (IOC). (2009) Determination of the content of waxes, fatty acid methyl esters and fatty acid ethyl esters by capillary gas chromatography. COI/T, 20/Doc. No. 28. IOC, Madrid, Spain.

International Olive Council (IOC). (2010) Determination of the difference between actual and theoretical content of triacylglycerols with ECN42. COI/T, 20/Doc. No. 20 Rev. 3. IOC, Madrid, Spain.

Jabeur, H., Zribi, A., Abdelhedi, R., & Bouaziz, M. (2015a) Effect of olive storage conditions on Chemlali olive oil quality and the effective role of fatty acids alkyl esters in checking olive oils authenticity. Food Chemistry 169, 289–296.

Jabeur, H., Zribi, A., & Bouaziz, M. (2015b) Extra-virgin olive oil and cheap vegetable oils: distinction and detection of adulteration as determined by GC and chemometrics. Food Analytical Methods. DOI:10.1007/s12161-015-0249-9

Jabeur, H., Zribi, A., Makni, J., Rebai, A., Abdelhedi, R., & Bouaziz, M. (2014) Detection of Chemlali extra-virgin olive oil adulteration mixed with soybean oil, corn oil, and sunflower oil by using GC and HPLC. Journal of Agricultural and Food Chemistry 62, 4893–4904.

Jafari, M., Kadivar, M., & Keramat, J. (2009) Detection of adulteration in Iranien olive oils using instrumental (CG, NMR, DSC) methods. Journal of the American Oils Chemists' Society 86, 103–110.

Kafatos, A., & Comas, G.E. (1991). Biological effects of olive oil on human health. In: Olive oil, ed. A. Kiritsakis. American Oil Chemists' Society, Champaign, IL, 81–157.

Lerma-García, M.J., Lusardi, R., Chiavaro, E., Cerretani, L., Bendini, A., Ramis-Ramos, G., & Simó-Alfonso, E.F. (2011) Use of triacylglycerol profiles established by high performance liquid chromatography with ultraviolet-visible detection to predict the botanical origin of vegetable oils. Journal of Chromatography A 1218, 7521–7527.

Lerma-García, M.J., Ramis-Ramos, G., Herrero-Martinez, J.M., & Simó-Alfonso, E.F. (2010) Authentication of extra virgin olive oils by Fourier-transform infrared spectroscopy. Food Chemistry 118, 78–83.

Lizhi, H., Toyoda, K., & Ihara I. (2010) Discrimination of olive oil adulterated with vegetable oils using dielectric spectroscopy. Journal of Food Engineering 96, 167–171.

Maggio, R.M., Cerretani, L., Chiavaro, E., Kaufman, T.S., & Bendini, A. (2010) A novel chemometric strategy for the estimation of extra virgin olive oil adulteration with edible oils. Food Control 21, 890–895.

Mannina, L., Dugo, G., Salvo, F., Cicero, L., Ansanelli, G., Calcagni, C., & Segre, A. (2003) Study of cultivar-composition relationship in Sicilian olive oils by GC, NMR, and statistical methods. Journal of Agricultural and Food Chemistry 51, 120–127.

Mariani, C., & Bellan, G. (2011) On the possible increase of the alkyl esters in extra virgin olive oil. La Rivista Italiana Delle Sostanze Grasse 88, 3–10.

Mavromoustakos, T., Zervou, M., Bonas, G., Kolocouris, A., & Petrakis, P. (2000) A novel analytical method to detect adulteration of virgin olive oil by other oils. Journal of the American Oil Chemists' Society 77, 405–411.

Méndez, A.I., & Falqué, E. (2007) Effect of storage time and container type on the quality of extra-virgin olive oil. Food Control 18, 521–529.

Miura, N., Yagihara, S., & Mashimo, S. (2003) Microwave dielectric properties of solid and liquid foods investigated by time-domain reflectometry. Journal of Food Science 68, 1396–1403.

Monfreda, M., Gobbi, L., & Grippa, A. (2014) Blends of olive oil and seeds oils: characterisation and olive oil quantification using fatty acids composition and chemometric tools. Food Chemistry 145, 584–592.

Nagy, K., Bongiorno, D., Avellone, G., Agozzino, P., Ceraulo, L., & Vékey, K. (2005) High performance liquid chromatography–mass spectrometry based chemometric characterization of olive oils. Journal of Chromatography A 1078, 90–97.

Ouesleti, I., Anniva, C., Daoud, D., Tsimidou, M.Z., & Zarrouk, M. (2009) Virgin olive oil (VOO) production in Tunisia: The commercial potential of the major olive varieties from the aride Tataouine zone. Food Chemistry 112, 733–741.

Ozen, B.F., Weiss, I., & Mauer, L.J. (2003) Dietary supplement oil classification and detection of adulteration using Fourier transform infrared spectroscopy. Journal of Agricultural and Food Chemistry 51, 5871–5876.

Papadopoulos, K., Triantis, T., Tzikis, C.H., Nikokavoura, A., & Dimotikali, D. (2002) Investigations of the adulteration of extra virgin olive oils with seed oils using their weak chemiluminescence. Analytica Chimica Acta 464, 135–140.

Parcerisa, J., Casals, I., Boatella, J., Codony, R., & Rafecas, M. (2000) Analysis of olive and hazelnut oil mixtures by high-performance liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry of triacylglycerols and gas-liquid chromatography of non-saponifiable compounds (tocopherols and sterols). Journal of Chromatography A 881, 149–158.

Pérez-Camino, M.C., Cert, A., Romero-Segura, A., Cert-Trujillo, R., & Moreda, W. (2008) Alkyl esters of fatty acids a useful tool to detect soft deodorized olive oils. Journal of Agricultural and Food Chemistry 56, 6740–6744.

Pérez-Camino, M.C., Moreda, W., Mateos, R., & Cert, A. (2002) Determination of esters of fatty acids with low molecular weight alcohols in olive oils. Journal of Agricultural and Food Chemistry 50, 4721–4725.

Popescu, R., Costinel, D., Dinca, O.R., Marinescu, A., Stefanescu, I., & Ionete, R.E. (2015) Discrimination of vegetable oils using NMR spectroscopy and chemometrics. Food Control 48, 84–90.

Ruiz-Samblás, C., Marini, F., Cuadros-Rodríguez, L., & González-Casado, A. (2012) Quantification of blending of olive oils and edible vegetable oils by triacylglycerol fingerprint gas chromatography and chemometric tools. Journal of Chromatography B 910, 71–77.

Saba, A., Mazzini, F., Raffaelli, A., Mattei, A., & Salvadori, P. (2005) Identification of 9(E),11(E)-18:2 fatty acid methyl ester at trace level in thermal stressed olive oils by GC coupled to acetonitrile CI-MS and CI-MS/MS, a possible marker for adulteration by addition of deodorized olive oil. Journal of Agricultural and Food Chemistry 53, 4867–4872.

Šmejkalová, D., & Piccolo, A. (2010) High-power gradient diffusion NMR spectroscopy for the rapid assessment of extra-virgin olive oil adulteration. Food Chemistry 118, 153–158.

Torrecilla, J.S., García, J., García, S., & Rodríguez, F. (2011) Quantification of adulterant agents in extra virgin olive oil by models based on its thermophysical properties. Journal of Food Engineering 103, 211–218.

Toyoda, K. (2003) The utilization of electric properties. In: The handbook of non-destructive detection, ed. K. Sumio. Science Forum, Tokyo, 108–126.

Valli, E., Bendini, A., Maggio, R.M., Cerretani, L., Toschi, T.G., Casiraghi, E., & Lercker, G. (2013) Detection of low-quality extra virgin olive oils by fatty acid alkyl esters evaluation: a preliminary and fast mid-infrared spectroscopy discrimination by a chemometric approach. International Journal of Food Science and Technology 48, 548–555.

Venkatesh, M.S., & Raghavan, G.S.V. (2004) An overview of microwave processing and dielectric properties of agri-food materials. Biosystems Engineering 88, 1–18.

Vigli, G., Philippidis, A., Spyros, A., & Dais, P. (2003) Classification of edible oils by employing 31P and 1H NMR spectroscopy in combination with multivariate statistical analysis. A proposal for the detection of seed oil adulteration in virgin olive oils. Journal of Agricultural and Food Chemistry 51, 5715–5722.

30 Authentication of olive oil based on minor components

Styliani Christophoridou

30.1 Introduction

Extra virgin olive oil (EVOO) is an important component of the Mediterranean diet, and it is known to have beneficial effects on human health. These health benefits lead to a high price for EVOO, which makes it a preferred target for adulteration. Moreover, protected denomination of origin (PDO) virgin olive oils (VOOs) challenge for markers that can adequately ensure a high-quality product for consumers in terms of cultivar, cultivation techniques, harvest periods, and methods of extraction. Thus, olive oil authenticity has become an important subject from both a commercial and a health perspective. The European Commission (Regulations No. 2568/91, 1991) and the International Olive Oil Council (IOOC) (COI/T.20/Doc. No. 19/Rev 2, 2008) have established official methods to characterize EVOO and guarantee its quality. Nevertheless, these methods cannot be used for oil traceability.

Functional micronutrients (minor components), apart from their distinct effect on the quality and nutritional value of EVOO, can contribute as markers for the authenticity of olive oil (Aparicio *et al.*, 2000). These micronutrients are mostly secondary metabolites of olive fruit, and thus their quantity and quality in VOO are greatly affected by the cultivar of olives used and their geographical origin. In recent years, intensive studies have been conducted for the establishment of EVOO authenticity using functional micronutrients in olive oils in combination with chemometrics. The term "authenticity of olive oil" refers to the classification of olive oil in terms of adulteration, cultivar, and geographical origin. Adulteration is usually accomplished by mixing of EVOOs either with lower quality olive oils such as refined (ROOs), pomace (POOs), and lampante olive oils (LOOs), or with other inferior edible vegetable oils (EOs) such as sunflower oil, soybean oil, corn oil, or the like. These oils are characterized as having lower quality due to the absence of minor components. Moreover, the micronutrient content and quality of olive oil are greatly affected by the cultivar of olives, whereas geographical origin may lead to distinct chemical characteristics reflecting specific environmental and soil conditions under which olives are grown. PDO VOOs and protected geographical indication (PGI) VOOs are characterized by cultivar, geographical, and cultivation differentiations leading to unique flavor and quality of olive oils.

Some functional micronutrients used in authentication of EVOOs are sterols, phenols, volatiles, tocopherols, and carotenoids, with remarkable results. In this chapter, the latest trends on the use of minor components composition of EVOOs for their authentication are described.

30.2 Sterols

Phytosterols comprise a major proportion of the unsaponified matter in vegetable oils, which is derived biosynthetically from squalene. The conventional method for phytosterol analysis is the hydrolysis of the esters and subsequent analysis by high-performance liquid chromatography (HPLC) or gas

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chromatography (GC). Major sterols of olive oil are by far β-sitosterol followed by Δ-5-avenasterol (Cañabate-Díaz *et al.*, 2007; Schröder & Vetter, 2012). Another group of compounds, the triterpene dialcohols erythrodiol and uvaol, which are co-chromatographed with 4-desmethylsterols, is usually studied simultaneously with sterols. Sterol composition and content of olive oil are affected by cultivar, crop year, degree of ripeness, storage time of fruits before oil extraction, and method of oil extraction. The purpose of this section is to focus on olive oil authentication on the bases of sterol content.

30.2.1 Adulteration tracing

Several studies have demonstrated the ability of sterol content and profile to discriminate EVOOs from EOs or lower quality olive oils, such as LOOs or ROOs, and for adulteration tracing (Bagur-González *et al.*, 2015). The 4-desmethylsterols fraction of EVOOs was found to be an important subclass for discriminating EVOOs from other EOs but not from ROOs, according to Gázquez-Evangelista *et al.* (2014). Al-Ismail *et al.* (2010) proposed an olive oil authenticity factor (A_f) based on the sum of campesterol and stigmasterol contents as an indicator of olive oil adulteration with vegetable oils, which is able to detect the presence of EOs as low as 5%.

For the detection of adulteration of olive oil with refined hazelnut oil, in which filbertone (the characteristic flavor compound in hazelnuts) is absent, sterol fraction has been used either by utilizing new factors (Vichi *et al.*, 2001; Mariani *et al.*, 2006), by applying more sensitive techniques (Purcaro *et al.*, 2015), or even by focusing in a sub-sterol fraction (Damirchi *et al.*, 2005).

30.2.2 Cultivar determination

The sterol content variation among different cultivars can be useful in order to authenticate olive oils according to their cultivar. The sterol and triterpene dialcohol (erythrodiol and uvaol) profiles were used to successfully discriminate EVOOs in accordance to cultivar (Sánchez Casas *et al.*, 2004; Casas *et al.*, 2009; Lerma-García *et al.*, 2011).

Lukić *et al.* (2013) found reliable sterol indicators for cultivar differentiation that were independent of the degree of ripening and storage conditions. As shown in Figure 30.1, EVOOs from three different cultivars, namely Buža, Črna, and Rosinjola, harvested at three different degrees of maturation and stored for 12 months, were separated according to their campesterol percentage, beta-sitosterol concentration, D^7-campesterol/$D^{5,24}$-stigmastadienol ratio, and 24-methylene-cholesterol/stigmasterol ratio. Moreover, the effect of harvest year on the sterol profile was low, compared to cultivar differentiation (Sena-Moreno *et al.*, 2015).

30.2.3 Geographical discrimination

The use of the sterol content and profile has been employed in several studies in order to highlight differences in the chemical composition of olive oils arising from origin differentiation reflecting mostly environmental conditions, agricultural practices, soil differences, and origin area (e.g., altitude) (Ferreiro & Aparicio, 1999; Ranalli *et al.*, 1999).

The geographical differentiation alone on the basis of sterol content was achieved by several authors (Alves *et al.*, 2005; Temime *et al.*, 2008; Casas *et al.*, 2009; Giacalone *et al.*, 2015).

When sterol content was used together with other chemical variables, in most cases, the sterol profile played a predominant role in achieving geographical discrimination (Ouni *et al.*, 2011a; Longobardi *et al.*, 2012a; Piravi-Vanak *et al.*, 2012), while in other cases their discriminating ability was limited (Petrakis *et al.*, 2008; Longobardi *et al.*, 2012b).

30.3 Vitamin E – tocopherols

Vitamin E is a collective name for eight compounds divided in two subcategories, tocopherols and tocotrienols, collectively known as tocols. In VOOs, the content of tocopherols varies from 52 to 87% for α-tocopherol followed by 15–20% and 7–23% for β- and γ-tocopherols, respectively, while tocotrienols are

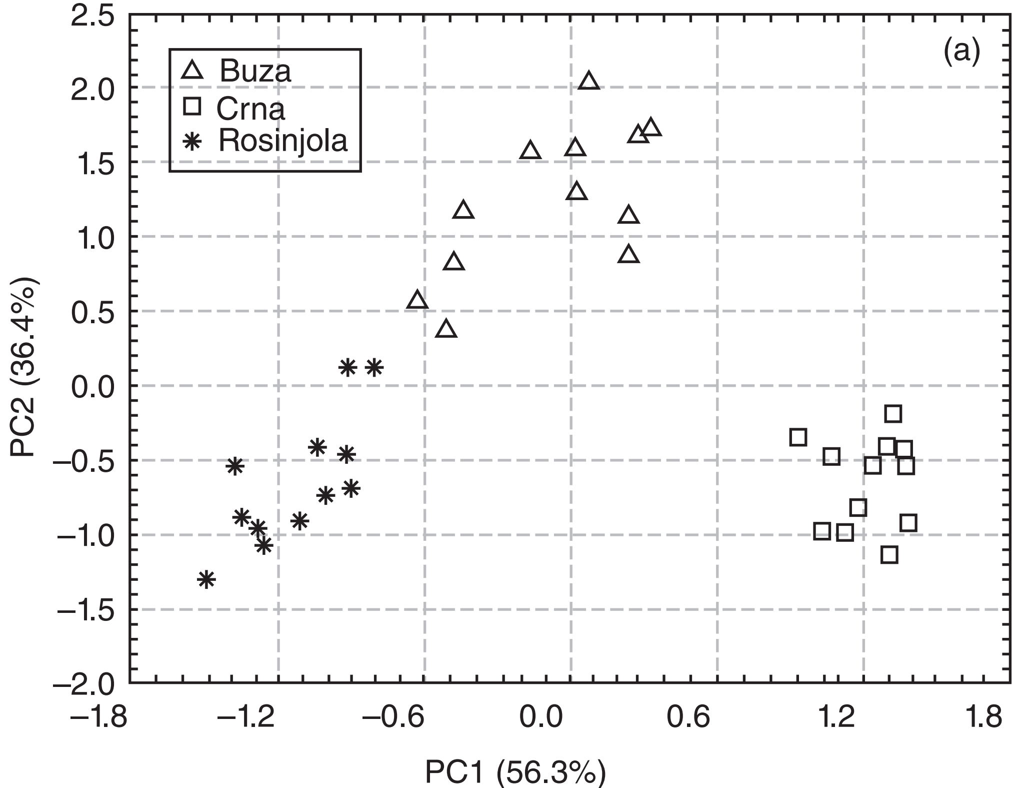

Figure 30.1 PCA separation of 36 samples and only four variables according to cultivar of olive oils from 'Buža', 'Črna', and 'Rosinjola' cultivars, harvested at three different ripening degrees and stored for 12 months, along the directions of principal components PC1 and PC2. *Source*: Lukić *et al.* (2013). Reproduced with permission of Elsevier.

not present (Sayago *et al.*, 2007). Reverse-phase high-performance liquid chromatography (RP-HPLC) is the most common method for their detection. The importance of tocols is due to their antioxidant effects in VOO, while their profile and composition are dependent on many variables, such as cultivar of olives, geographical origin, and environmental conditions (rainfall, sunshine, etc.), and thereby they are often criteria of purity and authenticity, although few authentication studies are based solely on tocopherol determination (Bramley *et al.*, 2000; Chen *et al.*, 2011).

30.3.1 Adulteration tracing

The tocopherol composition varies in different types of oil (Lerma-García *et al.*, 2007; Foo Wong *et al.*, 2014). Thereby, this class of compounds was used as screening factor for EVOO adulteration at concentrations as low as 5% (Bakre *et al.*, 2014). Due to their antioxidant properties, however, their concentration decreases during oil storage, so instead of the absolute value of tocopherol contents, the use of their ratios was proposed by Chen *et al.* (2011), who were able to successfully discriminate EVOOs from various EOs.

The multidimensional comprehensive gas chromatographic (GC×GC) method, which was employed by Parcerisa *et al.* (2000) and Purcaro *et al.* (2015), showed that hazelnut oil had a different tocopherol profile from EVOOs, confirming results from Morchio *et al.* (1999).

Tocotrienols are not present in EVOOs; thereby, their presence was used for EVOO adulteration tracing with oils of high levels of tocotrienols, such as palm and grapeseed oils at 1–2% (Dionisi *et al.*, 1995; Lerma-García *et al.*, 2007).

30.3.2 Cultivar determination

Tocopherol contents are highly cultivar dependent, and less dependent on the site of cultivation and environmental conditions (Tura *et al.*, 2007; Beltrán *et al.*, 2010). According to Dag *et al.* (2015), only α- and γ-tocopherols in EVOOs had significant discriminative effect, which is confirmed by Matos *et al.* (2007) and López-Cortés *et al.* (2013). Variation of tocopherols in terms of ripening degree was less notable than variation due to cultivar differences (Matos *et al.*, 2007; Franco *et al.*, 2014a; Sarolic *et al.*, 2014).

30.3.3 Geographical discrimination

Geographical discrimination of EVOOs on the basis of tocopherol content was found to be a difficult task (Ranalli *et al.*, 1999; Amaral *et al.*, 2010). Although distinct environmental differences in different origin areas of oils result in a wide variability of tocopherol content (Ben Temime *et al.*, 2006), tocopherols were found to be highly dependent on cultivar, whereas γ-tocopherol appeared to have a more consistent response with regard to geographical origin (Aguilera *et al.*, 2005; Tura *et al.*, 2007; Špika *et al.*, 2015). Alonso-Salces *et al.* (2010) studied the geographical discrimination of EVOOs from different countries (Spain, Italy, Greece, Tunisia, Turkey, and Syria) on the basis of their ^{1}H NMR fingerprint of their unsaponifiable matter. Many variables that contributed significantly to the classification model were found to originate from the tocopherol subfraction.

30.4 Phenols

The polyphenolic fraction of VOO is a complex mixture that is usually obtained from olive oil by extraction with a polar solvent. The total quantity and quality of polyphenols in EVOOs are very sensitive to variations in olive fruit cultivar, fruit maturity stage during harvesting time, environmental conditions, agricultural practices, and origin area, and therefore can be used for authentication of olive oils (Jiménez *et al.*, 2013).

30.4.1 Adulteration tracing

Few studies have reported on adulteration detection using polyphenols due to their high variety of profile and concentrations. Hirri *et al.* (2015) reported the limited ability of total polyphenol content to differentiate the VOO quality. Agiomyrgianaki *et al.* (2010), however, highlighted the polyphenol discriminating effect for the detection of adulteration of ROO with refined hazelnut oil at concentrations of up to 1%.

30.4.2 Cultivar determination

Polyphenols are remarkably variable according to cultivar (Alagna *et al.*, 2012; Franco *et al.*, 2014b). Cultivar exerted an important influence on the phenolic profiles compared to harvest year, geographical origin, and the ripening state (Douzane *et al.*, 2013; Chtourou *et al.*, 2013; De Medina *et al.*, 2014). El Riachy *et al.* (2012) found that differences among genotypes were significant for some phenols, while others were more affected by crop year. Agiomyrgianaki *et al.* (2012) reported that most of the phenolic substances were affected by harvest year, origin area, and cultivar, except for total hydroxytyrosol, which was mostly affected by harvest year and geographical origin, whereas total tyrosol was mostly affected by cultivar. According to De Medina *et al.* (2015), levels of secoiridoids and their derivatives were critical to establish differences between Spanish cultivars.

The classification of olive oils on the basis of a combination of polyphenol content with other chemical variables, such as fatty acids (FAs), tocopherols, sterols, carotenoids, and volatiles, among others, highlighted the effectiveness of polyphenols in discriminating olive oils in terms of cultivar (Rigane *et al.*, 2013; Franco *et al.*, 2014b; Hassine *et al.*, 2015).

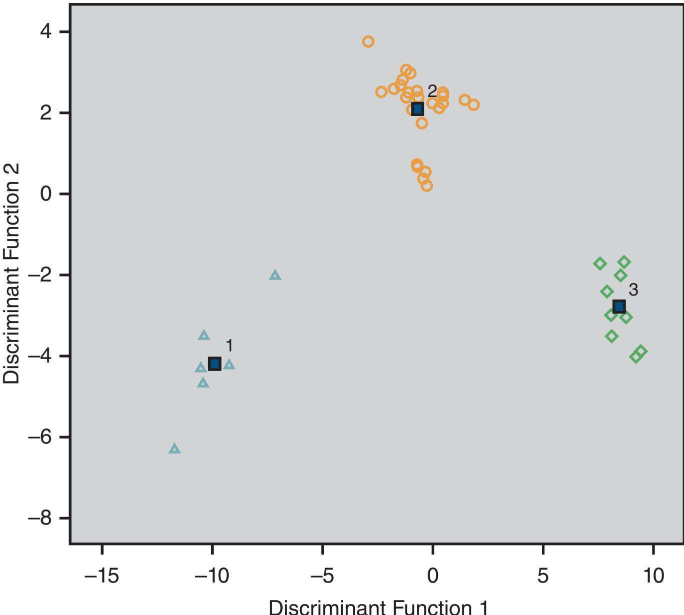

Figure 30.2 Score plot on the plane of the two canonical discriminant analysis functions obtained to predict the geographical origin of EVOOs from the same cultivar based on phenolic content. *Source*: Taamali *et al.* (2012). Reproduced with permission of Elsevier.

30.4.3 Geographical discrimination

Phenolics have evolved in plants because they play a crucial role in the plant response to environmental cues. The variation of phenolics of olive oil in different environments can be a useful tool for the geographical classification. Geographical discrimination of EVOOs was studied by several authors (Ouni *et al.* 2011b; Taamalli *et al.*, 2012; Bakhouche *et al.*, 2013; Bajoub *et al.*, 2015a). Figure 30.2 shows the canonical discriminant analysis of 13 monocultivar EVOOs, according to their geographical origin (north, center, and south of Tunisia) based on their phenolic profile (Taamalli *et al.*, 2012).

The geographical characterization of olive oils from different olive cultivars is a more challenging task as it incorporates many mutually dependent variables (Guerfel *et al.*, 2009; Lerma-García *et al.*, 2009; Gilbert-López *et al.*, 2014). PDO authentication of olive oils was achieved on the basis of phenolic compounds by several authors (Nescatelli *et al.*, 2014; Antonini *et al.*, 2015). However, Bajoub *et al.* (2014) and Romero *et al.* (2015) concluded that harvest year played a greater role in PDO EVOOs' polyphenols than origin.

30.5 Volatiles

The flavor of olive oil is derived from the evaporation and release of volatiles. Volatiles are low-molecular-weight compounds from various chemical classes such as alcohols, aldehydes, ketones, ethers, esters, furan, and thiophene derivatives (Kiritsakis, 1998). Conventional methods used for volatile quantification in olive oils are static and dynamic headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC-MS). The major volatile compounds of olive oil were reported to be hexanal, trans-2-hexenal, 1-hexanol, and 3-methylbutanol (Kalua *et al.*, 2007). The profile and content of volatiles in oils are dependent on the type of oil, cultivar, geographical origin, fruit maturity, processing methods, and microbiological activity (Angerosa *et al.*, 2004; Cayuela *et al.*, 2015).

30.5.1 Adulteration tracing

Olive oil adulteration with lower quality oils affects the organoleptic properties (sensory quality, taste, color, aroma, etc.) of the resulting blend. Flavor quality is reflected in the volatile chemical profile and can be used for adulteration tracing purposes. The discrimination of EVOOs from vegetable oils can be achieved on the basis of volatiles (Purcaro *et al.*, 2014; Vargas Jentzsch & Ciobotă, 2014).

Moreover, the adulteration of olive oils with hazelnut oils can be detected in low percentages by the presence of filbertone (Blanch *et al.*, 1998, 2000; Flores *et al.*, 2006).

30.5.2 Cultivar determination

Accumulation of volatiles in olive oil depends greatly on the cultivar, since climatic and environmental conditions affect their formation. Angerosa *et al.* (1999) and Sarolic *et al.* (2014) found that the concentration of (E)-hex-2-enal could be a useful tool for distinguishing different oil cultivars and that the level of each C6 compound depends on cultivar. Classification of EVOOs, according to geographical origin, was successful as reported by Pouliarekou *et al.* (1999) and de los Angeles Fernandez (2014).

30.5.3 Geographical discrimination

The formation of volatiles in olives is affected by both climatic factors and cultivation area characteristics, and therefore can be used for geographical discrimination of EVOOs. Indeed, significant differences were found in the composition of volatile compounds between oils from the same olive cultivar and from different geographic regions (Kiralan *et al.*, 2012; Bajoub *et al.*, 2015b), while according to Kandylis *et al.* (2011) volatile composition of olive oils seems to be primarily dependent on the degree of ripening and geographical region. Table 30.1 shows the contents of 20 volatile compounds used for the discrimination of olive oils from seven north Moroccan regions according to their geographical origin.

Geographical characterization was improved by the application of a combination of electronic nose and electronic tongue on Moroccan VOOs as reported by Haddi *et al.* (2013). Discrimination of PDO EVOOs by blind analysis of HS-SPME-GC profiles of volatiles revealed that the contents of only six volatiles were enough to successfully discriminate samples in terms of geographical origin (Pizarro *et al.*, 2011).

30.6 Olive oil pigments

Chlorophylls and carotenoids constitute the main colorants of olive oils (Moyano *et al.*, 2007), apart from polyphenols, which possess antioxidant and beneficial properties (Moyano *et al.*, 2010; Ghanbari, 2012). The main carotenoids in VOOs are lutein and β-carotene. Another pigment in olive oil is pheophytin-a, while pheophytin-b and chlorophyll-b are present in trace amounts. The routine method for carotene and chlorophyll content determination is HPLC, whereas total determination of each pigment can be achieved by ultraviolet-visible (UV-Vis) absorption spectra.

30.6.1 Adulteration tracing

Since the profile and contents of chlorophylls and carotenes alter easily during EVOO shelf life, only rarely can these compounds be considered markers of adulteration (Psomiadou & Tsimidou, 2002). Gandul-Rojas *et al.* (2000) proposed the ratios of chlorophyll/carotenoids and minor carotenoids/lutein as markers for EVOO authentication, independent of cultivar. The effectiveness of these ratios was verified by Roca *et al.* (2003), Aparicio-Ruiz *et al.* (2009), and Giuffrida *et al.* (2011). Cichelli and Pertesana (2004) proposed the use of the ratio between pheophytin epimer peaks for the identification of olive oil adulteration with natural or synthetic food colorants.

Table 30.1 Content (mean values and standard deviations [SD] in mg/kg) of 21 volatile compound (VOC) variables that presented high discrimination ability of olive oil samples from seven north Moroccan regions.

	Volatile compound	'Chefchaouane'	'Fès'	'Mèknes'	'Ouazzane'	'Sefrou'	'Taounate'	'Taza'
1	Pentan-2-one	0.10 ± 0.02	0.11 ± 0.03	0.16 ± 0.16	0.15 ± 0.04	0.20 ± 0.07	0.07 ± 0.02	0.09 ± 0.03
2	Pentanal	0.01 ± 0.00	0.02 ± 0.01	0.03 ± 0.02	0.03 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.01 ± 0.00
3	Pent-1-en-3-ol	0.50 ± 0.22	0.49 ± 0.21	0.88 ± 0.34	0.33 ± 0.15	0.36 ± 0.20	0.58 ± 0.14	0.30 ± 0.14
4	3-Ethyl-1,5-octadiene IS3	1.70 ± 0.77	1.81 ± 0.60	2.53 ± 0.74	1.16 ± 0.69	1.58 ± 0.55	1.94 ± 0.39	1.35 ± 0.57
5	(Z)-hex-3-enal	0.65 ± 0.47	0.47 ± 0.31	1.00 ± 0.45	0.16 ± 0.16	0.20 ± 0.16	0.52 ± 0.27	0.17 ± 0.16
6	1-Penten-3-ol	0.28 ± 0.11	0.49 ± 0.40	1.98 ± 0.74	0.45 ± 0.35	0.70 ± 0.34	2.08 ± 1.02	0.66 ± 0.47
7	Hexyl acetate	0.05 ± 0.03	0.05 ± 0.02	0.05 ± 0.02	0.07 ± 0.02	0.11 ± 0.05	0.07 ± 0.05	0.09 ± 0.03
8	Hexanal	0.96 ± 0.41	1.12 ± 0.41	1.52 ± 0.46	0.67 ± 0.41	0.71 ± 0.30	1.28 ± 0.28	0.71 ± 0.40
9	(Z)-3-hexen-1-ol acetate	0.84 ± 0.32	0.78 ± 0.23	1.13 ± 0.57	0.67 ± 0.18	0.73 ± 0.18	0.85 ± 0.19	0.59 ± 0.21
10	(E)-hex-3-enol	0.06 ± 0.03	0.06 ± 0.02	0.07 ± 0.06	0.07 ± 0.04	0.05 ± 0.01	0.09 ± 0.05	0.04 ± 0.01
11	(Z)-hex-2-enol	0.20 ± 0.09	0.18 ± 0.06	0.30 ± 0.07	0.13 ± 0.04	0.15 ± 0.06	0.24 ± 0.07	0.12 ± 0.05
12	Ethyl acetate	0.27 ± 0.22	0.22 ± 0.12	0.13 ± 0.08	0.63 ± 0.41	0.46 ± 0.22	0.18 ± 0.06	0.35 ± 0.25
13	2-Methyl-butanal	0.01 ± 0.00	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.07 ± 0.04	0.03 ± 0.01	0.04 ± 0.01
14	3-Methyl-butanal	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.05 ± 0.03	0.02 ± 0.01	0.02 ± 0.01
15	(E)-hex-3-enal	0.53 ± 0.26	0.33 ± 0.13	0.51 ± 0.13	0.21 ± 0.06	0.26 ± 0.07	0.74 ± 0.27	0.33 ± 0.19
16	Ethyl hexanoate	2.75 ± 0.75	3.63 ± 0.92	2.86 ± 1.17	2.41 ± 0.50	2.73 ± 0.63	3.95 ± 0.93	3.37 ± 1.09
17	3-Ethyl-1,5-octadiene IS2	0.29 ± 0.12	0.33 ± 0.08	0.54 ± 0.17	0.24 ± 0.10	0.36 ± 0.07	0.34 ± 0.08	0.21 ± 0.13
18	1-Pentanol	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.03	0.04 ± 0.02	0.05 ± 0.03	0.02 ± 0.01	0.02 ± 0.01
19	(Z)-2-pentenal	0.03 ± 0.01	0.02 ± 0.01	0.05 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.00	0.02 ± 0.00
20	(E)-pent-2-en-1-ol	0.05 ± 0.01	0.06 ± 0.01	0.08 ± 0.03	0.05 ± 0.01	0.07 ± 0.02	0.05 ± 0.01	0.05 ± 0.01

Source: Data acquired from Bajoub *et al.* (2015).

30.6.2 Cultivar determination

Pigment profile and content of EVOOs are highly dependent on the cultivar of source olives (Giuffrida *et al.*, 2007, 2011). Total carotenoids and total chlorophylls have been widely used for varietal discrimination purposes, whereas total chlorophylls presented limited discrimination effect (Sinelli *et al.*, 2010; Smaoui *et al.*, 2012; Sarolic *et al.*, 2014). The chlorophyll/carotenoid ratio and the ratio of minor carotenoids/lutein were found to differentiate among different cultivars (Psomiadou & Tsimidou, 2001).

A better insight on the pigment variability in terms of cultivar was studied by HPLC analysis of individual carotenes and chlorophylls. According to Cichelli and Pertesana (2004), different cultivars led to significant differences in the pigment composition of EVOOs, while geographical origin mainly affected the pigment contents. Gandul-Rojas *et al.* (2000) proposed the distinguishing of VOO cultivars on the basis of three variables: violaxanthin, lutein, and total pigment content. The origin dependence of the variables violaxanthin and lutein was verified by Aparicio-Ruiz *et al.* (2009).

30.6.3 Geographical discrimination

The discrimination efficiency of total carotenoid and chlorophyll contents of EVOOs in terms of geographical discrimination has been studied by several authors in conjunction with other chemical parameters. Significant differences between samples of the same cultivar from different locations were found, where the content of carotenes and chlorophylls was higher in oils originating from the lower altitude location (Dabbou *et al.*, 2010). Moreover, total carotenoid and chlorophyll contents were found to have a significant discriminative effect on western Greek EVOOs from various cultivars according to geographical origin as reported by Karabagias *et al.* (2013). On the contrary, no statistically significant differences were detected by Bijou *et al.* (2014).

30.7 Conclusion

Olive oil's minor components, apart from their nutritional and beneficial properties in human health, can be used for olive oil authenticity. Their presence, profile, and content are unique in olives, and this is reflected in the corresponding oil. Their profile is dictated by the olive cultivar used and can be employed for olive oil authentication in terms of cultivar. Minor components of functional micronutrients are secondary metabolic constituents that are produced as a response to environmental stimulations. Different cultivation areas of olives induce differences in the micronutrient content, which are then reflected in the corresponding olive oil. Moreover, PDO EVOOs are of importance mostly due to their distinct composition of minor components that attribute to olive oil a higher quality. This distinct composition can also be used for their authentication.

References

Agiomyrgianaki, A., Petrakis, P.V., & Dais, P. (2010) Detection of refined olive oil adulteration with refined hazelnut oil by employing NMR spectroscopy and multivariate statistical analysis. Talanta 80, 2165–2171.

Agiomyrgianaki, A., Petrakis, P.V., & Dais, P. (2012) Influence of harvest year, cultivar and geographical origin on Greek extra virgin olive oils composition: a study by NMR spectroscopy and biometric analysis. Food Chemistry 135, 2561–2568.

Aguilera, M.P., Beltrán, G., Ortega, D., Fernández, A., Jiménez, A., & Uceda, M. (2005) Characterisation of virgin olive oil of Italian olive cultivars: 'Frantoio' and 'Leccino', grown in Andalusia. Food Chemistry 89, 387–391.

Alagna, F., Mariotti, R., Panara, F., Caporali, S., Urbani, S., Veneziani, G., Esposto, S., Taticchi, A., Rosati, A., Rao, R., Perrotta, G., Servili, M., & Baldoni, L. (2012) Olive phenolic compounds: metabolic and transcriptional profiling during fruit development. BMC Plant Biology 12, 162–180.

Al-Ismail, K.M., Alsaed, A.K., Ahmad, R., & Al-Dabbas, M. (2010) Detection of olive oil adulteration with some plant oils by GLC analysis of sterols using polar column. Food Chemistry 121, 1255–1259.

Alonso-Salces, R.M., Héberger, K., Holland, M.V., Moreno-Rojas, J.M., Mariani, C., Bellan, G., Reniero, F., & Guillou, C. (2010) Multivariate analysis of NMR fingerprint of the unsaponifiable fraction of virgin olive oils for authentication purposes. Food Chemistry 118, 956–965.

Alves, M.R., Cunha, S.C., Amaral, J.S., Pereira, J.A., & Oliveira, M.B. (2005) Classification of PDO olive oils on the basis of their sterol composition by multivariate analysis. Analytica Chimica Acta 549, 166–178.

Amaral, S.J., Mafra, I., & Oliveira, P. (2010) Characterization of three Portuguese varietal olive oils based on fatty acids, triacylglycerols, phytosterols and vitamin E profiles: application of chemometrics. In: Olives and olive oil in health and disease prevention, ed. V.R. Preedy & R.R. Watson. Academic Press, Oxford, 581–589.

Angerosa, F., Basti, C., & Vito, R. (1999) Virgin olive oil volatile compounds from lipoxygenase pathway and characterization of some Italian cultivars. Journal of Agricultural and Food Chemistry 47, 836–839.

Angerosa, F., Servili, M., Selvaggini, R., Taticchi, A., Esposto, S., & Montedoro, G. (2004) Volatile compounds in virgin olive oil: Occurrence and their relationship with the quality. Journal of Chromatography A 1054, 17–31.

Antonini, E., Farina, A., Leone, A., Mazzara, E., Urbani, S., Selvaggini, R., Servili, M., & Ninfali, P. (2015) Phenolic compounds and quality parameters of family farming versus protected designation of origin (PDO) extra-virgin olive oils. Journal of Food Composition and Analysis 43, 75–81.

Aparicio, R., & Aparicio-Ruíz, R. (2000) Authentication of vegetable oils by chromatographic techniques. Journal of Chromatography A 881, 93–104.

Aparicio-Ruiz, R., Gandul-Rojas, B., & Roca, M. (2009) Pigment profile in non-Spanish olive varieties (*Olea europaea* L. Var. Coratina, Frantoio, and Koroneiki). Journal of Agricultural and Food Chemistry 57, 10831–10836.

Bagur-González, M.G., Pérez-Castaño, E., Sánchez-Viñas, M., & Gázquez-Evangelista, D. (2015) Using the liquid-chromatographic-fingerprint of sterols fraction to discriminate virgin olive from other edible oils. Journal of Chromatography A 1380, 64–70.

Bajoub, A., Carrasco-Pancorbo, A., Ajal, E.A., Beltrán Maza, G., Fernández-Gutiérrez, A., & Ouazzani, N. (2014) Contribution to the establishment of a protected designation of origin for Meknès virgin olive oil: a 4-year study of its typicality. Food Research International 66, 332–343.

Bajoub, A., Carrasco-Pancorbo, A., Ajal, E.A., Ouazzani, N., & Fernández-Gutiérrez, A. (2015a) Potential of LC-MS phenolic profiling combined with multivariate analysis as an approach for the determination of the geographical origin of north Moroccan virgin olive oils. Food Chemistry 166, 292–300.

Bajoub, A., Sánchez-Ortiz, A., Ajal, E.A., Ouazzani, N., Fernández-Gutiérrez, A., Beltrán, G., & Carrasco-Pancorbo, A. (2015) First comprehensive characterization of volatile profile of north Moroccan olive oils: A geographic discriminant approach. Food Research International 76, 410–417.

Bakhouche, A., Lozano-Sánchez, J., Beltrán-Debón, R., Joven, J., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2013) Phenolic characterization and geographical classification of commercial Arbequina extra-virgin olive oils produced in southern Catalonia. Food Research International 50, 401–408.

Bakre, S.M., Gadmale, D.K., Toche, R.B., & Gaikwad, V.B. (2014) Rapid determination of alpha tocopherol in olive oil adulterated with sunflower oil by reversed phase high-performance liquid chromatography. Journal of Food Science and Technology 52, 3093–3098.

Beltrán, G., Jiménez, A., del Rio, C., Sánchez, S., Martínez, L., Uceda, M., & Aguilera, M.P. (2010) Variability of vitamin E in virgin olive oil by agronomical and genetic factors. Journal of Food Composition and Analysis 23, 633–639.

Ben Temime, S., Baccouri, B., Taamalli, W., Abaza, L., Daoud, D., & Zarrouk, M. (2006) Location effects on oxidative stability of Chétoui virgin olive oil. Journal of Food Biochemistry 30, 659–670.

Blanch, G.P., Caja, M.M., León, M., & Herraiz, M. (2000) Determination of (E)-5-methylhept-2-en-4-one in deodorised hazelnut oil. Application to the detection of adulterated olive oils. Journal of the Science of Food and Agriculture 80, 140–144.

Blanch, G.P., Del Mar Caja, M., Del Castillo, M.L.R., & Herraiz, M. (1998) Comparison of different methods for the evaluation of the authenticity of olive oil and hazelnut oil. Journal of Agricultural and Food Chemistry 46, 3153–3157.

Bramley, P., Elmadfa, I., Kafatos, A., Kelly, F., Manios, Y., Roxborough, H., Schuch, W., Sheehy, P., & Wagner, K.H. (2000) Vitamin-E. Journal of the Science of Food and Agriculture 80, 913–938.

Cañabate-Díaz, B., Segura Carretero, A., Fernández-Gutiérrez, A., Belmonte Vega, A., Garrido Frenich, A., Martínez Vidal, J.L., & Duran Martos, J. (2007) Separation and determination of sterols in olive oil by HPLC-MS. Food Chemistry 102, 593–598.

Casas, J.S., De Miguel Gordillo, C., Osorio Bueno, E., Marín Expósito, J., Mendoza, M.F., Hierro, T.A., González, L.G., & Martínez Cano, M. (2009) Characteristics of virgin olive oils from the olive zone of Extremadura (Spain), and an approximation to their varietal origin. Journal of the American Oils Chemists' Society 86, 933–940.

Cayuela, J.A., Gómez-Coca, R.B., Moreda, W., & Pérez-Camino, M.C. (2015) Sensory defects of virgin olive oil from a microbiological perspective. Trends in Food Science and Technology 43, 227–235.

Chen, H., Angiuli, M., Ferrari, C., Tombari, E., Salvetti, G., & Bramanti, E. (2011) Tocopherol speciation as first screening for the assessment of extra virgin olive oil quality by reversed-phase high-performance liquid chromatography/fluorescence detector Food Chemistry 125, 1423–1429.

Chtourou, M., Gargouri, B., Jaber, H., Abdelhedi, R., & Bouaziz, M. (2013) Comparative study of olive oil quality from Chemlali Sfax versus Arbequina cultivated in Tunisia European. Journal of Lipid Science and Technology 115, 631–640.

Cichelli, A., & Pertesana, G.P. (2004) High-performance liquid chromatographic analysis of chlorophylls, pheophytins and carotenoids in virgin olive oils: chemometric approach to variety classification. Journal of Chromatography A 1046, 141–146.

Dabbou, S., Sifi, S., Rjiba, I., Esposto, S., Taticchi, A., Servili, M., Montedoro, G.F., & Hammami, M. (2010) Effect of pedoclimatic conditions on the chemical composition of the Sigoise olive cultivar. Chemistry and Biodiversity 7, 898–908.

Dag, C., Demirtas, I., Ozdemir, I., Bekiroglu, S., & Ertas, E. (2015) Biochemical characterization of Turkish extra virgin olive oils from six different olive varieties of identical growing conditions. Journal of the American Oils Chemists' Society 92, 1349–1356.

Damirchi, S.A., Savage, G.P., & Dutta, P.C. (2005) Sterol fractions in hazelnut and virgin olive oils and 4,4′-dimethylsterols as possible markers for detection of adulteration of virgin olive oil. Journal of the American Oils Chemists' Society 82, 717–725.

De los Angeles Fernandez, M., Assof, M., Jofre, V., & Silva, M.F. (2014) Volatile profile characterization of extra virgin olive oils from Argentina by HS-SPME/GC-MS and multivariate pattern recognition tools. Food Analytical Methods 7, 2122–2136.

Dionisi, F., Prodolliet, J., & Tagliaferri, E. (1995) Assessment of olive oil adulteration by reversed-phase high-performance liquid chromatography amperometric detection of tocopherols and tocotrienols. Journal of the American Oils Chemists' Society 72, 1505–1511.

Douzane, M., Tamendjari, A., Abdi, A.K., Daas, M-S., Mehdid, F., & Bellal, M.M. (2013) Phenolic compounds in mono-cultivar extra virgin olive oils from Algeria. Grasas y Aceites 64, 285–294.

El Riachy, M., Priego-Capote, F., León, L., Luque de Castro, M.D., & Rallo, L. (2012) Virgin olive oil phenolic profile and variability in progenies from olive crosses. Journal of the Science of Food and Agriculture 92, 2524–2533.

Ferreiro, L., & Aparicio, R. (1992) Influencia de la altitud en la composición química de los aceites de oliva vírgenes de Andalucía. Ecuaciones matemáticas de clasificación. Grasas y Aceites 43, 149–156.

Flores, G., Ruiz Del Castillo, M.L., Herraiz, M., & Blanch, G.P. (2006) Study of the adulteration of olive oil with hazelnut oil by on-line coupled high performance liquid chromatographic and gas chromatographic analysis of filbertone. Food Chemistry 97, 742–749.

Foo Wong, Y., Makahleh, A., Saad, B., Ibrahim, M.N.M., Abdul Rahim, A., & Brosse, N. (2014) UPLC method for the determination of vitamin e homologues and derivatives in vegetable oils, margarines and supplement capsules using pentafluorophenyl column. Talanta 130, 299–306.

Franco, M.N., Galeano-Díaz, T., López, O., Fernández-Bolaños, J.G., Sánchez, J., De Miguel, C., Gil, M.V., & Martín-Vertedor, D. (2014a) Phenolic compounds and antioxidant capacity of virgin olive oil. Food Chemistry 163, 289–298.

Franco, M.N., Galeano-Díaz, T., Sánchez, J., De Miguel, C., & Martín-Vertedor, D. (2014b) Total phenolic compounds and tocopherols profiles of seven olive oil varieties grown in the south-west of Spain. Journal of Oleo Science 63, 115–125.

Gandul-Rojas, B., Cepero, M.R.-L., & Mínguez-Mosquera, M.I. (2000) Use of chlorophyll and carotenoid pigment composition to determine authenticity of virgin olive oil. Journal of the American Oil Chemists' Society 77, 853–858.

Gázquez-Evangelista, D., Pérez-Castaño, E., Sánchez-Viñas, M., & Bagur-González, M.G. (2014) Using offline HPLC-GC-FID 4-desmethylsterols concentration profiles, combined with chemometric tools, to discriminate different vegetable oils. Food Analytical Methods 7, 912–925.

Ghanbari, R., Anwar, F., Alkharfy, K.M., Gilani, A.-H., & Saari, N. (2012) Valuable nutrients and functional bioactives in different parts of olive (*Olea europaea* L.): a review. International Journal of Molecular Sciences, 13, 1291–1340.

Giacalone, R., Giuliano, S., Gulotta, E., Monfreda, M., & Presti, G. (2015) Origin assessment of EV olive oils by esterified sterols analysis. Food Chemistry 188, 279–285.

Gilbert-López, B., Valencia-Reyes, Z.L., Yufra-Picardo, V.M., García-Reyes, J.F., Ramos-Martos, N., & Molina-Díaz, A. (2014) Determination of polyphenols in commercial extra virgin olive oils from different origins (Mediterranean and South American countries) by liquid chromatography–electrospray time-of-flight mass spectrometry. Food Analytical Methods 7, 1824–1833.

Giuffrida, D., Salvo, F., Salvo, A., Cossignani, L., & Dugo, G. (2011) Pigments profile in monovarietal virgin olive oils from various Italian olive varieties. Food Chemistry 124, 1119–1123.

Giuffrida, D., Salvo, F., Salvo, A., Pera, L.L., & Dugo, G. (2007) Pigments composition in monovarietal virgin olive oils from various Sicilian olive varieties. Food Chemistry 101, 833–837.

Guerfel, M., Ouni, Y., Taamalli, A., Boujnah, D., Stefanoudaki, E., & Zarrouk, M. (2009) Effect of location on virgin olive oils of the two main Tunisian olive cultivars. European Journal of Lipid Science and Technology 111, 926–932.

Haddi, Z., Alami, H., El Bari, N., Tounsi, M., Barhoumi, H., Maaref, A., Jaffrezic-Renault, N., & Bouchikhi, B. (2013) Electronic nose and tongue combination for improved classification of Moroccan virgin olive oil profiles. Food Research International 54, 1488–1498.

Hassine, K.B., Taamalli, A., Slama, M.B., Khouloud, T., Kiristakis, A., Benincasa, C., Perri, E., Malouche, D., Hammami, M., Bornaz, S., & Grati-Kammoun, N. (2015) Characterization and preference mapping of autochthonous and introduced olive oil cultivars in Tunisia. European Journal of Lipid Science and Technology 117, 112–121.

Hirri, A., Bassbasi, M., Platikanov, S., Tauler, R., & Oussama, A. (2015) FTIR spectroscopy and PLS-DA classification and prediction of four commercial grade virgin olive oils from Morocco. Food Analytical Methods 8. Article in Press DOI:10.1007/s12161-015-0255-y

Jiménez, B., Sánchez-Ortiz, A., Lorenzo, M.L., & Rivas, A. (2013) Influence of fruit ripening on agronomic parameters, quality indices, sensory attributes and phenolic compounds of Picudo olive oils. Food Research International 54, 1860–1867.

Jukić Špika, M., Kraljić, K., Koprivnjak, O., Škevin, D., Žanetić, M., & Katalinić, M. (2015) Effect of Agronomical Factors and Storage Conditions on the Tocopherol Content of Oblica and Leccino Virgin Olive Oils. Journal of the American Oil Chemists' Society 92, 1293–1301.

Kalua, C.M., Allen, M.S., Bedgood Jr., D.R., Bishop, A.G., Prenzler, P.D., & Robards, K. (2007) Olive oil volatile compounds, flavour development and quality: a critical review. Food Chemistry 100, 273–286.

Kandylis, P., Vekiari, A.S., Kanellaki, M., Grati Kamoun, N., Msallem, M., & Kourkoutas, Y. (2011) Comparative study of extra virgin olive oil flavor profile of Koroneiki variety (*Olea europaea* var. Microcarpa alba) cultivated in Greece and Tunisia during one period of harvesting. LWT – Food Science and Technology 44, 1333–1341.

Karabagias, I., Michos, C., Badeka, A., Kontakos, S., Stratis, I., & Kontominas, M.G. (2013) Classification of Western Greek virgin olive oils according to geographical origin based on chromatographic, spectroscopic, conventional and chemometric analyses Food Research International 54, 1950–1958.

Kiralan, M., Ozkan, G., Koyluoglu, F., Ugurlu, H.A., Bayrak, A., & Kiritsakis, A. (2012) Effect of cultivation area and climatic conditions on volatiles of virgin olive oil. European Journal of Lipid Science and Technology 114, 552–557.

Kiritsakis, A.K. (1998) Flavor components of olive oil – a review. Journal of the American Oils Chemists' Society 75, 673–681.

Lerma-García, M.J., Lantano, C., Chiavaro, E., Cerretani, L., Herrero-Martínez, J.M., & Simó-Alfonso, E.F. (2009) Classification of extra virgin olive oils according to their geographical origin using phenolic compound profiles obtained by capillary electrochromatography. Food Research International 42, 1446–1452.

Lerma-García, M.J., Simó-Alfonso, E.F., Méndez, A., Lliberia, J.L., & Herrero-Martínez, J.M. (2011) Classification of extra virgin olive oils according to their genetic variety using linear discriminant analysis of sterol profiles established by ultra-performance liquid chromatography with mass spectrometry detection. Food Research International 44, 103–108.

Lerma-García, M.J., Simó-Alfonso, E.F., Ramis-Ramos, G., & Herrero-Martínez, J.M. (2007) Determination of tocopherols in vegetable oils by CEC using methacrylate ester-based monolithic columns. Electrophoresis 28, 4128–4135.

Longobardi, F., Ventrella, A., Casiello, G., Sacco, D., Catucci, L., Agostiano, A., & Kontominas, M.G. (2012a) Instrumental and multivariate statistical analyses for the characterisation of the geographical origin of Apulian virgin olive oils. Food Chemistry 133, 579–584.

Longobardi, F., Ventrella, A., Casiello, G., Sacco, D., Tasioula-Margari, M., Kiritsakis, A.K., & Kontominas, M.G. (2012b) Characterisation of the geographical origin of Western Greek virgin olive oils based on instrumental and multivariate statistical analysis. Food Chemistry 133, 169–175.

López-Cortés, I., Salazar-García, D.C., Velázquez-Martí, B., & Salazar, D.M. (2013) Chemical characterization of traditional varietal olive oils in East of Spain. Food Research International 54, 1934–1940.

Lukić, M., Lukić, I., Krapac, M., Sladonja, B., & Piližota, V. (2013) Sterols and triterpene diols in olive oil as indicators of variety and degree of ripening. Food Chemistry 136, 251–258.

Mariani, C., Bellan, G., Lestini, E., & Aparicio, R. (2006) The detection of the presence of hazelnut oil in olive oil by free and esterified sterols. European Food Research and Technology 223, 655–661.

Matos, L.C., Cunha, S.C., Amaral, J.S., Pereira, J.A., Andrade, P.B., Seabra, R.M., & Oliveira, B.P.P. (2007) Chemometric characterization of three varietal olive oils (Cvs. Cobrançosa, Madural and Verdeal Transmontana) extracted from olives with different maturation indices. Food Chemistry 102, 406–414.

Morchio G., A. Pellegrino, C. Mariani, & Bellan, G. (1999) Detection of hazelnut oil in olive oil. Note II. Rivista Italiana delle Sostanze Grasse 76, 115–127.

Moyano, M.J., Heredia, F.J., & Meléndez-Martínez, A.J. (2010) The color of olive oils: The pigments and their likely health benefits and visual and instrumental methods of analysis. Comprehensive Reviews in Food Science and Food Safety 9, 278–291.

Moyano, M.J., Meléndez-Martínez, A.J., Alba, J., & Heredia, F.J. (2008) A comprehensive study on the colour of virgin olive oils and its relationship with their chlorophylls and carotenoids indexes (I): CIEXYZ non-uniform colour space. Food Research International 41, 505–512.

Nescatelli, R., Bonanni, R.C., Bucci, R., Magrì, A.L., Magrì, A.D., & Marini, F. (2014) Geographical traceability of extra virgin olive oils from Sabina PDO by chromatographic fingerprinting of the phenolic fraction coupled to chemometrics. Chemometrics and Intelligent Laboratory Systems 139, 175–180.

Ouni, Y., Flamini, G., Youssef, N.B., Guerfel, M., & Zarrouk, M. (2011a) Sterolic composition and triacylglycerols of Oueslati virgin olive oil: Comparison among different geographic areas. International Journal of Food Science and Technology 46, 1747–1754.

Ouni, Y., Taamalli, A., Gómez-Caravaca, A.M., Segura-Carretero, A., Fernández-Gutiérrez, A., & Zarrouk, M. (2011b) Characterisation and quantification of phenolic compounds of extra-virgin olive oils according to their geographical origin by a rapid and resolutive LC-ESI-TOF MS method. Food Chemistry 127, 1263–1267.

Parcerisa, J., Casals, I., Boatella, J., Codony, R., & Rafecas, M. (2000) Analysis of olive and hazelnut oil mixtures by high-performance liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry of triacylglycerols and gas-liquid chromatography of non-saponifiable compounds (tocopherols and sterols). Journal of Chromatography A 881, 149–158.

Petrakis, P.V., Agiomyrgianaki, A., Christophoridou, S., Spyros, A., & Dais, P. (2008) Geographical characterization of Greek virgin olive oils (cv. Koroneiki) using 1H and 31P NMR fingerprinting with canonical discriminant analysis and classification binary trees. Journal of Agricultural and Food Chemistry 56, 3200–3207.

Piravi-Vanak, Z., Ghasemi, J.B., Ghavami, M., Ezzatpanah, H., & Zolfonoun, E. (2012) The influence of growing region on fatty acids and sterol composition of iranian olive oils by unsupervised clustering methods. Journal of the American Oils Chemists' Society 89, 371–378.

Pizarro, C., Rodríguez-Tecedor, S., Pérez-del-Notario, N., & González-Sáiz, J.M. (2011) Recognition of volatile compounds as markers in geographical discrimination of Spanish extra virgin olive oils by chemometric analysis of non-specific chromatography volatile profiles. Journal of Chromatography A 1218, 518–523.

Pouliarekou, E., Badeka, A., Tasioula-Margari, M., Kontakos, S., Longobardi, F., & Kontominas, M.G. (2011) Characterization and classification of Western Greek olive oils according to cultivar and geographical origin based on volatile compounds. Journal of Chromatography A 1218, 7534–7542.

Psomiadou, E., & Tsimidou, M. (2002) Stability of virgin olive oil. 1. Autoxidation studies. Journal of Agricultural and Food Chemistry 50, 716–721.

Purcaro, G., Barp, L., Beccaria, M., & Conte, L.S. (2015) Fingerprinting of vegetable oil minor components by multidimensional comprehensive gas chromatography with dual detection. Analytical and Bioanalytical Chemistry 407, 309–319.

Purcaro, G., Cordero, C., Liberto, E., Bicchi, C., & Conte, L.S. (2014) Towards a definition of blueprint of virgin olive oil by comprehensive two-dimensional gas chromatography. Journal of Chromatography A 1334, 101–111.

Ranalli, A., De Mattia, G., Patumi, M., & Proietti, P. (1999) Quality of virgin olive oil as influenced by origin area. Grasas y Aceites 50, 249–259.

Rigane, G., Boukhris, M., Bouaaziz, M., Sayadi, S., & Salem, R.B. (2013) Analytical evaluation of two monovarietal virgin olive oils cultivated in the south of Tunisia: Jemri-Bouchouka and Chemlali-Tataouin cultivars. Journal of the Science of Food and Agriculture 93, 1242–1248.

Roca, M., Gandul-Rojas, B., Gallardo-Guerrero, L., & Mínguez-Mosquera, M.I. (2003) Pigment parameters determining Spanish virgin olive oil authenticity: stability during storage. Journal of the American Oils Chemists' Society 80, 1237–1240.

Romero, N., Saavedra, J., Tapia, F., Sepúlveda, B., & Aparicio, R. (2015) Influence of agroclimatic parameters on phenolic and volatile compounds of Chilean virgin olive oils and characterization based on geographical origin, cultivar and ripening stage. Journal of the Science of Food and Agriculture. DOI:10.1002/jsfa.7127

Sánchez Casas, J., Osorio Bueno, E., Montaño García, A.M., & Martinez Cano, M. (2004) Sterol and erythrodiol + uvaol content of virgin olive oils from cultivars of Extremadura (Spain). Food Chemistry 8, 225–230.

Sánchez De Medina, V., Priego-Capote, F., & De Castro, M.D.L. (2015) Characterization of monovarietal virgin olive oils by phenols profiling. Talanta 132, 424–432.

Sánchez de Medina, V., Priego-Capote, F., & Luque de Castro, M.D. (2014) Phenolic profile and fatty acid composition influence the quality of virgin olive oils from olive breeding programs. European Journal of Lipid Science and Technology 117, 954–966.
Sarolic, M., Gugic, M., Tuberoso, C.I.G., Jerkovic, I., Suste, M., Marijanovic, Z., & Kus, P.M. (2014) Volatile profile, phytochemicals and antioxidant activity of virgin olive oils from Croatian autochthonous varieties masnjaca and krvavica in comparison with Italian variety leccino. Molecules 19, 881–895.
Sayago, A., Marín, M.I., Aparicio, R., & Morales, M.T. (2007) Vitamin E and vegetable oils. Grasas y Aceites 58, 74–86.
Schröder, M., & Vetter, W. (2012) Investigation of unsaponifiable matter of plant oils and isolation of eight phytosterols by means of high-speed counter-current chromatography. Journal of Chromatography A 1237, 96–105.
Sena-Moreno, E., Alvarez-Ortí, M., Zied, D.C., Pardo-Giménez, A., & Pardo, J.E. (2015) Olive oils from Campos de Hellin (Spain) exhibit significant varietal differences in fatty acid composition, sterol fraction, and oxidative stability. European Journal of Lipid Science and Technology 117, 967–975.
Sinelli, N., Casale, M., Di Egidio, V., Oliveri, P., Bassi, D., Tura, D., & Casiraghi, E. (2010) Varietal discrimination of extra virgin olive oils by near and mid infrared spectroscopy. Food Research International 43, 2126–2131.
Smaoui, S., Hlima, H.B., Jarraya, R., Kamoun, N.G., Ellouze, R., & Damak, M. (2012) Physico-chemical characterization and analysis of various Tunisian olive oil varieties: Minor compounds and fatty acids. Journal of the Chemical Society of Pakistan 34, 491–498.
Taamalli, A., Arráez Román, D., Zarrouk, M., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2012) Classification of 'Chemlali' accessions according to the geographical area using chemometric methods of phenolic profiles analysed by HPLC-ESI-TOF-MS. Food Chemistry 132, 561–566.
Temime, S.B., Manai, H., Methenni, K., Baccouri, B., Abaza, L., Daoud, D., Casas, J.S., Bueno, E.O., & Zarrouk, M. (2008) Sterolic composition of Chétoui virgin olive oil: Influence of geographical origin. Food Chemistry 110, 368–374.
Tura, D., Gigliotti, C., Pedò, S., Failla, O., Bassi, D., & Serraiocco, A. (2007) Influence of cultivar and site of cultivation on levels of lipophilic and hydrophilic antioxidants in virgin olive oils (*Olea europea* L.) and correlations with oxidative stability. Scientia Horticulturae 112, 108–119.
Vargas Jentzsch, P., & Ciobotă, V. (2014) Raman spectroscopy as an analytical tool for analysis of vegetable and essential oils. Flavour and Fragrance Journal 29, 287–295.
Vichi, S., Pizzale, L., Toffano, E., Bortolomeazzi, R., & Conte, L. (2001) Detection of hazelnut oil in virgin olive oil by assessment of free sterols and triacylglycerols. Journal of AOAC International 84, 1534–1541.

31 New analytical trends for the measurement of phenolic substances of olive oil and olives with significant biological and functional importance related to health claims

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31.1 Introduction

Olive oil and table olives are basic ingredients of the traditional Mediterranean diet, and a major reason for the interest of the scientific community is their health-protecting activities (Pérez-Jiménez *et al.*, 2007; Frankel, 2011; Visioli & Bernardini, 2011).

One of the most important changes in the olive and related products in the recent past is that the European Union (EU) legislation, based on the scientific opinion of the European Food Safety Authority (EFSA) (EFSA, 2011), has permitted specific health claims related to the levels of specific phenolic compounds found in olive oil (5 mg per 20 g dose, or 250 mg/kg). The key compounds that are responsible for the recognized health claim of EFSA related to the protection of blood lipids from oxidative stress are hydroxytyrosol, tyrosol, and their derivatives.

The EU legislation about the health claim of olive oil requires accurate measurement of the levels of specific bioactive compounds in olive oil. However, as of today, there is no officially adopted method for their measurement because of well-known technical difficulties that will be discussed in this chapter.

It should briefly be mentioned that there are several works concerning the chromatographic analysis of bioactive compounds (using high-performance liquid chromatography [HPLC] or gas chromatography [GC]) (Beauchamp *et al.*, 2005; Impellizzeri & Lin, 2005; Bendini *et al.*, 2007; Kanakis *et al.*, 2013), but their accuracy is questionable (Karkoula *et al.*, 2012, 2014) because oleocanthal, oleacein, and the oleuropein and ligstroside aglycons react with the stationary phases as well as with methanol and water, which are commonly and officially used for extraction of polyphenols and as the mobile phase during their analysis, according to the International Olive Council (IOC, 2009). These reactions lead to the formation of several artifacts that make analysis very difficult. To overcome these problems that make chromatographic analysis complicated, other alternative methods have recently been developed based on quantitative nuclear magnetic resonance (qNMR) (Karkoula *et al.*, 2012, 2014) or new colorimetric reactions (Magiatis & Melliou, 2015).

31.2 Phenolic compounds of olive oil with special importance

Figure 31.1 (**1**–**14**) shows the different compounds of olive oil with the recognized protection effect of low-density lipoprotein (LDL) oxidation. Hydroxytyrosol (**1**) and tyrosol (**2**) are found in olive oil, mainly as the

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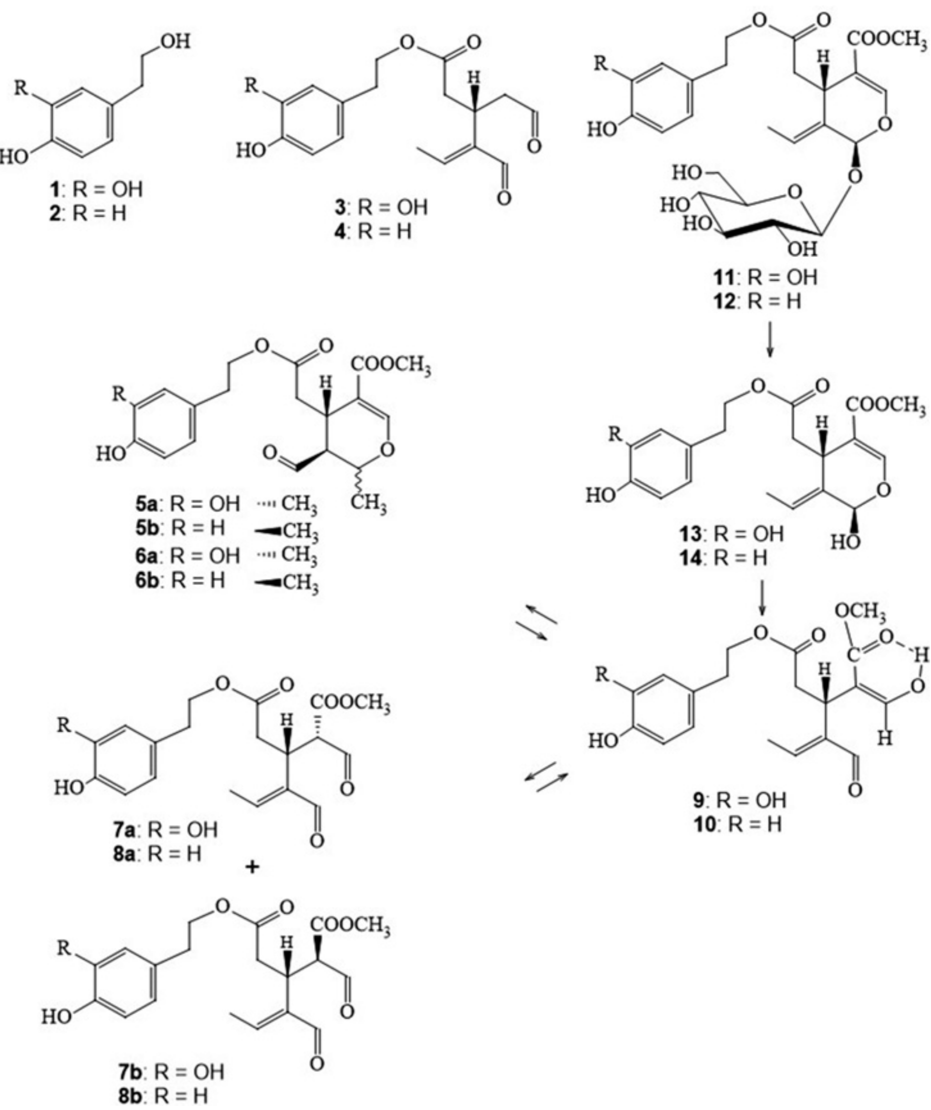

Figure 31.1 Different compounds of olive oil with recognized protection effect of low-density lipoprotein (LDL) oxidation.

esterified derivatives as oleacein (3,4-DHPEA-EDA) (**3**), oleocanthal (p-HPEA-EDA) (**4**), the monoaldehydic form of oleuropein aglycon (3,4-DHPEA-EA) (**5a–b**), the monoaldehydic form of ligstroside aglycon (p-HPEA-EA) (**6a–b**) (Karkoula *et al.*, 2012, 2014) and the recently described (Diamantakos *et al.*, 2015) oleuropeindial (dialdehydic form of oleuropein aglycon) (**7a–b**) and ligstrodial (dialdehydic form of ligstroside aglycon) (**8a–b**), oleomissional (enolic form of oleuropein aglycon) (**9**), and oleokoronal (enolic form of ligstroside aglycon) (**10**). All secoiridoid phenolic derivatives in olive oil originate from oleuropein (**11**) and ligstroside (**12**), which are the major secoiridoids in the olive fruit. During crushing and malaxation in the production of olive oil, those two compounds come in contact with β-glucosidase (Koudounas *et al.*, 2015) and are initially transformed to the corresponding real aglycons (**13** and **14**). These two forms are unstable and have been observed only under very specific conditions (Christophoridou & Dais, 2009). Inside the matrix of olive oil or generally in a non-aqueous medium, the real aglycons (**13** and **14**) do not exist and

are mainly transformed to the more stable closed-ring monoaldehydic forms (**5a** and **6a**) by rearrangement (Limiroli *et al.*, 1995) or to open-ring dialdehydic forms (**7**–**10**). Meanwhile, demethylation and decarboxylation of the dialdehydic forms during malaxation lead to oleacein (**3**) and oleocanthal (**4**).

All of the compounds discussed here show a significant effect in protecting LDL against oxidation, but some of them, especially oleocanthal and oleacein, possess other significant biological activities (e.g. anti-inflammatory and neuroprotective), as summarized by Cicerale *et al.* (2012).

The different methods of extraction and determination of olive oil phenolic compounds are described in the subsequent sections of this chapter.

31.2.1 Extraction methods of phenolic compounds from olive oil

The extraction of phenolic compounds from a sample of olive oil is a very important step for subsequent analyses. The oil extracts usually contain a complex and heterogeneous mixture of phenolic constituents, which makes the selection of the appropriate extraction process a critical step. The aim of the extraction procedure is to obtain the desired polar phenolic fraction from the total mixture with the highest possible efficiency, and to avoid possible structural changes that can occur in the compounds initially present in the extract due to hydrolysis, oxidation, or isomerization.

Many methods have been proposed for the extraction of the polar phenolic constituents of the oil, most of which are based on liquid–liquid extraction (LLE) and solid phase extraction (SPE). These methods have been used with many variations by different researchers, who use a variety of extraction solvent systems depending on the objective of their studies.

31.2.1.1 Liquid–liquid extraction

LLE has commonly been used for the extraction of the phenolic fraction of the oil. In a typical protocol, a quantity of oil is dissolved in a lipophilic solvent, usually hexane, and the phenolic fraction is extracted with methanol or a methanol–water mixture (water concentration from 0 to 40%). Extraction is carried out either by simple stirring or by sonication for a few minutes. The lipophilic and the methanolic–aqueous phases are separated by centrifugation. The two phases are separated, and the methanolic–aqueous phase is evaporated using a rotary evaporator. The extraction with hexane is repeated to achieve better degreasing, and the extract is obtained again after evaporation. The use of a suitable solvent system is a first step that greatly affects the performance of the method as well as the nature of the solvent selected, and the relative proportions of the solvents determine the recovery of polyphenols in the final extract. In addition, other solvents such as petroleum ether and chloroform have been suggested. However, the addition of hexane or other organic solvents in the oil prior to extraction does not lead to a significant improvement in the recovery of the polyphenolic components (Tasioula-Margari & Okogeri, 2001).

The use of ultrasound as compared with the simple stirring has been studied by Jerman-Klen and Mozetič-Vodopivec (2012). The results showed that although the simple stirring gave satisfactory results, the use of ultrasound during the extraction led to optimum recovery of the phenolic fraction. In the same contribution, a variant of this method by cooling the sample for 2–3 h to −25 °C was presented. Using this modification, a better removal of the nonpolar fraction was achieved. Furthermore, Montedoro *et al.* (1992) reported several extraction methods for nonhydrolyzable and hydrolyzable phenolic compounds in virgin olive oil, using different solvent systems to test the material ratio and other conditions. It was concluded that the optimal solvent system was methanol–water (80:20, v/v). However, Angerosa *et al.* (1995) later showed opposite results. The incomplete recovery of some components and the formation of emulsion between water and methanol resulted in selection of pure methanol for extraction.

Cortesi *et al.* (1995) tried to extract the polar components of the oil using tetrahydrofuran (THF)–water (80:20, v/v) followed by centrifugation; thus, the recovery was five times greater for hydroxytyrosol and twice for tyrosol relative to the methanol–water system (60:40, v/v). The use of N,N-dimethylformamide was shown to have interesting results regarding the recovery of phenolic components. After the process of LLE and in order to isolate the desired analytes from interfering species, the residual oil was removed by storing at ambient temperature overnight and further centrifugation or extraction with hexane.

Karkoula *et al.* (2012, 2014) used cyclohexane as a lipophilic solvent and acetonitrile for the recovery of the phenolic fraction. In this protocol, 5 g of oil were dissolved in 20 ml cyclohexane, and the solution was agitated for 1 min. Twenty-five milliliters of acetonitrile were then added, and the mixture was agitated for 1 min. The separation of the two phases was achieved by centrifugation for 5 min. The use of acetonitrile, as will be explained later in this chapter, overrides the problem of the artificial formation of derivatives that may be produced by reaction with methanol or water.

31.2.1.2 Solid phase extraction

The great flexibility of SPE led researchers to use this technique to recover phenolic compounds from olive oil. The widespread use of this technique also provided opportunity for use of many solvent systems, both for isolation and for purification of the extract. SPE includes two modes, the normal phase and the reversed phase. Different sorbents have been used, with C18 cartridges being the most common. Ion exchange cartridges have also been used for the isolation of phenolic fractions from various oils, but the recoveries obtained were quite low (53–62%). Amino phase and diol-bonded cartridges, which are in the middle between silica normal and reverse phase and also are compatible with a wide range of solvents, have been used (Pirisi *et al*., 2000; Mateos *et al*., 2001; Rios *et al*., 2005). C18 is a reliable material for the isolation of simple phenolic substances. However, the recovery is low in the case of secoiridoid derivatives and mainly their dialdehyde forms. In a recent study, 15 phenolic compounds were isolated for studying their recoveries for each analyte, and it was found that LLE gave the best recovery of phenolics. Liberatore *et al.* (2001) compared different sorbents of SPE with LLE.

31.2.1.3 Liquid-liquid microextraction

In an effort to compare the methods based on LLE, liquid-liquid microextraction (LLME), and SPE, Pizarro *et al.* (2012) compared these methods with ultrasonication extraction (USE). The results showed that the application of USE had the highest rate of recovery; however, they observed that the differences of the respective results from the LLME process were not significant. Taking into account that microextraction has less solvent consumption and needs less time, this method seems to be most suitable for the extraction of phenolic fractions of olive oil (Pizarro *et al.*, 2012).

31.2.2 Quantitative measurement of phenolic compounds in olive oil

The next step in oil analysis is a qualitative and/or quantitative determination of the phenolic constituents of the polar extract. The various methods that have been developed are mainly based on spectrophotometry or chromatography, as detailed in this section.

31.2.2.1 Photometric measurement

A most popular method that is commonly used for quantitation of total phenolics in olive oil is based on the Folin-Ciocalteu reagent that reacts with the hydroxyl groups of the phenolic compounds (Singleton & Rossi, 1965; Gutfinger, 1981). The method includes calibration using a pure standard (e.g., caffeic acid or gallic acid), extracting phenols from the sample, and measuring the absorbance at 500–750 nm after the colorimetric reaction. The widespread use of this method is attributed to its simplicity, ease, and speed of analysis (Blekas *et al.*, 2002). However, the drawback of this colorimetric method is its low selectivity, as the color reaction is performed with any phenolic hydroxyl group of the oil components or other oxidizable functional groups. For this reason, the Folin-Ciocalteu method cannot discriminate oils that contain free or esterified forms of tyrosol or hydroxytyrosol. This is a very important drawback because high levels of free phenols are present in oils that are aged, due to hydrolysis of the original esterified forms.

Although it is clear that the molecular absorption is related to the reaction of hydroxyls per molecule (Singleton *et al.*, 1999), the possible interfering role of the aldehyde groups in compounds **3**–**10** (see Figure 31.1) has not been studied. In fact, the Folin-Ciocalteu reagent is a mixture of phosphomolybdate and

phosphotungstate, which participate in an oxidation reaction. After the reaction is complete, the reagent is reduced to a product with blue color that is measured at 500–750 nm. The assay measures all compounds readily oxidizable under the reaction conditions, and this cannot exclude the participation of the dialdehydes in the same reaction. Moreover, the expression of the results as gallic acid or caffeic acid equivalents is arbitrary and cannot be directly correlated with the requirements of the EU health claim.

31.2.2.2 Chromatographic analysis of the phenolic content of olive oil

Chromatography is the most common method for qualitative and quantitative determination of the phenolic content of olive oil. Several techniques like thin-layer chromatography (TLC), GC, and liquid chromatography (LC) have been used with several types of detectors. High-performance liquid chromatography–ultraviolet (HPLC-UV) remains the most frequently used method despite the problems that make it inappropriate for quantitative measurement of specific compounds (e.g., oleocanthal). Significant improvements have been achieved using liquid chromatography–mass spectrometry (LC-MS), but still there are ingredients that cannot be measured chromatographically.

31.2.2.2.1 Thin-layer chromatography

The older chromatographic techniques that were used for the analysis of olive oil phenolics included TLC as well as paper chromatography. Normal and reverse phase TLC using silica, polyamide, or cellulose have been reported for the separation of a series of phenolics (Ragazzi *et al.*, 1973; Vasquez-Roncero, 1974) in one or two dimensions using a variety of mobile phases. Today, these techniques are used mainly for final purification of phenolics (Karkoula *et al.*, 2012, 2014; Diamantakos *et al.*, 2015).

31.2.2.2.2 Gas chromatography and GC-MS

The qualitative and quantitative determination of several phenolic constituents of the oil can be achieved using GC. The first work concerning the separation of phenolic compounds from olive oil by GC was published by Janer del Valle and Vazquez-Roncero (1980). At about the same time, Solinas and Cichelli (1982) also used the GC method for determining mixtures of virgin olive oils and refined olive oils. Although the method allowed the characterization of simple components, other phenols that were present in large quantities could not be detected. Improvements were made using more sophisticated analytical techniques such as GC-MS and GC–tandem MS (MS/MS), which are mainly applied to silylated derivatives of the phenolic components (Angerosa, 1996; Saitta *et al.*, 2002). Although there are several analytical and applied works using this technique, other chromatographic techniques like HPLC are more commonly used because they avoid the derivatization step and the high temperatures that can decompose some of the analytes.

31.2.2.2.3 High-pressure liquid chromatography

The low volatility of many phenolic constituents of olive oil has limited the use of the GC method for their qualitative and quantitative determination, and led to the use of the HPLC method as the more popular technique for the analysis of olive oil phenolics. Numerous mobile phases have been used, but the binary systems consisting of an aqueous component and a less polar organic component such as acetonitrile or methanol remain the most common. Acids (acetic, formic, or phosphoric) are commonly added to the two components to maintain a constant acid concentration during fractionation. The decrease in pH helps prevent the degradation of phenolic compounds while improving the shape of the peaks and reducing their width. For example, a common method for the analysis of the phenolic fraction uses reverse phase HPLC with isocratic elution with an aqueous solution of sulfuric acid–acetonitrile, methanol–aqueous acetic acid, or acetonitrile–aqueous acetic acid (Graciani-Constante & Vazquez-Roncero, 1980; Nergiz & Unal, 1991).

The routine detection by HPLC is based on the UV absorption at 240, 250, or 280 nm (Tsimidou *et al.*, 1992). The most common wavelength used for detection is 280 nm, which is a convenient representative one, although the detection of other wavelengths and a dual detection wavelength have also been used.

The identification of phenols by HPLC (and GC) is based on the correlation between retention times with standards. The limited commercial availability of appropriate standards for quantitation is an obstacle that can be overcome, in part, by the use of standards isolated by preparative chromatography (Mateos *et al.*, 2001). In many cases, the quantitation by UV peaks corresponding to standards that are unavailable is based on calibration curves that are constructed with related compounds, such as tyrosol (Pirisi *et al.*, 2000) or oleuropein. However, all phenols possess a strong chromophore system that is particularly informative, providing important information on the structure, which can reveal the category to which they belong.

Fluorescence is an alternative method of detection complementary to UV. In fact, lignans are a class of phenolics that can be successfully detected by fluorescence, as was shown by Brenes *et al.* (2002). Although the fluorescence detection shows some advantages in comparison with UV with respect to improved selectivity and sensitivity, there are phenolics that cannot be detected by fluorescence.

Coulometric detection coupled with HPLC has also been used for quantitation of the phenolic compounds in olive oil. The advantage of this method, apart from easy sample preparation, is also the chance to separate co-eluting components with different potential (Achilli *et al.*, 1993). The detector measures the different potentials in which the phenolic compounds are oxidized.

31.2.2.2.4 Liquid chromatography–mass spectrometry

The combination of LC with MS has made a significant change in the analysis of polar components of the oil, as evidenced by the increased number of publications that have used this technique during recent years (Segura-Carretero *et al.*, 2010). For example, LC-MS/MS with atmospheric pressure chemical ionization (APCI) has been used for the analysis of phenolic acids, tyrosol, and oleuropein (Bianco *et al.*, 2001). Electrospray ionization (ESI) in negative mode is more sensitive for the majority of phenolic ingredients and has also been extensively used (De la Torre-Carbot *et al.*, 2005; Jerman-Klen *et al.*, 2015).

By using LC-MS/MS, a serious overlapping problem observed in HPLC-UV methods has been overcome; however, the method still suffers from lack of commercial standards, possible formation of artifacts, and a significant amount of effort is currently spent to override such problems. To avoid problems related to the reaction of some constituents (e.g., oleocanthal or oleacein) with the chromatographic solvents (see Section 31.2.3), an interesting approach using derivatization reactions combined with ultra (U)-HPLC-ESI-MS/MS has been reported (Di Donna *et al.*, 2011).

31.2.2.2.5 Capillary electrophoresis (CE)

CE combines reduced analysis time and satisfactory separation of the phenolic fraction. CE is an alternative or additional method of HPLC. The speed, resolution, and ease of use combined with the low cost of the device make this technique a useful option for the development and improvement of methods of analysis of olive oil. Vulcano *et al.* (2015) used CE for quantitation of oleocanthal and oleacein.

31.2.3 The problems related to chromatographic measurement

The characterization and quantitation of the phenolic compounds of olive oil by HPLC require the use of not easily available standards, demand accurate sample preparation, and are generally time-consuming processes. In fact, the entire analysis of phenols in olive oil by HPLC can last more than 90 min. However, the most critical problems are related to the reaction of some important phenolic ingredients with the mobile or the stationary phase that leads to the formation of artifacts.

The first attempt for quantitation of oleocanthal in olive oil extracts was performed following previous works (Beauchamp *et al.*, 2005; Impellizzeri & Lin, 2006) using HPLC-UV with reverse phase columns and aqueous mobile phase; it was observed that pure oleocanthal was not giving a single sharp peak. This problem, which had previously been observed by others (Di Donna *et al.*, 2011), prompted us to investigate in more detail the reaction of oleocanthal and oleacein with water, methanol, acetonitrile, dimethyl sulfoxide (DMSO), or their mixtures. The study was performed by NMR using deuterated solvents and monitoring *in situ* the formation of the corresponding derivatives. We found that both compounds react spontaneously

with water or methanol to give mixtures of hemiacetals or acetals that were characterized using 1D and 2D NMR spectra. Interestingly, oleocanthal and oleacein gave NMR spectra with each corresponding to a single molecule in the case of pure $CDCl_3$, d-ACN, and DMSO.

These findings confirm that the classic chromatographic determination of these compounds in aqueous media is problematic and that many of the previous measurements reported in literature are more or less questionable. For example, the proportion between the aldehydic and the hydrated forms in water–acetonitrile mixtures is time and solvent ratio dependent, making the accurate measurement very difficult. In addition, during chromatography, E-oleocanthal is transformed to Z-oleocanthal, making the analysis even more complicated (Adhami *et al.*, 2015).

Isomerization problems during chromatography also occur for oleuropein and ligstroside aglycons, and this leads to the artificial formation of minor isomers. This problem was observed by Karkoula *et al.* (2014) when the extract from an olive oil sample was submitted to reverse chromatography by using RP18 silica and a mixture of acetonitrile and water as mobile phase. In that case, the ratio between the isomers of oleuropein and ligstroside aglycons (Figure 31.1, **5a–b** and **6a–b**) changed dramatically during chromatography from 1:0 to an even 1:1.

Another similar problem is that the open-ring dialdehydic forms of oleuropein or ligstroside aglycons (**5**–**10**) during chromatography are transformed to the closed-ring forms (**3** and **4**) and their stereoisomers, as reported in a recent work by Diamantakos *et al.* (2015).

All of the problems described here explain why the HPLC-UV chromatograms of the olive oil extracts appear more complicated than they really are. The formation of artificial derivatives or isomers makes the HPLC-UV methods inappropriate for quantitation of tyrosol and hydroxytyrosol derivatives in olive oil.

31.2.4 Quantification using 1D qNMR

To overcome quantitation problems, it is necessary to develop a method for olive oil extraction and analysis without involving any reacting mobile or stationary phase. qNMR appeared as an attractive alternative solution to this complicated problem. NMR spectroscopy is well suited for quantitative analyses of complex chemical mixtures. Among the available qNMR methods, 1D ^{1}H NMR typically provides the highest sensitivity with excellent linear response to component concentrations.

31.2.4.1 Selection of extraction solvent

The selection of acetonitrile as a solvent for extraction of olive oil was based on the observation that it does not react with the analytes. In contrast, methanol, which is commonly used for the extraction of phenolics from olive oil (as discussed in this chapter), reacts immediately with the aldehydic form of oleocanthal or oleacein, leading to the corresponding acetals or hemiacetals. Evaporation of methanol and re-dilution in chloroform afford again the aldehydic form. Moreover, one extraction with acetonitrile was sufficient for more than 85% recovery of both studied compounds.

31.2.4.2 NMR spectral analysis of oleocanthal and oleacein in extra virgin olive oil

This method is based on the observation that the ^{1}H-NMR spectrum of olive oil acetonitrile extracts when recorded in $CDCl_3$ and in magnetic fields of 600 or 800 MHz presented a very well-resolved set of peaks corresponding to the aldehydic protons of the target compounds between 9.1 and 9.8 ppm (Figure 31.2). This spectrum region in all the studied samples was clearly resolved, making the integration of the corresponding peaks and their comparison with the peak of the internal standard feasible. Oleocanthal (**4**), oleacein (**3**), oleuropein aglycon (**5a**), and ligstroside aglycon (**6a**) were quantified by integrating the peaks noted in Figure 31.2 and by using calibration curves constructed with pure compounds added to oils of zero phenolic content, as described by Karkoula *et al.* (2012, 2014). The peaks corresponding to compounds **7a–b,9** and **8a–b,10** were integrated as two total sets corresponding to oleuropein aglycon dialdehyde and enolic forms and to ligstroside aglycon dialdehyde and enolic forms, respectively.

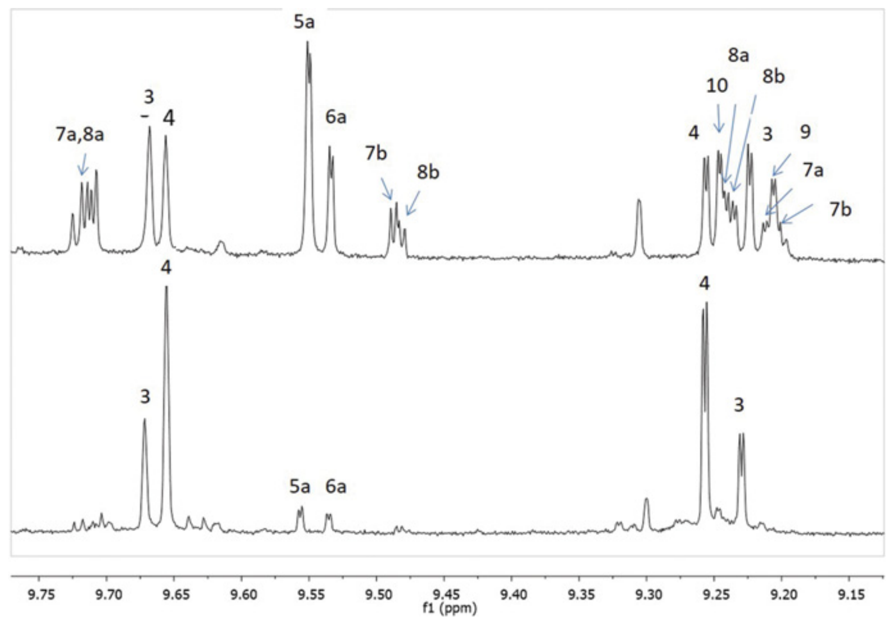

Figure 31.2 ^{1}H NMR spectrum of the aldehydic region of a (a) 'Mission' olive oil extract sample and (b) typical 'Koroneiki' showing the peaks corresponding to compounds **3–10**.

31.2.4.2.1 Selective excitation pulse

As explained, the 1D qNMR method was applied to olive oil extracts that were prepared in such a way that the majority of lipids were removed while the polar phenolics were retained. A recent interesting modification of the qNMR method was the use of a selective excitation pulse that permitted the analysis of the target phenolics without extraction and in the presence of the dominating olive oil lipids.

Quantitation of key trace analytes in the presence of very strong signals from the bulk matrix is problematic or even impossible, depending on the concentration, using typical broadband excitation. This is due to dynamic range limitations of the analog-to-digital converter (ADC), especially on older instruments. These limitations can be overcome by the use of band-selective shaped pulses to excite only the region containing the minor analytes while excluding the regions containing strong matrix signals that would exceed the ADC range. Region-selective 1D ^{1}H-NMR methods for quantitation of aldehydes in honey and terpenes in olive oil have been described (Rastrelli *et al.*, 2009). These utilize double-pulsed field gradient spin echo (DPFGSE) sequences with band-selective refocusing pulses. In cases where the selectively excited region contains two or more J-coupled spins, significant antiphase magnetization can occur, reducing the integrated signal intensities of these relative to uncoupled spins. This coupling evolution can be removed by utilizing the reported "perfect echo" (PE) sequence (Adams *et al.*, 2013).

In this framework, a selective excitation with a double-pulsed field gradient perfect echo method (SELDPFGPE) was utilized to analyze the aldehydes in olive oil without the need for previous extraction of these analytes. The method was developed to target all the major secoiridoid derivatives of hydroxytyrosol and tyrosol (Magiatis *et al.*, 2015).

An analysis of the aldehyde region in Sicilian extra virgin olive oils utilizing DPFGSE has been reported (Rotondo *et al.*, 2011), although the authors did not identify oleocanthal, oleacein, or the aglycons of oleuropein and ligstroside as the observed components, nor were the components quantified.

The developed method in combination with the previously reported qNMR method was applied to the study of 100 commercial olive oil samples from all the major brands available in supermarkets in California, offering a good estimation of the average levels of the secoiridoid aldehydes that are available to the consumers (Magiatis *et al.*, 2015).

The selective excitation pulse method offers quantitative results using only 225 mg of olive oil (ca. 250 μL). The oil is mixed with 500 μL $CDCl_3$ containing syringaldehyde as an internal standard (50 μg/mL). The results are obtained with just 16 scans collected during a total experiment time of 3 min. The experiments were performed using a Bruker Avance 600 MHz with cryoprobe.

The selective pulse method presents important advantages in comparison to alternative chromatographic or spectroscopic methods. The oil sample can be analyzed quantitatively without any treatment (e.g., extraction, derivatization, or separation), without the use of standards, and without the risk of decomposition or isomerization, as these happen during chromatographic analysis. Moreover, it is the fastest available method, with a total analysis time of less than 5 min for sample preparation, spectrum acquisition, integration, and quantitation. However, the method presents some overlapping limitations that in some cases may lead to overestimation of some compounds (Figure 31.3a and 31.3b). For this reason, this method is currently useful mainly as a screening tool. More specifically, it is the rapidest and easiest way to discriminate the oils

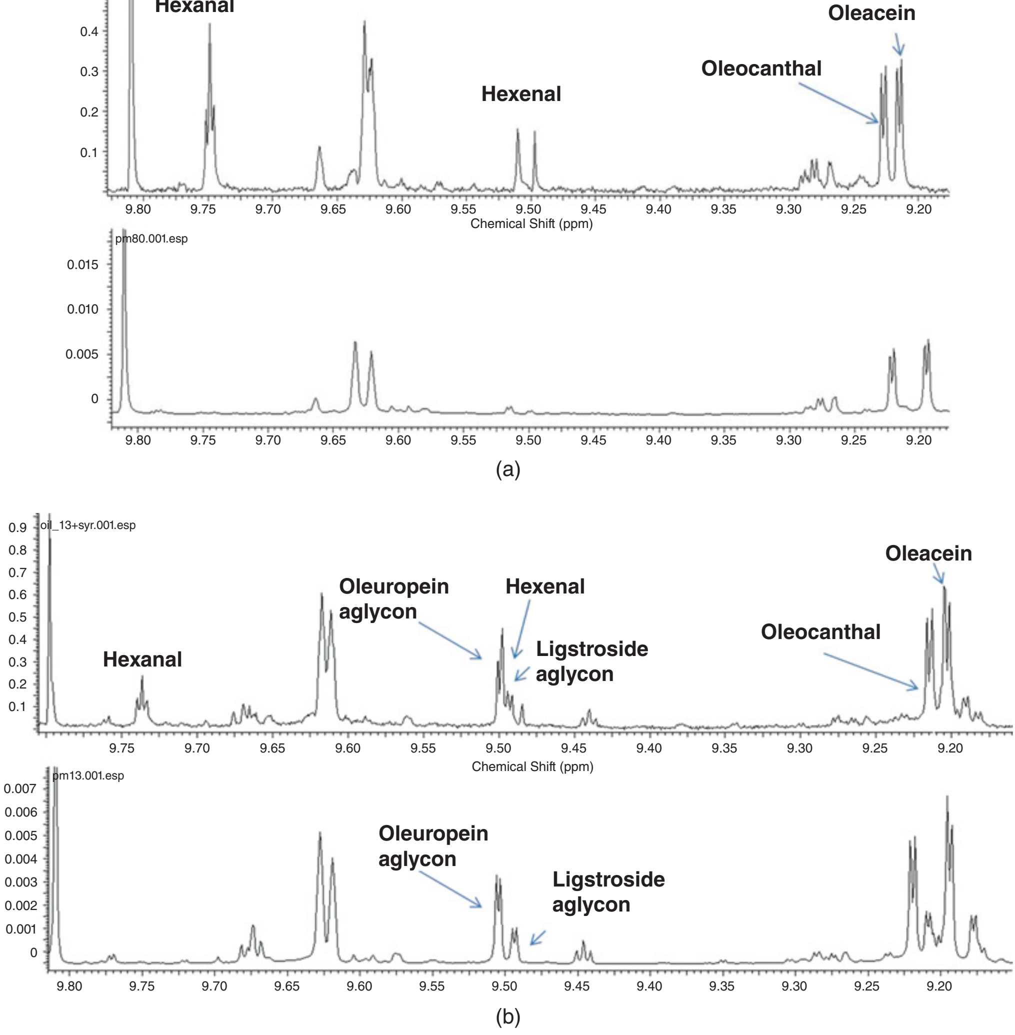

Figure 31.3 (a) Comparison of spectra using selective pulse without extraction (up) and after extraction (down) of the same oil. (b) Comparison of spectra using selective pulse without extraction (up) and after extraction (down) of the same oil; this case presents two types of overlapping limitations.

that do not fulfill the EU criteria for polyphenol content and health claims. Even if it does not give accurate results for all oils, it can definitely be used to exclude oils from further evaluation.

31.2.4.3 2D NMR quantitation

The quantitation of secoiridoid phenolics by the 1D NMR method in some cases presents limitations due to partial overlapping of peaks corresponding to some ingredients (e.g., **8a–b** with **10** or **7a–b** with **9** in Figure 31.2). This problem becomes more intense in the case where the spectra are recorded at 400 and not at 600 MHz or higher.

To override this overlapping, we have developed an alternative method for quantitation that is based on 2D qNMR and specifically on the heteronuclear single quantum coherence (HSQC) experiment. The use of two dimensions (^{1}H and ^{13}C) achieves the complete disappearance of the overlapping problem as shown in Figure 31.4.

Using Top-Spin software, we integrated the cross-peaks as noted on Figure 31.4 and correlated this with known concentrations of pure compounds (**3, 4, 7–10**) added to zero-phenolics oil. The integration values (expressed as a ratio to the internal standard) presented excellent linearity ($r^2 > 0.99$) and were in the range between 10 and 500 mg/kg. Construction of calibration curves for all ingredients (**3, 4, 7**–**10**) permitted the ingredients' accurate and precise measurement.

It should be noted that the spectrum presented in Figure 31.4 was recorded in only 10 min, revealing that the developed method is not only accurate but also time efficient. The 2D qNMR method permits the unambiguous measurement of compounds **3, 4, 7–10**, which constitute over 99% of the total tyrosol and hydroxytyrosol derivatives in all samples that have been analyzed until now (>2000 samples from all major varieties and all major oil-producing countries).

31.2.4.4 Analysis of the oil used for the EUROLIVE Project by qNMR

Lately, there has been a discussion about the interpretation of EU Regulation 432/2012 concerning the health claim of olive oil. The question is about the measurement of the tyrosol derivatives. The exact wording of the health claim refers to hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol). One issue that has arisen is the meaning of the legislation with respect to the use of the term "oleuropein complex" and whether it includes tyrosol derivatives.

To answer these questions, we carefully studied the analytical works that were behind the oils that had been used in the EUROLIVE trial and were the basis for the expression of the claim.

The oils used in the EUROLIVE project had been studied by LC-MS, and interestingly the tyrosol and hydroxytyrosol derivatives in the oils were quantified using oleuropein as the standard for calibration curves due to the lack of standards of the target compounds. For this reason, it can be understood why all the complex mixtures of oleuropein and ligstroside derivatives were described using the term "oleuropein complex."

To give an undoubtful answer about the quantitative data, we analyzed the oils that were used in the EUROLIVE study. The oils were kept at −80 °C and were provided by Dr. María-Isabel Covas. The two oils were analyzed with the 1D qNMR method, and the results are shown in Table 31.1.

Oil with medium phenolic content was used to set the limit of active oil at 5 mg/20g or 250 mg/kg. Although the LCMS methods could not give precise results for each type of ligstroside or oleuropein derivative, both methods give the same total amounts for the measured compounds. For this reason, it can be stated clearly that all the tyrosol and hydroxytyrosol esterified forms found in olive oil should account for the total amount of derivatives used as the criterion for the health claim. If the tyrosol derivatives were excluded, then the limit for the health claim should be 3 mg/20 g and not 5 mg/20 g.

31.2.5 Colorimetric quantitation of oleocanthal and oleacein

A new colorimetric method for the measurement of oleocanthal and oleacein in olive oil has recently been reported (Magiatis & Melliou, 2015). The method is known as Melliou-Magiatis or "Aristoleo" from the name of the commercial kit. The measuring method is based on the selective reaction of the polar aldehydes found in olive oil toward the formation of green-colored water-soluble derivatives (Schiff bases) that can be

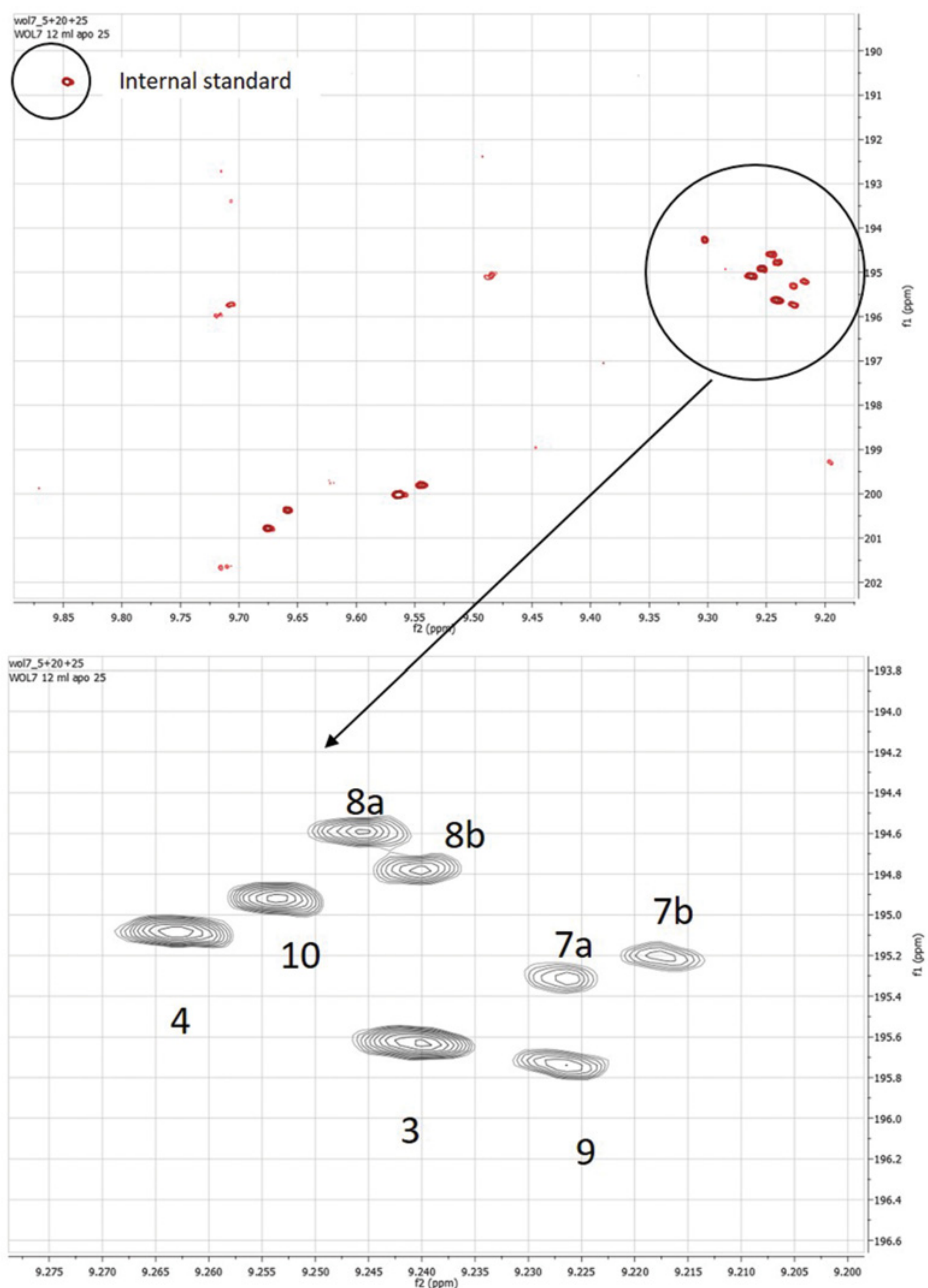

Figure 31.4 Example of HSQC experiment used for 2D qNMR.

Table 31.1 Analysis of phenolic compounds of two oils of the EUROLIVE study by the 1D qNMR method.

Compounds	High phenolics oil, mg/kg	Medium phenolics oil, mg/kg
Oleocanthal	80	43
Oleacein	82	45
Oleuropein aglycon (monoaldehyde form)	80	42
Oleuropein aglycon (dialdehyde forms)	53	28
Ligstroside aglycon (monoaldehyde form)	37	20
Ligstroside aglycon (dialdehyde forms)	59	32
Free tyrosol	43	21
Free hydroxytyrosol[a]	40	20
Total hydroxytyrosol derivatives	255	135
Total tyrosol derivatives	219	116
Total of analyzed compounds	474	251

[a]Not measured by qNMR but with HPLC.

quantified in the aqueous phase either by the intensity of the color or by measurement of the absorbance in the visible spectrum. The reaction is achieved under acidic conditions using aniline derivatives bearing polar groups (e.g., carboxyls). As an example of an appropriate reagent, *p*-hydroxyanthranilic acid is reported in combination with glacial acetic acid.

In contrast with the older method, which quantifies aldehydes in olive oil using *p*-anisidine, the Melliou-Magiatis method uses a biphasic system (lipid and aqueous). This crucial modification permits the selective measurement of the water-soluble colored derivatives and avoids overlapping with the coexisting lipophilic aldehydes, which are products of the oxidative degradation of lipids. As an example of the method, it is reported that olive oil (e.g., 5 ml) is mixed with a solution of *p*-hydroxyanthranilic acid in glacial acetic acid, agitated until it becomes homogeneous (30 sec), and then water is added and it is agitated again. The mixture is allowed to stand for 45 min, and then the mixture is separated in two phases. The lower aqueous phase has a color ranging from clear or light yellow to intense green. The intensity of the color is directly related with the concentration of oleocanthal and oleacein in the olive oil. The concentration is evaluated by comparing the color with a printed colorimetric index that has been accurately established using the NMR method. Alternatively, the aqueous phase can be obtained, and the absorbance at 635 nm can be read photometrically. The absorbance at the specific wavelength is linearly related with the D1 index (oleocanthal + oleacein).

31.2.5.1 Advantages and limitations of the Melliou-Magiatis method in comparison with Folin-Ciocalteu

The Melliou-Magiatis method is highly selective and can accurately quantify the oleocanthal and oleacein levels in olive oil. In a large number of olive oils (especially of Greek origin), oleocanthal and oleacein were the dominating tyrosol and hydroxytyrosol derivatives, and consequently their colorimetric quantitation can provide a fast and cheap way to predict if an oil qualifies for the EU health claim criteria. Using a database with >2000 samples analyzed during the 2010–2015 period, we have estimated that in 65% of the olive oil samples, oleocanthal + oleacein account for >50% of the total tyrosol and hydroxytyrosol derivatives. Moreover, in a portion of 50% of the samples, oleocanthal + oleacein account for >67% of the total tyrosol and hydroxytyrosol derivatives. In contrast, in 10% of the samples, oleocanthal + oleacein account for <33% of the above total. In these limited cases, the Melliou-Magiatis method underestimates the total content of tyrosol and hydroxytyrosol derivatives, and this may lead to negative results.

The Folin-Ciocalteu method is nonselective for tyrosol and hydroxytyrosol derivatives and can give false-positive results, especially in cases where the oil contains significant amounts of other phenolics (e.g., lignans, flavonoids, or phenolic acids) that do not account for the EU health claim. The addition of equivalent amounts of pure oleocanthal and oleacein in zero-phenolic olive oil revealed that the two oils do not behave as expected using the Folin-Ciocalteu method.

31.3 Analysis of table olives

Table olives from *Olea europaea* L. are a traditional product and an important component of the Mediterranean diet. World consumption of table olives was approximately 2,521,500 tons in 2013 (IOC, 2013). There are three main categories of table olives based upon the trade preparation methods, including green (or Spanish-style), natural black (or Greek-style), and California-style black ripe olives (Garrido Fernández *et al.*, 1997). Olive fruit is a rich source of compounds with health-protecting activities, including hydroxytyrosol, oleuropein, and many other related biophenols and secoiridoid derivatives (Boskou, 2008). The complement of bioactives in the final olive products is influenced by the olive variety and the debittering method used in preparing them (Charoenprasert & Mitchell, 2012).

The major bitter component of olives is oleuropein and its derivatives (Romero *et al.*, 2004). Oleuropein is water-soluble and is not normally found in olive oil. Oleuropein is removed during debittering (e.g., using dry salt, lye, or brine) (Ramírez *et al.*, 2013). In Greece, olives are produced primarily using brine (known as Greek-style), dry salt, and lye (i.e., a 1–2% sodium hydroxide solution). In other countries, such as Spain, Italy, and the USA, most producers rely on lye to assist in debittering olives (Spanish-style and California-style black ripe olives).

Rapid and reliable methods for quantitation of hydroxytyrosol, oleuropein, and their derivatives are of special interest since these compounds have recently been recognized by the EU as agents protecting LDL against oxidation and thus offering cardiovascular protection. Currently, this health claim is recognized only for olive oil. To date, several GC-MS, HPLC-UV, and LC-MS methods are available for monitoring phenolic and secoiridoid compounds in olive products (e.g. olive oil, olive leaves, and olive fruits) (Segura-Carretero

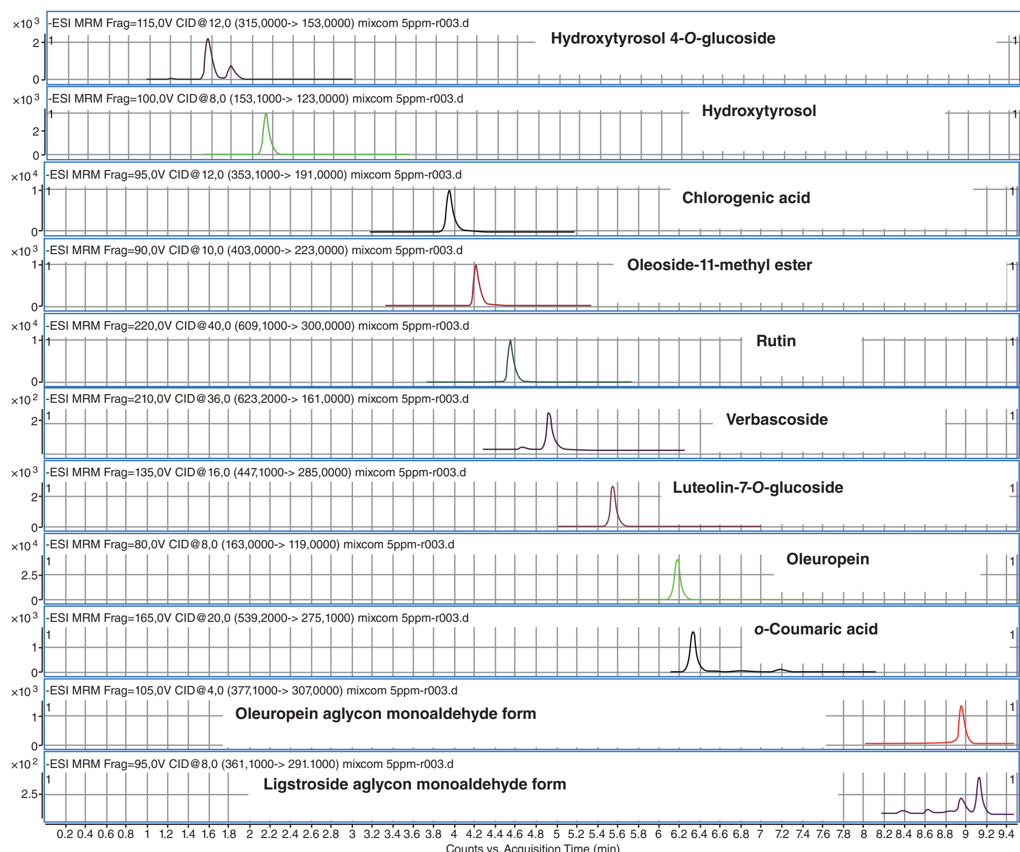

Figure 31.5 MRM extracted chromatograms for each quantified compound from a 'Mission' olive sample.

et al., 2010). The GC-MS methods, as in the case of olive oil, require derivatization and present a long analysis time (30–120 min). The HPLC-UV methods have low sensitivity (e.g., limit of quantitation [LOQ] = 1 μg/mL for hydroxytyrosol) (Zoidou *et al.*, 2010) and are limited by long chromatographic separation times (20–100 min) (Segura-Carretero *et al.*, 2010). Additionally, in some cases, HPLC-UV methods are unable to resolve overlapped peaks (Vinha *et al.*, 2005), making difficult the simultaneous quantitation of numerous compounds, especially in short separation times. Rapid LC-MS methods have been applied to unprocessed olives but are restricted to the quantitation of oleuropein and hydroxytyrosol (Bouaziz *et al.*, 2009; Jemai *et al.*, 2009). An HPLC-Orbitrap MS/MS method for quantitation of nine compounds in fresh olives was recently reported (Kanakis *et al.*, 2013). A rapid UHPLC triple quadruple MS/MS (QqQ MS/MS) method utilizing dynamic multiple reaction monitoring (dMRM) for the measurement of a range of key bitter and bioactive constituents in olives was recently developed (Melliou *et al.*, 2015). The method was applied for the simultaneous quantitation of hydroxytyrosol, oleuropein, hydroxytyrosol-4-*O*-glucoside, luteolin-7-*O*-glucoside, rutin, verbascoside, oleoside-11-methyl ester, 2,6-dimethoxy-*p*-benzoquinone, phenolic acids (chlorogenic acid amd *o*-coumaric acid), oleuropein, and ligstroside aglycon monoaldehydic forms in three different varieties of fresh and processed olives, cured using either dry salt or California-style black ripe processing methods. This method offers improved sensitivity, selectivity, and throughput (<10 min/per run) in comparison with previously reported methods as reviewed by Segura-Carretero *et al.* (2010). The usefulness of the new method was demonstrated in the simultaneous quantitation of a broad range of phenolic and secoiridoid compounds in fresh and finished olive products generated by dry salt or California-style debittering methods.

The HPLC-Orbitrap and the UHPLC QqQ methods present similar limits of detection (LODs) and LOQs for oleuropein, hydroxytyrosol, oleuropein aglycon, and ligstroside aglycon, the only four common compounds studied using the two methods. The Orbitrap method, however, needed a longer analysis time (31 min) and the quantitation range was restricted to higher concentrations (e.g., 1000–50,000 ng/mL vs. 100–12,500 ng/mL for oleuropein aglycon and 500–20,000 ng/mL vs. 50–6250 ng/mL for hydroxytyrosol). A representative MRM extracted chromatogram of 'Mission' olives obtained using the UHPLC QqQ method is presented in Figure 31.5 and demonstrates clearly resolved peaks.

31.4 Conclusion

Several qualitative and quantitative methods for the analysis of the phenolic content of olive oil and table olives have been reported. Especially, the derivatives of hydroxytyrosol and tyrosol found in olive oil require new alternative analysis methods as the widely used chromatographic methods present several problems. The new qNMR and colorimetric methods can potentially become the standard method in the future for the evaluation of the health claim criteria for olive oil.

References

Achilli, G., Cellerino, G.P., Gamache, P.H., & Deril, G. (1993) Identification and determination of phenolic constituents in natural beverages and plant-extracts by means of a coulometric electrode array system. Journal of Chromatography A 632, 111–117.

Adams, R.W., Holroyd, C.M., Aguilar, J.A., Nilsson, M., & Morris, G.A. (2013) "Perfecting" WATERGATE: clean proton NMR spectra from aqueous solution. Chemical Communications 49, 358–360.

Adhami, H.R., Zehl, M., Dangl, C., Dorfmeister, D., Stadler, M., Urban, E, Hewitson, P., Ignatova, & S., Krenn, L. (2015) Preparative isolation of oleocanthal, tyrosol, and hydroxytyrosol from olive oil by HPCCC. Food Chemistry 170, 154–159.

Angerosa, F. (1996) Characterization of phenolic and secoiridoid aglycons present in virgin olive oil by gas chromatography-chemical ionization mass spectrometry. Journal of Chromatography A 736, 195–203.

Angerosa, F., D'Alessandro, N., Konstantinidou, P., & Di Giacinto, L. (1995) GC-MS evaluation of phenolic compounds in virgin olive oil. Journal of Agricultural and Food Chemistry 43, 1802–1807.

Beauchamp, G.K., Keast, R.S.J., Morel, D., Lin, J., Pika, J., Han, Q., Lee, C., Smith, A.B., & Breslin, P.A.S. (2005) Phytochemistry: ibuprofen-like activity in extra-virgin olive oil. Nature 437, 45–46.

Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gómez-Caravaca, A.M., Segura-Carretero, A., Fernández-Gutiérrez, A., & Lercker, G. (2007) Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. Molecules 12, 1679–1719.

Bianco, A., Buiarelli, F., Cartoni, G., Coccioli, F., Muzzalupo, I., Polidori, A., & Uccella, N. (2001) Analysis by HPLCMS/MS of biophenolic components in olives and oils. Analytical Letters 34, 1033–1051.

Blekas, G., Psomiadou, E., Tsimidou, M., & Boskou, D. (2002) On the importance of total polar phenol measurement for monitoring the stability of Greek virgin olive oils. European Journal of Lipid Science 104, 340–346.

Boskou, D. (2008) Phenolic compounds in olives and olive oil. In Olive oil: minor constituents and health. CRC Press, Boca Raton, FL.

Bouaziz, M., Jemai, H., Khabou, W., & Sayadi, S. (2010) Oil content, phenolic profiling and antioxidant potential of Tunisian olive drupes. Journal of the Science of Food and Agriculture 90, 1750–1758.

Brenes, M., Garcia, A., Rios, J.J., García, P., & Garrido, A. (2002) Use of 1-acetoxypinoresinol to authenticate Picual olive oils. International Journal of Food Science and Technology 37, 615–625.

Charoenprasert, S., & Mitchell, A. (2012) Factors influencing phenolic compounds in table olives (*Olea europaea*). Journal of Agricultural and Food Chemistry 60, 7081–7095.

Christophoridou, S., & Dais, P. (2009) Detection and quantification of phenolic compounds in olive oil by high resolution 1H nuclear magnetic resonance spectroscopy. Analytica Chimica Acta 633, 283–292.

Cicerale, S., Lucas L.J., & Keast R.S.J. (2012). Oleocanthal: a naturally occurring anti-inflammatory agent in virgin olive oil, olive oil – constituents, quality, health properties and bioconversions, ed. D. Boskou Dimitrios. In Tech, Rijeka, Croatia. Available from: http://www.intechopen.com/books/olive-oil-constituents-quality-health-properties-and-bioconversions/oleocanthal-a-naturally-occurring-anti-inflammatory-agent-in-virgin-olive-oil

Cortesi, N., Azzolini, M., Rovelini, P., & Fedelii, E. (1995) Minor polar components of virgin olive oils: a hypothetical structure by LC-MS. Rivista Italiana delle Sostanze Grasse 72, 241–251.

De la Torre-Carbot, K., Jauregui, O., Gimeno, E., Castellote, A.I., Lamuela-Raventós, R.M., & López-Sabater, M.C. (2005) Characterization and quantification of phenolic compounds in olive oils by solid-phase extraction, HPLC-DAD, and HPLC-MS/MS. Journal of Agricultural and Food Chemistry 53, 4331–4340.

Diamantakos, P., Velkou, A., Killday, K., Brian, Gimisis, T., Melliou, E., & Magiatis, P. (2015) Oleokoronal and oleomissional: new major phenolic ingredients of extra virgin olive oil. Olivae 122, 22–35.

Di Donna, L., Benabdelkamel, H., Mazzotti, F., Napoli, A., Nardi, M., & Sindona, G. (2011) High-throughput assay of oleopentanedialdheydes in extra virgin olive oil by the UHPLC-ESI-MS/MS and isotope dilution methods. Analytical Chemistry 83, 1990–1995.

Frankel, E.N. (2011) Nutritional and biological properties of extra virgin olive oil. Journal of Agricultural and Food Chemistry 59, 785–792.

Garrido Fernández, A., Fernández Diez, M.J., & Adams, M.R. (1997) Table olives. Chapman & Hall, London.

Graciani-Constante, E., & Vazquez-Roncero, A. (1980) A study of the polar compounds in olive oil by high performance liquid chromatography 2. chromatography in inverse phase. Grasas y Aceites 31, 237–243.

Gutfinger, T. (1981) Polyphenols in olive oils. Journal of the American Oils Chemists' Society 58, 966–968.

Impellizzeri, L., & Lin, J. (2006) A simple high-performance liquid chromatography method for the determination of throat-burning oleocanthal with probated antiinflammatory activity in extra virgin olive oils. Journal of Agricultural and Food Chemistry 54, 3204–3208.

International Olive Council (IOC). (2009, November) Determination of biophenols in olive oil by HPLC. Testing methods. COI/T.20/Doc No. 29. IOC, Madrid, Spain.

International Olive Council (IOC). (2013, June) Focus on table olives: world estimates for 2012/13. Market Newsletter 73, 1.

Janer del Valle, C., & Vazquez-Roncero, A. (1980) A study of the polar compounds in olive oil. Grasas y Aceites 31, 309–316.

Jemai, H., Bouaziz, M., & Sayadi, S. (2009) Phenolic composition, sugar contents and antioxidant activity of Tunisian sweet olive cultivar with regard to fruit ripening. Journal of Agricultural and Food Chemistry 57, 2961–2968.

Jerman-Klen, T., Golc-Wondra, A., Vrhovšek, U., & Mozetič-Vodopivec, B. (2015) Phenolic profiling of olives and olive oil process-derived matrices using UPLC-DAD-ESI-QTOF-HRMS analysis. Journal of Agricultural and Food Chemistry 63, 3859–3872.

Jerman-Klen, T., & Mozetič-Vodopivec, B. (2012) Optimisation of olive oil phenol extraction conditions using a high-power probe ultrasonication. Food Chemistry 134, 2481–2488.

Kanakis, P., Termentzi, A., Michel, T., Gikas, E., Halabalaki, M., & Skaltsounis, A.L. (2013) From olive drupes to olive oil: an HPLC-Orbitrap-based qualitative and quantitative exploration of olive key metabolites. Planta Medica 79, 1576–1587.

Karkoula, E., Skantzari, A., Melliou, E., & Magiatis, P. (2012) Direct measurement of oleocanthal and oleacein levels in olive oil by quantitative 1H NMR: establishment of a new index for the characterization of extra virgin olive oils. Journal of Agricultural and Food Chemistry 60, 11696–11703.

Karkoula, E., Skantzari, A., Melliou, E., & Magiatis, P. (2014) Quantitative measurement of major secoiridoid derivatives in olive oil using qNMR: proof of the artificial formation of aldehydic oleuropein and ligstroside aglycon isomers. Journal of Agricultural and Food Chemistry 62, 600–607.

Koudounas, K., Banilas, G., Michaelidis, C., Demoliou, C., Rigas, S., & Hatzopoulos, P. (2015) A defence-related *Olea europaea* β-glucosidase hydrolyses and activates oleuropein into a potent protein cross-linking agent. Journal of Experimental Botany. DOI:10.1093/jxb/erv002

Liberatore, L., Procida, G., D'Alessandro, A., & Cichelli, A. (2001) Solid-phase extraction and gas chromatographic analysis of phenolic compounds in virgin olive oil. Food Chemistry 73, 119–124.

Limiroli, R., Consonni, R., Ottolina, G., Marsilio, V., Bianchi, G., & Zetta, L. (1995) 1H and 13C NMR characterisation of new oleuropein aglycone. Journal of the Chemical Society, Perkin Transactions 1, 1519–1523.

Magiatis, P., & Melliou, E. (2015) Method of colorimetric determination of oleocanthal and oleacein in olive oil – treatment and measurement kit. GR1008569 (B) 2015-09-04.

Magiatis, P., Melliou, E., & Killday, B. (2015) A new ultra rapid screening method for olive oil health claim evaluation using selective pulse NMR spectroscopy. In: Magnetic resonance in food science: defining food by magnetic resonance, ed. F. Capozzi, L. Laghi, & P.S. Belton. Royal Society of Chemistry, London, 84–92.

Mateos, R., Espartero, J.L., Trujillo, M., Ríos, J.J., León-Camacho, M., Alcudia, F., & Cert, A. (2001) Determination of phenols, flavones, and lignans in virgin olive oils by solid-phase extraction and high-performance liquid chromatography with diode array ultraviolet detection. Journal of Agricultural and Food Chemistry 49, 2185–2192.

Melliou, E., Zweigenbaum, J.A., & Mitchell, A.E. (2015) Ultrahigh-pressure liquid chromatography triple-quadrupole tandem mass spectrometry quantitation of polyphenols and secoiridoids in California-style black ripe olives and dry salt-cured olives. Journal of Agricultural and Food Chemistry 63, 2400–2405.

Montedoro, G.F., Servilli, M., Bardioli, M., & Miniati, E. (1992) Simple and hydrolyzable phenolic compounds in virgin olive oil 2. Initial characterization of the hydrolyzable fraction. Journal of Agricultural and Food Chemistry 40, 1571–1576.

Nergiz, C., & Unal, K, (1991) Determination of phenolic acids in virgin olive oil. Food Chemistry 39, 237–240.

Pérez-Jiménez, F., Ruano, J., Perez-Martinez, P., Lopez-Segura, F., & Lopez-Miranda, J. (2007) The influence of olive oil on human health: not a question of fat alone. Molecular Nutrition & Food Research 51, 1199–1208.

Pirisi, F.M., Cabras, P., Falqui Cao, C., Migliorini, M., & Mugelli, M. (2000) Phenolic compounds in virgin olive oil 2. Reappraisal of the extraction, HPLC separation, and quantification procedures. Journal of Agricultural and Food Chemistry 48, 1191–1196.

Pizarro, M. Luz., Becerra, M., Sayago, A., Beltrán, M., & Beltrán, R. (2012) Comparison of different extraction methods to determine phenolic compounds in virgin olive oil. Food Analytical Methods 6(1), 123–132.

Ragazzi, E. (1973) Quantitative analysis of phenolic compounds after thin-layer chromatographic separation. Journal of Chromatography 77, 369–375.

Ramírez, E., García-García, P., de Castro, A., Romero, C., & Brenes, M. (2013) Debittering of black dry-salted olives. European Journal of Lipid Science and Technology 11, 1319–1324.

Rastrelli, F., Schievano, E., Bagno, A., & Mammi, S. (2009) NMR quantification of trace components in complex matrices by band-selective excitation with adiabatic pulses. Magnetic Resonance in Chemistry 47, 868–872.

Rios, J.J., Gil, M.J., & Gutierrez-Rosales, F. (2005) Solid-phase extraction gas chromatography-ion trap-mass spectrometry qualitative method for evaluation of phenolic compounds in virgin olive oil and structural confirmation of oleuropein and ligstroside aglycons and their oxidation products. Journal of Chromatography A 1093, 167–176.

Romero, C., Brenes, M., Yousfi, K., García, P., García, A., & Garrido, A. (2004) Effect of cultivar and processing method on the contents of polyphenols in table olives. Journal of Agricultural and Food Chemistry 52, 479–484.

Rotondo, A., Salvo, A., Giuffrida, D., Dugo, G., & Rotondo, E. (2011) Atti Accad. Pelorit. Pericol. Cl. Sci. Fis. Mat. Nat. 89, C1A 8901002.

Saitta, M., Lo Curto, S., Salvo, F., Di Bella, G., & Dugo, G. (2002) Gas chromatographic-tandem mass spectrometric identification of phenolic compounds in Sicilian olive oils. Analytica Chimica Acta 466, 335–344.

Segura-Carretero, A., Carrasco-Pancorbo, A., Bendini, A., Cerretani, L., & Fernández-Gutiérrez, A. (2010) Analytical determination of polyphenols in olive oil. In: Olives and olive oil in health and disease prevention, ed. V.R. Preedy & R.R. Watson. Academic Press, San Diego, CA, 509–523.

Singleton, V.L., Orthofer, R., & Lamuela-Raventos, R.M. (1999) Methods in Enzymology. Packer L 299, 152–178.

Singleton, V.L., & Rossi, J.A., Jr. (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Society for Enology and Viticulture 16, 144–158.

Solinas, L., & Cichelli, A. (1982) Determination of phenolic substances in olive oil be GLC and HPLC: possible role of tyrosol in the determination of the quantity of olive oil blends with refined oil. La Rivista di Scienza dell'Alimentazione 11, 223–230.
Tasioula-Margari, M., & Okogeri, O. (2001) Simultaneous determination of phenolic compounds and tocopherols in virgin olive oil using HPLC and UV detection. Food Chemistry 74, 377–383.
Tsimidou, M., Papadopoulos, G., & Boskou, D. (1992) Determination of phenolic compounds in virgin olive oil by reversed-phase HPLC with emphasis on UV detection. Food Chemistry 44, 53–60.
Vazquez-Roncero, A. (1974) Phenolic compounds in olive fruits. Grasas y Aceites 25, 269–279.
Vinha, A.F., Ferreres, F., Silva, B.M., Valentao, P., Goncalves, A., Pereira, J.A., Oliveira, M.B., Seabra, R.M., & Andrade, P.B. (2005) Phenolic profiles of Portuguese olive fruits *Olea europaea* L.: influences of cultivar and geographical origin. Food Chemistry 89, 561–568.
Visioli, F., & Bernardini, E. (2011) Extra virgin olive oil's polyphenols: biological activities. Current Pharmaceutical Design 17, 786–804.
Vulcano, I., Halabalaki, M., Skaltsounis, L., & Ganzera, M. (2015) Quantitative analysis of pungent and anti-inflammatory phenolic compounds in olive oil by capillary electrophoresis. Food Chemistry 169, 381–386.
Zoidou, E., Melliou, E., Gikas, E., Tsarbopoulos, A., Magiatis, P., & Skaltsounis, A.L. (2010) Identification of Throuba Thassos, a traditional Greek table olive variety, as a nutritional rich source of oleuropein. Journal of Agricultural and Food Chemistry 58, 46–50.

32 DNA fingerprinting as a novel tool for olive and olive oil authentication, traceability, and detection of functional compounds

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32.1 Introduction

The need for accurate and reliable methods for plant species identification in nature and in food products has steadily increased during the past decades, particularly in relation to recent food scares, international trade, and technological progress in food production. Moreover, the development of value-added products based on plants has raised concerns about adulteration. Thus, reliable methods to protect the producer, the company, and the customer are needed. Fresh food products without any processing are suitable for many types of analytical or molecular analyses. As most foodstuffs nowadays are processed to some extent, extensive research has been performed to establish the best method to authenticate processed food. Although DNA is usually degraded and cut into small fragments, still DNA-based methods for food authenticity remain the methods of choice (Madesis *et al.*, 2014).

Olive tree (*Olea europaea* L.) is one of the oldest and most important crops in the Mediterranean region, as much evidence from ancient Greece and other Mediterranean countries manifests. *Olea europaea* includes both cultivated (var. *sativa*) and wild-type (var. *oleaster*) cultivars. The cultivated olive cultivars are evergreen trees; they are outcrossing, can be interfertile, and are interconnected by sporadic, spontaneous hybridization (Zohary & Spiegel-Roy, 1975). Olive tree cultivars have a high genetic diversity as a result of both plant longevity and the low number of genotypes that resulted through centuries of cultivation. There are many different olive cultivars worldwide (Kiritsakis, 1991, 1998). Some of these cultivars have the same or similar names, while others, although identical, have different names in different regions and countries, which hinders the description and classification of olive cultivars (Fabbri *et al.*, 2009). As a result, the Food and Agriculture Organization (FAO) olive germplasm database includes about 1250 cultivars, cultivated in 54 countries and conserved in over 100 collections. The size of this germplasm is somewhat controversial (Bartolini, 2008). It is also certain that the number is even higher because of the lack of information on many local cultivars and ecotypes (Cantini *et al.*, 1999). The majority of these cultivars originate from southern European countries such as Italy (538 cultivars), Spain (183), France (88), and Greece (52) (Baldoni & Belaj, 2010). Thus, there is a high biodiversity in the olive germplasm that represents a rich source of genetic variability that could be used for breeding superior cultivars (Baldoni & Belaj, 2010).

Olive oil could be characterized as a functional food, constituting the major ingredient of the Mediterranean diet. Olive oil is used in cooking as well as in pharmaceuticals, soaps, and cosmetics. Furthermore, it is used by the food industry for preparation of products, as a replacement of solid fat, and as an additive to improve the fatty acid profile in terms of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids, as well as the ω-6 to ω-3 ratio and even as a pharmaceutical (Reddy *et al.*, 2015).

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Moreover, *in vitro* and *in vivo* studies revealed that olive oil has many health-promoting attributes, like lowering the risk of cardiovascular diseases and cancer risks, and being anti-inflammatory, antimicrobial, antioxidant, and other health benefits (Reddy *et al.*, 2015). In addition, it has been shown that olive oil contains active compounds like phenolics, squalene, and the important oleic acid (Bonanome *et al.*, 2005). The main ingredients in olive oil are derivatives of secoiridoids, followed by flavonoids and phenolic alcohols. The quality parameters of olive oil are its sensory attributes and oxidative stability, which depend on the quantity and quality of the active compounds present in oils. The rate of oleate, linoleate, and linoleneate autoxidation is 1:12:25 (Frankel, 2010). The oxidation process depends, first, on the chemical structure and, second, on the degree of unsaturation of fatty acids. The ratio of MUFAs to PUFAs or oleic to linoleic acids in olive oil determines its oxidative stability. Approximately 72% of oleic acid is present in olive oil, and olive oil consumption has been correlated with the prevention of the aromas and the development of thrombosis in humans, arising from the higher ratios of MUFAs to PUFAs and MUFAs to SFAs. Oleic acid has also been linked to positive health effects like inhibition of the progression of adrenoleukodystrophy (ALD), and this is responsible for reducing blood pressure (Terés *et al.*, 2008). Oleic acid has also been involved with preventing the negative effects of low-density lipoprotein (LDL). Moreover, Kontogianni *et al.* (2007) showed that the use of olive oil with a higher percentage of oleic acid in food preparation has a positive effect on protection from ischemic heart disease. Thus, extra virgin olive oil (EVOO) could be considered as an ideal functional food, having good polyphenolic components and important therapeutic value. The most important polyphenolic compounds of olive oil that make major contributions toward health benefits are tyrosol derivatives, squalene, and oleuropein (Visioli *et al.*, 2002).

Olive oil is a product of high economic importance for the Mediterranean countries. For example, Spain, Italy, and Greece are the first, second, and third producers of olive oil in the world (FAO, 2015; http://faostat3.fao.org/home/E), respectively, and olive oil is the number two agricultural export product in Greece in terms of financial value (www.minagric.gr). For this reason, the olive oil industry, nurseries, and olive growers are interested in methods that will allow the accurate identification of olive cultivars. Quality of olive oil is determined by the specific cultivar that it is produced from. Certain olive oil cultivars, in fact, are recognized as high quality due to the well-defined geographical area where they grow, and consequently they impose higher market prices. The European Union has established specific rules about olive oil production and its market through European Commission (EC) Regulation No. 865/2004 on the Protected Designation of Origin (PDO) mark and Protected Geographical Indication (PGI) mark (No. 510/2006) (EC, 2004, 2006).

Furthermore, considering the complexity of the germplasm and the high economic importance of olives and olive oil, it is becoming essential to establish a method for EVOO authentication and detection of possible adulterations. Although several different analytical techniques have been developed, most of the chemical analyses are of limited impact due to the environmentally imposed high variability of oil components, such as fatty acids and secondary metabolites. Official methods, which measure the physicochemical parameters (free acidity, peroxide value, fatty acids, and specific ultraviolet [UV] absorbance) of olive oil in order to characterize it as EVOO, have been established by the EC (Regulations No. 2568/91, 1991) and the International Olive Council (IOC) (COI/T.20/Doc. No. 19/Rev 2, 2008). However, these methods are not sufficient to provide reliable data for oil traceability (Christopoulou *et al.*, 2004; Ben-Ayed *et al.*, 2013). Instead, DNA-based technologies are gaining increasing popularity in the food authenticity field (Rasmussen & Morrissey, 2008). There are many examples of successful application of DNA-based markers, in order to overcome problems associated with differences due to environmental growth conditions, or to verify food authenticity and traceability (Martins-Lopes *et al.*, 2008; Montemurro *et al.*, 2008; Pafundo *et al.*, 2010; Ganopoulos *et al.*, 2012a, 2013; Madesis *et al.*, 2013a, 2014). The application of DNA markers in characterizing olive oil offers a number of advantages compared to biochemically based markers, as they provide increased specificity, sensitivity, and reliable performance with highly processed samples (Montealegre *et al.*, 2009).

32.2 DNA-based fingerprinting

Molecular markers are investigated as a diagnostic tool for food authenticity and traceability of the cultivar or type composition of complex food matrices in an increasing number of projects (Palmieri *et al.*, 2004;

Ganopoulos *et al.*, 2011, 2012a, 2012b, 2013; Bosmali *et al.*, 2012; Madesis *et al.*, 2012, 2013a). DNA-based methods make an important contribution to test and help in protecting the high quality of olive oils.

Significant amounts of DNA are present in olive oil obtained by cold pressing (Consolandi *et al.*, 2008). However, the filtration process lowers DNA concentrations, which tend to disappear due to nuclease degradation (Muzzalupo & Perri, 2002; de la Torre *et al.*, 2004). On the other hand, the length of storage time after oil milling can affect the use of DNA as an analyte for molecular traceability. Pafundo *et al.* (2010) observed a significant decrease in the quality of DNA extracted from olive oil, with a consequent loss of information, a month after olive oil production. Significant amounts of DNA are present in olive oil obtained by cold pressing (Consolandi *et al.*, 2008).

During the determination of the cultivar origin of olive oil (Spaniolas *et al.*, 2008b), the length of DNA fragments present in olive oil during storage (which can be used to determine degradation) was estimated via the use of Lambda DNA (Spaniolas *et al.*, 2008b). Lambda DNA is a linear molecule of approximately 50 kb, a length that might be that of olive DNA present in olive oil. Thus, having Lambda DNA as a marker allows the estimation of successful application of PCR-based fingerprinting techniques, which require templates longer than 100 base pairs (bp), as smaller fragments might not be able to successfully amplify the target sequences from olive oil samples stored for several months, in which the concentration of DNA is low. The presence of DNA fragments shorter than 100 bp might increase the application range of DNA fingerprinting in olive oil.

As seen in this chapter, the main obstacle in olive oil genotyping and fingerprinting is DNA extraction. However, there are several methods that allow the successful extraction of DNA from olive oil (Busconi *et al.*, 2003; Doveri *et al.*, 2006; Pasqualone *et al.*, 2007; Consolandi *et al.*, 2008). In addition to "homemade" DNA extraction protocols, there are several commercial kits, which provide adapted protocols for olive oil DNA extraction and have been successfully used (Martins-Lopes *et al.*, 2008; Spaniolas *et al.*, 2008a; Ayed *et al.*, 2009; Pafundo *et al.*, 2010). Once there is DNA available, then there are several options regarding the DNA markers that could be used in order to identify the olive cultivar from which an olive oil is originated (Consolandi *et al.*, 2008). The first attempts, however, were carried out using genomic DNA extracted from drupes, which had a good potential to be amplified correctly, using random amplified polymorphic DNA (RAPD) markers (Cresti *et al.*, 1997). Despite the fact that different methods have been developed, DNA could be extracted from oil and subsequently be used via markers for olive oil authenticity. Doveri *et al.* (2006) raised the concern on the use of DNA markers for cultivar discrimination and authenticity of olive oil. Their concerns focus on discrepancies between the genetic profiles of olive oil and olive fruit. They suggested that this might be the effect of DNA originating from pollen donors present in the extract from the paste obtained by crushing whole fruits. Thus, they suggested that special care should be taken in the interpretation of DNA profiles obtained from DNA extracted from oil, for identifying the varietal origin, provenance, and authenticity issues (Martins-Lopes *et al.*, 2008). It should also be noted here that one should pay attention when comparing the amplification profiles of leaves with the corresponding oils for cultivar traceability purposes, as the possible presence of additional alleles due to paternal contribution in oils extracted from entire drupes might be significant (Alba *et al.*, 2009).

The determination of cultivar(s) of origin is a decisive parameter regarding the authenticity of olive oil, as the cultivar determines its special characteristics. Olive tree includes a considerably high number of different cultivars, which possess significant differences in terms of chemical composition and sensory characteristics. These unique characteristics in many cases are also affected by climatic conditions and agronomic techniques, together with olive ripeness and the olive extraction system (Arvanitoyannis & Vlachos, 2007). However, the vast number of cultivars and the longevity of the species have resulted in that the same cultivar (being genetically identical) is cultivated in different countries with different names (Matos *et al.*, 2007). Certain olive oils have acquired the PDO and PGI awards because of their unique characteristics, ensuring both producers and consumers. In this context, the determination of olive cultivar(s) used in olive oil production is extremely important for the authentication of the final product as, depending on the PDO olive oil, only certain cultivars are allowed to be used, while at the same time some of them are monovarietal. Several analytical techniques have been developed in order to ensure the PDO olive oil authenticity in terms of the cultivar used. In this context, the analysis of different olive oil chemical components coupled to chemometric techniques for data exploitation has been reported as a possible approach (Christy *et al.*, 2004).

Recently, DNA-based marker techniques have been successfully applied to authenticate products, to overcome problems associated with differences in terms of chemical components due to environmental

conditions during growth, and to function as a diagnostic tool for food discrimination, traceability, and authenticity of a cultivar or type composition of complex food matrices in an increasing number of worldwide cases (Consolandi *et al.*, 2008; Martins-Lopes *et al.*, 2008; Montemurro *et al.*, 2008; Pafundo *et al.*, 2010).

A number of different DNA markers have been proposed to be used for the identification of olive cultivars in olive oils. These markers include amplified fragment length polymorphisms (AFLPs), RAPDs, microsatellites, inter-simple sequence repeats (ISSRs), and single nucleotide polymorphisms (SNPs). Many studies have been performed using different marker systems, for example the efficiency of RAPDS, ISSR, and SSR markers in identifying the varietal origin of olive oils was assessed by Martins-Lopes *et al.* (2008), while later their possible use and application in certification processes were evaluated by Bracci *et al.* (2011). Specifically, we describe in this section many of these studies. However, there is an innate problem arising from the olive oil production procedure, which is the multicultivar nature of many olive oils. This fact increases tremendously the complexity and the usefulness of DNA-based techniques for their distinction.

32.2.1 Amplified fragment length polymorphisms

AFLPs can be used in order to simultaneously screen a large number of loci without any need for preliminary sequence knowledge. They are considered to be the most efficient markers in revealing polymorphic bands in a single assay. Moreover, they are also considered as highly reliable, and so they have been used for genotyping in a large number of crops and wild species, including olives (Angiolillo *et al.*, 1999; Sanz-Cortés *et al.*, 2003). Busconi *et al.* (2003) showed that the DNA extracted from monovarietal olive oil was suitable for AFLP analysis. The profiles obtained by olive oil were highly similar to the profiles obtained by the DNA from the leaves of the same cultivar. This result allowed cultivar identification used for olive oil production via the AFLP analysis. Furthermore, Pafundo *et al.* (2005), apart from showing that DNA extraction is a critical step for the success of the AFLP application, have also presented high correspondence (70%) between AFLP profiles in four cultivars and their olive oils. However, this correspondence was present only on fragments below 250 bp.

AFLP markers were the system of choice for Montemurro *et al.* (2008) in order to test ten virgin monovarietal olive oils. AFLP markers used were able to discriminate all the olive oils examined, even though for some samples only a partial correspondence with the AFLP profile from the leaves was acquired.

AFLP markers also allowed the development of sequence-characterized amplified region (SCAR) markers. These markers were produced from AFLP reproducible fragments during fingerprinting of monocultivar oil (Pafundo *et al.*, 2005). This method allowed the development of a specific single-locus polymerase chain reaction (PCR)-based marker that can be used in complex food matrices. Fifty-six olive oil cultivars were classified using the chloroplast region CP-rp116T. However, as seen before, fragments should be less than 300 bp when DNA is extracted from olive oil.

Busconi *et al.* (2003), using SCAR and AFLP markers on DNA recovered from olive oil, showed that the DNA had both organellar and nuclear origin. AFLPs have also been used in order to identify the cultivar composition of monocultivar olive oils, while they have also recognized that DNA isolation is the most critical step (Pafundo *et al.*, 2005). Later, Pafundo *et al.* (2010) used again AFLPs to amplify DNA isolated from olive oils, but they have also developed SCAR markers in order to successfully amplify the olive oil–extracted DNA.

32.2.2 Random amplified polymorphic DNA

RAPD markers are widely applied to plant research, such as phylogenetic studies, genome mapping, and population genetic studies, as well as to cultivar identification. The advantages of this technique rely on the simplicity of its use, being low cost and needing a small amount of plant material. Muzzalupo and Perri (2002) reported the possibility of using RAPD to analyze DNA from sediments of olive oil, previously treated with proteinase K during the oil production (malaxation). Moreover, they verified some differences between the leaves and the oil profiles, assigning this discrepancy to the cross-pollination process observed in olive trees. Busconi *et al.* (2003) also applied RAPD to obtain fingerprint profiles from 15 olive cultivars, from which they selected two fragments that, after cloning and sequencing, were transformed into more

reliable SCAR markers, as seen in AFLPs. A similar approach is the development of specific SCAR markers for olive tree that produced amplification for olive sediments, and filtered and unfiltered olive oil (de la Torre *et al.*, 2004). The differences found in six SCAR patterns of three olive oil cultivars were considered as characteristic fingerprints. Nonetheless, whenever possible, the use of DNA from sediments is recommended. Martins-Lopes *et al.* (2008) tested 11 RAPD primers, two of which produced reproducible bands in all olive oil samples under study. Among the RAPD markers obtained, seven of nine bands were considered as polymorphic, reporting a mean level of polymorphism of 78%. This finding is in agreement with the general lack of reproducibility attributed to RAPD markers (Jones *et al.*, 1997), which are not adequate markers for olive oil fingerprinting.

32.2.3 Microsatellites

Microsatellites are simple sequence repeats (SSRs) and are considered to be the most reliable markers, since they present a high polymorphic level due to variations of the number of repeats. Several studies have reported the successful use of microsatellites in plant genotyping and plant-based products authentication (Madesis *et al.*, 2013b).

Genomic microsatellites (Belaj *et al.*, 2003; Bandelj *et al.*, 2004; de la Torre *et al.*, 2004; Breton *et al.*, 2008; Do Val *et al.*, 2012; Trujillo *et al.*, 2014) as well as chloroplast-SSR markers (Hannachi *et al.*, 2010; Mariotti *et al.*, 2010) have been used to differentiate and characterize the olive cultivars (Belaj *et al.*, 2003; Mookerjee *et al.*, 2005; Khadari *et al.*, 2008; Marra *et al.*, 2013; Linos *et al.*, 2014; Muzzalupo *et al.*, 2014; Trujillo *et al.*, 2014; Xanthopoulou *et al.*, 2014; Abdessemed *et al.*, 2015; Beghè *et al.*, 2015).

Pasqualone *et al.* (2004), using SSR markers, succeeded in amplifying DNA from olive oil, DNA with patterns identical to those of leaves and drupes of the same cultivar. Six out of the seven primer sets used for SSR markers were polymorphic, producing fragments of different lengths for each olive oil type they studied. Thus, through their results, they showed that SSR DNA microsatellites were able to distinguish virgin olive oils from different cultivars. The SSR markers showed good discriminating ability, and, finally, this technique might be also used in mixtures of 3–4 cultivars, such as those usually adopted in PDO oils. Testolin and Lain (2005) used six SSR primer sets with DNA from olive oil samples; all sets produced DNA amplicons of the expected size. The authors faced two problems: (a) low DNA yield and (b) DNA degradation. In the case of low DNA yield, the use of nested PCR improved the amount of amplified DNA. In the case of degradation, the fragments of up to 188 bp enabled consistent amplification of SSR from low starting amounts of oil. Thus, the authors concluded that the use of microsatellite polymorphisms for olive oil cultivar identification might be a promising tool (Testolin & Lain, 2005). SSR markers in the case of olive oil should be used with care, as paternal alleles could contribute to the band pattern. Therefore, care should also be taken during interpretation of results (Doveri *et al.*, 2006). When DNA was extracted from maternal tissues (leaves and olive pulp), identical genetic profiles by means of SSR markers were obtained. However, additional alleles were identified in embryos (stone), in the paste obtained by crushing whole fruits, and even from oil obtained by whole fruits. Thus, the band pattern obtained using DNA from olive oil might be an amalgamation of the maternal alleles, with alleles contributed by different pollen donors. Yet, Muzzalupo *et al.* (2007) proved that DNA purified from olive oil is suitable to be applied in microsatellite analysis, showing that the profile of DNA purified from monocultivar oil corresponded to the profile of DNA originating from the leaves of the same cultivar. Pasqualone *et al.* (2007) have used SSR markers to prove that microsatellites might be useful for the identification of the presence of a specific cultivar in PDO oil, thus authenticating the product. However, they have been able to obtain a marker profile only of the main cultivar in the oil, while no signal was detected of the other cultivars present. Moreover, Ayed *et al.* (2009), evaluated the potential applicability of microsatellites in order to trace Tunisian olive oil cultivars. They compared the genetic profiles obtained when they used DNA extracted from oil and leaves of two cultivars. In this case, certain SSR markers showed alleles of the pollinators in oil samples and were able to distinguish them from alleles of tree somatic tissues, suggesting that they are reliable and could be used for olive oil traceability. Furthermore, Alba *et al.* (2009) stretssed the importance of low concentration and high degradation of DNA, and the putative presence of additional paternal alleles in oils from entire drupes, and raised the issue of conscious consideration when comparing SSR profiles from leaves with the corresponding oils for cultivar traceability purposes. The SSRs used had a success rate of 85.7% while showing that 90% of them had identical patterns between leaves and oil DNA. Capillary electrophoresis (CE) coupled by an

Table 32.1 Application of microsatellite molecular markers in *Olea europea* L. studies.

Microsatellites	Application in *Olea europea* L.	References
✓	DNA fingerprinting of cultivars	Sefc *et al.* (2000), Donini *et al.* (2006, 2009), Xanthopoulou *et al.* (2014)
✓	Estimation of genetic diversity	Belaj *et al.* (2003), Mookerjee *et al.* (2005), Khadari *et al.* (2008), Marra *et al.* (2013), Linos *et al.* (2014), Muzzalupo *et al.* (2014), Trujillo *et al.* (2014), Xanthopoulou *et al.* (2014), Abdessemed *et al.* (2015), Beghè *et al.* (2015)
✓	Paternity analysis	De la Rosa *et al.* (2004), Mookerjee *et al.* (2005), Díaz *et al.* (2007)
✓	Cultivar traceability in olive oil	Pasqualone *et al.* (2007, 2015), Martins-Lopes *et al.* (2008), Alba *et al.* (2009), Xanthopoulou *et al.* (2014), Montemurro *et al.* (2015)
✓	Phylogenetic studies	Belaj *et al.* (2007), Linos *et al.* (2014), Noormohammadi *et al.* (2014), Diez *et al.* (2015)

automatic sequencer enabled the identification of specific alleles, even for those that had weak signals, thus confirming that DNA microsatellites were able to distinguish and identify olive oils from different cultivars. Table 32.1 shows the main application of microsatellite molecular markers in *Olea europaea* L. studies.

32.2.4 Inter-simple sequence repeat markers

ISSR markers refer to PCR amplicons of DNA regions situated between adjacent and inversely oriented sequence repeats. Due to microsatellite loci's ample presence in plant genomes, the use of ISSR primers often results in multiple bands, which might be useful for genotyping and mapping and also for developing SSR markers. Yet, this technique is not used so often in food authentication studies. Nevertheless, Pasqualone *et al.* (2001) used ISSR markers in order to discriminate drupes from different cultivars, combining two ISSR markers that were the most polymorphic. They could amplify bands from DNA extracted from olive oil; thus, they suggested that ISSR markers could be an alternative tool for cultivar identification purposes when pressing also the drupes for the production of PDO oils. Later, Martins-Lopes *et al.* (2008) tested ISSR markers on leaves and olive oil samples, successfully obtaining a total of 18 reproducible ISSR fragments from the two most informative sets of primers. They concluded that ISSR markers are more informative compared to RAPD markers. Yet, this statement is problematic, since other authors could not produce bands when they had used DNA from olive oil; thus, ISSR markers should be used with caution.

32.2.5 Single nucleotide polymorphisms

SNPs are usually abundant in the genome and genetically stable when compared to other genetic markers. They have been effectively applied in order to genotype olive cultivars (Reale *et al.*, 2006). Consolandi *et al.* (2008) also adopted the "ligation detection reaction-universal array" (LDR-UA), described by Gerry *et al.* (1999), and applied it in olive oil to genotype a panel of 49 cultivars with respect to 17 SNPs. Twelve amplicons out of the 13 that contained these SNPs were successfully amplified from oil-derived templates, and their profiles were fully consistent with the amplicons obtained from leaf-derived DNA. The assay

developed proved to be adequate and with enough discriminating power to distinguish all 49 olive cultivars. It could also be applied as a high-throughput method, if used as a semi-automated SNP genotyping assay to identify the origin of monocultivar olive oils. This is an important and very promising tool to discriminate olive oil cultivars, considering the great number of olive oil cultivars and thus the need for authentication.

Recently, Uncu *et al.* (2015) sequenced short fragments from five olive genes for SNP identification and developed CAPS (cleaved amplified polymorphic DNA) assays for SNPs that alter restriction enzyme recognition motifs. They applied it on the oils of 17 olive cultivars and suggested that a maximum of five CAPS assays were necessary to discriminate the cultivar origin of the samples. They concluded that the SNP-based CAPS assays developed could be used for testing and verification of the authenticity of Turkish monocultivar olive oils, for olive tree certification, and also in germplasm characterization and preservation studies.

Furthermore, a multiplex SNP genotyping assay for olive oil cultivar identification, performed on a suspension of fluorescence-encoded microspheres, has been recently developed (Kalogianni *et al.*, 2015). According to the protocol developed, it is suggested that up to 100 sets of microspheres, with unique "fluorescence signatures," were available. Out of these, a model panel of three SNPs was chosen that enabled identification of six common Greek olive cultivars ('Adramytini', 'Chondrolia', 'Chalkidikis', 'Kalamon', 'Koroneiki', and 'Valanolia').

Another method based on differences in the DNA sequence was developed by Consolandi *et al.* (2008), who have developed a semi-automated SNP genotyping assay that is able to identify the origin of, and authenticate, EVOOs. The method that Consolandi and coworkers have developed is using a ligation detection reaction–universal array (LDR-UA) platform combined with several olive SNPs. They have shown that 13 correctly selected SNPs were sufficient to unambiguously discriminate 49 different cultivars (Bracci *et al.*, 2011).

32.2.6 Chloroplast genome sequencing

Mariotti *et al.* (2010) published the annotated sequence of the plastome of the Italian cultivar 'Frantoio'. This is an important achievement in *Olea europaea* L genomic studies. This olive plastid genome has a size of 155,889 bp and showed the organization and the gene order that are conserved among the angiosperms. The olive chloroplast genome was found to contain 130 genes and 644 repetitive sequences (among which they have identified 633 mononucleotide SSRs, six di-, three tetra-, and two pentanucleotide SSRs) (Bracci *et al.*, 2011).

The annotated sequence was used to assess the content of coding genes, the extent and distribution of repeated and long dispersed sequences, as well as the nucleotide composition pattern. Thus, vital information was obtained for structural, functional, and comparative genomic studies in olive plastids. Furthermore, the alignment of the olive plastome sequence to those of other cultivars and species identified 30 new organellar polymorphisms within the cultivated olive. Chloroplast DNA polymorphisms have also been used as molecular markers to identify cultivars of *Olea europaea* L. (Intrieri *et al.*, 2007). Spaniolas *et al.* (2008a) developed a CE method that detected chloroplast polymorphisms for the authentication of plant oils. Universal primers were used to amplify different-length plastid DNA sequences from each plant species. Two years later, Spaniolas *et al.* (2010) described another PCR-based method that could differentiate a range of 11 plant species commonly used for oil extraction. This method was also based on polymorphisms of the plastid genome that were detected by the use of CE on the basis of different lengths of the corresponding PCR amplicons. CE methods' major disadvantage is the need for gel electrophoresis and thus the use of UV luminescence and ethidium bromide. Moreover, CE methods have a high risk of contamination, another major problem.

32.2.7 Real-time PCR

The detection of adulterations and possible frauds, caused either due to mixtures with oils of other species (e.g., corn or rapeseed) or to the wrong certification of PDOs, compels absolute detection and quantification of the adulteration (Madesis *et al.*, 2014). Conventional PCR, being the technique of choice for absolute detection of species, still remains a semiquantitative technique, and, therefore, it is not the optimal technique for authentication purposes when quantification is needed (Giménez *et al.*, 2010). Real-time PCR (RT-PCR) has also been applied as a tool for authentication of olive oil (Giménez *et al.*, 2010). The authors also

supported that quantitative RT-PCR (qRT-PCR) was useful to quantify DNA extracted from oil, and therefore to evaluate the yields of different methods of extraction. However, they found that the size of the *rbcL* amplification product used in their method was critical for the success of the analysis.

32.2.8 Taqman probe

Taqman probes, designed on SNPs specific for cultivars forming the oil composition, were also used in the application of qRT-PCR for the quantitation of cultivars in PDO or PGI oils (Marmiroli *et al.*, 2009).

32.2.9 High resolution melting (HRM) analysis

Xanthopoulou *et al.* (2013) presented a distinct genetic fingerprint produced by five microsatellite markers from 47 Greek olive cultivars and allowed their discrimination based on their HRM profiles. They suggested that three microsatellite markers (DCA03, DCA09, and DCA17), which generated 29 HRM profiles, were sufficient to genotype all of the 47 olive cultivars studied, highlighting their potential use for cultivar identification.

Montemurro *et al.* (2015) verified the applicability of microsatellite markers in HRM analysis for the identification of the olive cultivars used in "Terra di Bari" PDO EVOO. They tested a panel of nine cultivars, widespread in the Apulia region, with 17 SSR primers. They obtained an identification key for the nine cultivars, which showed an unambiguous discrimination among the cultivars constituting the Terra di Bari PDO EVOO: 'Cima di Bitonto', 'Coratina', and 'Ogliarola'.

Recently, Pasqualone *et al.* (2015) verified the effect of talc (hydrated magnesium silicate) addition on olive oil DNA by targeting four selected microsatellites: DCA03, DCA16, DCA18 (Sefc *et al.*, 2000), and GAPU103A (Carriero *et al.*, 2002). Olive-processing trials were carried out at two different levels of talc (1 and 2%) and without talc (control). They showed that the DNA microsatellites analyzed identical HRM profiles in all the samples, excluding any effect of talc and confirming the genetic homogeneity of the olive lot processed (cv. 'Coratina').

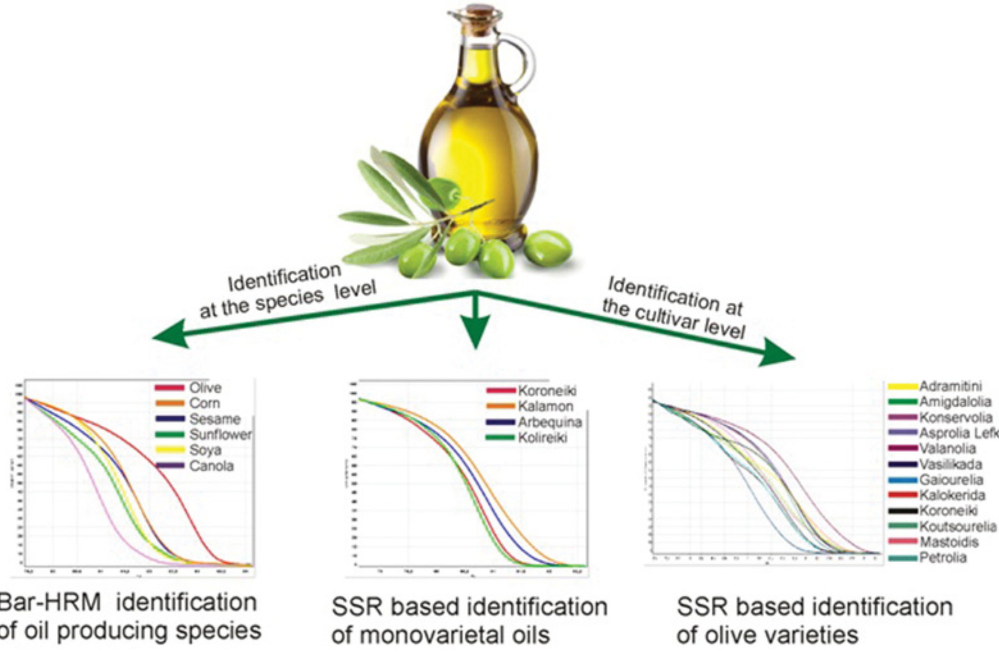

Figure 32.1 Workflow of HRM analysis for olive and olive oil identification and adulteration.

Bar-HRM has been used for the analysis and detection of different plant species but also on vegetable oils (Bosmali *et al.*, 2012; Madesis *et al.*, 2012, 2013a; Ganopoulos *et al.*, 2012a 2012b, 2013; Kalivas *et al.*, 2014; Osathanunkul *et al.*, 2015; Singtonat & Osathanunkul, 2015). In the case of oils, Ganopoulos *et al.* (2013) have used the universal chloroplast *rbcL* region in order to identify the botanical origin of the main vegetable oil species. Furthermore, they have used the same *rbcL* region for their quantitative detection in mixed oils. Using the *rbcL* region, the authors could detect the presence of 1% canola oil (*Brassica napus*) as an adulterant of olive oil (*Olea europaea*). It had also been shown earlier that the plastid-based molecular DNA technology has a great potential to be used for rapid detection of adulteration, up to 5% in olive oil easily (Kumar *et al.*, 2011). A general scheme regarding the application of HRM and microsatellites in the identification and discrimination of olives and olive oils can be found in Figure 32.1.

Vietina *et al.* (2013) used Bar-HRM analysis and produced melting profiles for each plant species identical in oils, leaves, or seeds. HRM was performed with primers for the chloroplast *rbcL* region (rbcL-DQ336704) (Wu *et al.*, 2011). They suggested that with HRM analysis, it was possible to detect alien oils (hazelnut, maize, sunflower, peanut, sesame, soybean, rice, and pumpkin) in all mixes, based on 'Leccino' monocultivar olive oil, even when the alien component was as low as 10%.

32.2.10 Microarrays

Recently, another application of peptide nucleic acid (PNA) microarrays was reported for the specific detection of DNA from olive oil using PNA probes (Rossi *et al.*, 2012). By applying this method, the presence of 5% refined hazelnut (*Corylus avellana*) oil could be detected in EVOO (*Olea europaea L.*). A set of two SNPs from the *Actin* gene of olive was selected for the evaluation of PNA probes as a discriminating tool for olive cultivars. Microarray is a powerful method that is able to detect multiple adulterations and discriminate a high number of species and cultivars, but it is expensive and difficult to develop, while at the same time the level of detection provided is not as low as in other methods described (Tedeschi *et al.*, 2011).

32.3 Omics approaches in olive and detection of functional compounds

One of the most important oil-producing plant species worldwide is the olive tree. Yet, and despite the recent advantages in omics technologies, a complete and fully annotated olive genome sequence is still not available (Muleo *et al.*, 2012). Alagna *et al.* (2009), using comparative 454-pyrosequencing from two olive genotypes during fruit development, supplied information on the structure and putative function of gene transcripts that accumulate during fruit development. They also reported differentially expressed genes between the two cultivars, with potential relevance in regulating the fruit metabolism and phenolic content during ripening. However, different attempts to exploit transcriptomic approaches are under development. For example, transcriptomics analysis on olive fruits sampled at three different stages showed the metabolic pathways and transcriptional aspects responsible for carbohydrates, fatty acids, secondary metabolites, transcription factors, and hormones, as well as a response to biotic and abiotic stresses throughout olive drupe development (Galla *et al.*, 2009). Ozdemir Ozgenturk *et al.* (2010) generated ESTs from two complementary DNA (cDNA) libraries from young olive leaves and immature olive fruits, which serve as a valuable source for further functional studies. Later, Bazakos *et al.* (2012) using Sanger sequencing and further microarray analysis, identified differentially expressed transcripts in salt-tolerant and salt-sensitive olive cultivars; and Gil-Amado and Gomez-Jimenez (2013), using high-throughput sequencing, studied the olive abscission zone during cell separation in order to understand mature fruit abscission control and thus help in current olive-breeding programs. Recently, 12 cDNA libraries from olive fruit, seeds, young stems, leaves, buds, and roots were sequenced, assembled, and annotated, offering the scientific community more data, which could be extremely useful for understanding metabolic pathways of olive in the production of components with healthy attributes (Muñoz-Mérida *et al.*, 2013).

The appearance of studies on genomics and transcriptomics of *Olea europaea* L. and in many cases metabolomics is both hopeful and promising (Carmona *et al.*, 2015). We should mention here the Olive Genome Project (OLEA), which is expected to offer transcriptomic data, molecular markers, and genomic

information regarding the 'Leccino' cultivar (http://www.oleagenome.org). Another project concerning genomic analysis of olive is the International Olive (*Olea europaea*) Genome Consortium (IOGC). The goal of the project (http://olivegenome.karatekin.edu.tr) is whole-genome sequencing of olive and identification of the biological nature of this plant. Currently, the annotated genome sequence of wild olive can be downloaded from IOGC, and the website harbors several basic genome analysis tools.

Despite both the economic and nutritional importance of olive oil, the genomic information on the species is hardly surprising. Special attention has been given to genes involved in fatty acid biosynthesis, including enoyl-ACP reductase, stearoyl-ACP desaturase, omega-6 cytoplasmatic desaturase, omega-6 plastidial desaturase, omega-3 cytoplasmic desaturase, omega-3 plastidial desaturase, cytochrome b5, acyl-CoA diacylglycerol acyltransferase, and oleosin enzymes (Giannoulia *et al.*, 2002). Other studies have also been performed earlier, concerning the clarification of the biosynthetic pathways for antioxidant biosynthesis (Shibuya *et al.*, 1999; Saimaru *et al.*, 2007).

In addition, the cloning, characterization, and spatial/temporal activation of genes involved in these pathways have been extensively studied (Poghosyan *et al.*, 1999; Banilas *et al.*, 2005; Hernández *et al.*, 2005; Giannoulia *et al.*, 2007). Banilas *et al.* (2011) have studied the triacylglycerols (TAGs) biosynthesis. Through their studies, they showed that DGAT1 and DGAT2, which belong to two families of diacylglycerol acyltransferase, the last and key enzyme of the TAG pathway, have a differential contribution to TAG storage in the olive tissues, with DGAT2 mainly involved in oil accumulation in the mesocarp. Besides the well-known fatty acids in olive oil, other components are present in low concentration, which also have great value for human health, in terms of protecting DNA as well as proteins and lipids from oxidative damage. Among these components, phenolics have been studied more than any other in olive oil.

Olive oil is one of the most important commodities and foodstuffs, both for the economy of the producing countries and for the consumers, due to its healthy attributes. Yet, up to the present and despite the progress in the omics field, there is still little information regarding the genomic sequence of *Olea europaea*. However, a continuously increasing number of expressed gene functions have been described, using the next-generation sequencing approaches as well (Chiappetta *et al.*, 2015). The recent advantages in omics technologies suggest that we will soon be able to identify the molecular pathways of all the important components of olives and olive oils, which will allow the efficient breeding and usage of the vast olive germplasm for the production of even healthier products. Omics technologies will also allow us not only to breed better cultivars but also be able to identify how the functional constituents of olives and olive oil influence human health, what mechanisms are involved, and how we can use them to live a healthier life.

References

Abdessemed, S., Muzzalupo, I., & Benbouza, H. (2015) Assessment of genetic diversity among Algerian olive (*Olea europaea* L.) cultivars using SSR marker. Scientia Horticulturae 192, 10–20.

Alagna, F., D'Agostino, N., Torchia, L., Servili, M., Rao, R., Pietrella, M., Giuliano, G., Chiusano, M.L., Baldoni, L., & Perrotta, G. (2009) Comparative 454 pyrosequencing of transcripts from two olive genotypes during fruit development. BMC Genomics 10, 399–414.

Alba, V., Sabetta, W., Blanco, A., Pasqualone, A., & Montemurro, C. (2009) Microsatellite markers to identify specific alleles in DNA extracted from monovarietal virgin olive oils. European Food Research and Technology 229, 375–382.

Angiolillo, A., Mencuccini, M., & Baldoni, L. (1999) Olive genetic diversity assessed using amplified fragment length polymorphisms. Theoretical and Applied Genetics 98, 411–421.

Arvanitoyannis, I.S., & Vlachos, A. (2007) Implementation of physicochemical and sensory analysis in conjunction with multivariate analysis towards assessing olive oil authentication/adulteration. Critical Reviews in Food Science and Nutrition 47, 441–498.

Ayed, R.B., Grati-Kamoun, N., Moreau, F., & Rebao, A. (2009) Comparative study of microsatellite profiles of DNA from oil and leaves of two Tunisian olive cultivars. European Food Research and Technology 229, 757–762.

Baldoni, L., & Belaj, A. (2010) Olive: oil crops. Springer, Berlin, 397–421.

Bandelj, D., Jakše, J., & Javornik, B. (2004) Assessment of genetic variability of olive varieties by microsatellite and AFLP markers. Euphytica 136, 93–102.

Banilas, G., Karampelias, M., Makariti, I., Kourti, A., & Hatzopoulos, P. (2011) The olive *DGAT2* gene is developmentally regulated and shares overlapping but distinct expression patterns with DGAT1. Journal of Experimental Botany 62, 521–532.

Banilas, G., Moressis, A., Nikoloudakis, N., & Hatzopoulos, P. (2005) Spatial and temporal expressions of two distinct oleate desaturases from olive (*Olea europaea* L.). Plant Science 168, 547–555.

Bartolini, G. (2008) Olea databases. Cultivar sheet Leucocarpa Sesto Fiorentino. National Research Council of Italy, Rome.

Bazakos, C., Dulger, A.O., Uncu, A.T., Spaniolas, S., Spano, T., & Kalaitzis, P. (2012) A SNP-based PCR–RFLP capillary electrophoresis analysis for the identification of the varietal origin of olive oils. Food Chemistry 134, 2411–2418.

Beghè, D., Molano, J.F.G., Fabbri, A., & Ganino, T. (2015) Olive biodiversity in Colombia: a molecular study of local germplasm. Scientia Horticulturae 189, 122–131.

Belaj, A., Muñoz-Diez, C., Baldoni, L., Porceddu, A., Barranco, D., & Satovic, Z. (2007) Genetic diversity and population structure of wild olives from the north-western Mediterranean assessed by SSR markers. Annals of Botany 100, 449–458.

Belaj, A., Satovic, Z., Cipriani, G., Baldoni, L., Testolin, R., Rallo, L., & Trujillo, I. (2003) Comparative study of the discriminating capacity of RAPD, AFLP and SSR markers and of their effectiveness in establishing genetic relationships in olive. Theoretical and Applied Genetics 107, 736–744.

Ben-Ayed, R., Kamoun-Grati, N., & Rebai, A. (2013) An overview of the authentication of olive tree and oil. Comprehensive Reviews in Food Science and Food Safety 12, 218–227.

Bonanome, A., Colomer, R., Corella-Piquer, D., Covas, I., Chamorro-Quiros, J., Escrich, E., Lairon, D., Lamuela-Raventos, R., Lopez-Miranda, J., & Lopez-Segura, F. (2005) virgin olive oil. European Journal of Clinical Investigation 35, 421–424.

Bosmali, I., Ganopoulos, I., Madesis, P., & Tsaftaris, A. (2012) Microsatellite and DNA-barcode regions typing combined with high resolution melting (HRM) analysis for food forensic uses: a case study on lentils (*Lens culinaris*). Food Research International 46, 141–147.

Bracci, T., Busconi, M., Fogher, C., & Sebastiani, L. (2011) Molecular studies in olive (*Olea europaea* L.): overview on DNA markers applications and recent advances in genome analysis. Plant Cell Reports 30, 449–462.

Breton, C., Pinatel, C., Mídail, F., Bonhomme, F., & Berville, A. (2008) Comparison between classical and Bayesian methods to investigate the history of olive cultivars using SSR-polymorphisms. Plant Science 175, 524–532.

Busconi, M., Foroni, C., Corradi, M., Bongiorni, C., Cattapan, F., & Fogher, C. (2003) DNA extraction from olive oil and its use in the identification of the production cultivar. Food Chemistry 83, 127–134.

Cantini, C., Cimato, A., & Sani, G. (1999) Morphological evaluation of olive germplasm present in Tuscany region. Euphytica 109, 173–181.

Carmona, R.M., Zafra, A., Seoane, P., Castro, A.J., Guerrero-Fernández, D., Castillo-Castillo, T., Medina-García, A., Cánovas, F.M., Aldana-Montes, J., & Navas-Delgado, I. (2015) ReprOlive: a database with linked data for the olive tree (*Olea europaea* L.) reproductive transcriptome. Frontiers in Plant Science 6, 625.

Carriero, F., Fontanazza, G., Cellini, F., & Giorio, G. (2002) Identification of simple sequence repeats (SSRs) in olive (*Olea europaea* L.). Theoretical and Applied Genetics 104, 301–307.

Chiappetta, A., Muto, A., Bruno, L., Woloszynska, M., Van Lijsebettens, M., & Bitonti, M.B. (2015) A dehydrin gene isolated from feral olive enhances drought tolerance in *Arabidopsis* transgenic plants. Frontiers in Plant Science 6.

Christopoulou, E., Lazaraki, M., Komaitis, M., & Kaselimis, K. (2004) Effectiveness of determinations of fatty acids and triglycerides for the detection of adulteration of olive oils with vegetable oils. Food Chemistry 84, 463–474.

Christy, A.A., Kasemsumran, S., Du, Y., & Ozaki, Y. (2004) The detection and quantification of adulteration in olive oil by near-infrared spectroscopy and chemometrics. Analytical Sciences 20, 935–940.

Consolandi, C., Palmieri, L., Severgnini, M., Maestri, E., Marmiroli, N., Agrimonti, C., Baldoni, L., Donini, P., De Bellis, G., & Castiglioni, B. (2008) A procedure for olive oil traceability and authenticity: DNA extraction, multiplex PCR and LDR–universal array analysis. European Food Research and Technology 227, 1429–1438.

Cresti, M., Linskens, H.F., Mulcahy, D.L., Bush, S., Di Stilio, V., Xu, M.Y., Vignani, R., & Cimato, A. (1997) Comunicación preliminar sobre la identificación del DNA de las hojas y el aceite de oliva de *Olea europaea*. Olivae 36–37.

De la Rosa, R., James, C.M., & Tobutt, K.R. (2004) Using microsatellites for paternity testing in olive progenies. HortScience 39, 351–354.

de la Torre, F., Canovas, F.M., & Claros, M.G. (2004) Isolation of DNA from olive oil and oil sediments: application in oil fingerprinting. Journal of Food Agriculture and Environment 2, 84–89.

Díaz, A., Martín, A., Rallo, P., & De la Rosa, R. (2007) Cross-compatibility of the parents as the main factor for successful olive breeding crosses. Journal of the American Society for Horticultural Science 132, 830–835.

Diez, C.M., Trujillo, I., Martinez-Urdiroz, N., Barranco, D., Rallo, L., Marfil, P., & Gaut, B.S. (2015) Olive domestication and diversification in the Mediterranean Basin. New Phytologist 206, 436–447.

Do Val, A.D., Ferreira, J.L., Vieira Neto, J., Pasqual, M., de Oliveira, A.F., Borím, A., & Canηado, G.M.A. (2012) Genetic diversity of Brazilian and introduced olive germplasms based on microsatellite markers. Genetics and Molecular Research 11, 556–571.

Donini, P., Muleo, R., Colao, M.C., Miano, D., Cirilli, M., Intrieri, M.C., Baldoni, L., & Rugini, E. (2009) Mutation scanning and genotyping by high-resolution DNA melting analysis in olive germplasm. Genome 52, 252–260.

Donini, P., Sarri, V., Baldoni, L., Porceddu, A., Cultrera, N.G.M., Contento, A., Frediani, M., Belaj, A., Trujillo, I., & Cionini, P.G. (2006) Microsatellite markers are powerful tools for discriminating among olive cultivars and assigning them to geographically defined populations. Genome 49, 1606–1615.

Doveri, S., O'Sulliva, D.M., & Lee, D. (2006) Non-concordance between genetic profiles of olive oil and fruit: a cautionary note to the use of DNA markers for provenance testing. Journal of Agricultural and Food Chemistry 54, 9221–9226.

Fabbri, A., Lambardi, M., & Ozden-Tokatli, Y. (2009) Olive breeding. Breeding plantation tree crops: tropical species. Springer, Berlin, 423–465.

Frankel, E.N. (2010) Chemistry of extra virgin olive oil: adulteration, oxidative stability, and antioxidants. Journal of Agricultural and Food Chemistry 58, 5991–6006.

Galla, G., Barcaccia, G., Ramina, A., Collani, S., Alagna, F., Baldoni, L., Cultrera, N.G.M., Martinelli, F., Sebastiani, L., & Tonutti, P. (2009) Computational annotation of genes differentially expressed along olive fruit development. BMC Plant Biology 9, 128.

Ganopoulos, I., Argiriou, A., & Tsaftaris, A. (2011) Microsatellite high resolution melting (SSR-HRM) analysis for authenticity testing of protected designation of origin (PDO) sweet cherry products. Food Control 22, 532–541.

Ganopoulos, I., Bazakos, C., Madesis, P., Kalaitzis, P., & Tsaftaris, A. (2013) Barcode DNA high-resolution melting (Bar-HRM) analysis as a novel close-tubed and accurate tool for olive oil forensic use. Journal of the Science of Food and Agriculture 93, 2281–2286.

Ganopoulos, I., Madesis, P., Darzentas, N., Argiriou, A., & Tsaftaris, A. (2012a) Barcode High Resolution Melting (Bar-HRM) analysis for detection and quantification of PDO "Fava Santorinis" (*Lathyrus clymenum*) adulterants. Food Chemistry 133, 505–512.

Ganopoulos, I., Madesis, P., & Tsaftaris, A. (2012b) Universal ITS2 barcoding DNA region coupled with high-resolution melting (HRM) analysis for seed authentication and adulteration testing in leguminous forage and pasture species. Plant Molecular Biology Reporter 30, 1322–1328.

Gerry, N.P., Witowski, N.E., Day, J., Hammer, R.P., Barany, G., & Barany, F. (1999) Universal DNA microarray method for multiplex detection of low abundance point mutations. Journal of Molecular Biology 292, 251–262.

Giannoulia, K., Banilas, G., & Hatzopoulos, P. (2007) Oleosin gene expression in olive. Journal of Plant Physiology 164, 104–107.

Giannoulia, K., Gazis, F., Nikoloudakis, N., Milioni, D., & Haralampidis, K. (2002) Breeding, molecular markers and molecular biology of the olive tree. European Journal of Lipid Science and Technology 104, 574–586.

Gil-Amado, J.A., & Gomez-Jimenez, M.C. (2013) Transcriptome analysis of mature-fruit abscission control in olive. Plant and Cell Physiology, pcs179.

Giménez, M.J., Pistón, F., Martín, A., & Atienza, S.G. (2010) Application of real-time PCR on the development of molecular markers and to evaluate critical aspects for olive oil authentication. Food Chemistry 118, 482–487.

Hannachi, H., Breton, C., Msallem, M., El Hadj, S.B., El Gazzah, M., & Bervillí, A. (2010) Genetic Relationships between cultivated and wild olive trees (*Olea europaea* L. var. europaea and var. sylvestris) based on nuclear and chloroplast SSR markers. Natural Resources 1, 95.

Hernández, M.L., Mancha, M., & Martínez-Rivas, J.M. (2005) Molecular cloning and characterization of genes encoding two microsomal oleate desaturases (FAD2) from olive. Phytochemistry 66, 1417–1426.

Intrieri, M.C., Muleo, R., & Buiatti, M. (2007) Chloroplast DNA polymorphisms as molecular markers to identify cultivars of *Olea europaea* L. Journal of Horticultural Science and Biotechnology 82, 109–113.

Jones, C.J., Edwards, K.J., Castaglione, S., Winfield, M.O., Sala, F., Van de Wiel, C., Bredemeijer, G., Vosman, B., Matthes, M., & Daly, A. (1997) Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. Molecular Breeding 3, 381–390.

Kalivas, A., Ganopoulos, I., Xanthopoulou, A., Chatzopoulou, P., Tsaftaris, A., & Madesis, P. (2014) DNA barcode ITS2 coupled with high resolution melting (HRM) analysis for taxonomic identification of *Sideritis* species growing in Greece. Molecular Biology Reports 41, 5147–5155.

Kalogianni, D.P., Bazakos, C., Boutsika, L.M., Targem, M.B., Christopoulos, T.K., Kalaitzis, P., & Ioannou, P.C. (2015) Olive oil DNA fingerprinting by multiplex SNP genotyping on fluorescent microspheres. Journal of Agricultural and Food Chemistry 63, 3121–3128.

Khadari, B., Charafi, J., Moukhli, A., & Ater, M. (2008) Substantial genetic diversity in cultivated Moroccan olive despite a single major cultivar: a paradoxical situation evidenced by the use of SSR loci. Tree Genetics & Genomes 4, 213–221.

Kontogianni, M.D., Panagiotakos, D.B., Chrysohoou, C., Pitsavos, C., Zampelas, A., & Stefanadis, C. (2007) The impact of olive oil consumption pattern on the risk of acute coronary syndromes: the CARDIO2000 case–control study. Clinical Cardiology 30, 125–129.

Kumar, S., Kahlon, T., & Chaudhary, S. (2011) A rapid screening for adulterants in olive oil using DNA barcodes. Food Chemistry 127, 1335–1341.

Linos, A., Nikoloudakis, N., Katsiotis, A., & Hagidimitriou, M. (2014) Genetic structure of the Greek olive germplasm revealed by RAPD, ISSR and SSR markers. Scientia Horticulturae 175, 33–43.

Madesis, P., Ganopoulos, I., Anagnostis, A., & Tsaftaris, A. (2012) The application of Bar-HRM (Barcode DNA-High Resolution Melting) analysis for authenticity testing and quantitative detection of bean crops (Leguminosae) without prior DNA purification. Food Control 25, 576–582.

Madesis, P., Ganopoulos, I., Bosmali, I., & Tsaftaris, A. (2013a) Barcode High Resolution Melting analysis for forensic uses in nuts: A case study on allergenic hazelnuts (*Corylus avellana*). Food Research International 50, 351–360.

Madesis, P., Ganopoulos, I., Sakaridis, I., Argiriou, A., & Tsaftaris, A. (2014) Advances of DNA-based methods for tracing the botanical origin of food products. Food Research International 60, 163–172.

Madesis, P., Ganopoulos, I., & Tsaftaris, A. (2013b) Microsatellites: evolution and contribution. Microsatellites. Springer, Berlin, 1–13.

Mariotti, R., Cultrera, N.G.M., Díez, C.M., Baldoni, L., & Rubini, A. (2010) Identification of new polymorphic regions and differentiation of cultivated olives (*Olea europaea* L.) through plastome sequence comparison. BMC Plant Biology 10, 211.

Marmiroli, N., Maestri, E., Pafundo, S., & Vietina, M. (2009) Molecular traceability of olive oil: From plant genomics to food genomics. Advances in Olive Resources 157–172.

Marra, F.P., Caruso, T., Costa, F., Di Vaio, C., Mafrica, R., & Marchese, A. (2013) Genetic relationships, structure and parentage simulation among the olive tree (*Olea europaea* L. subsp. *europaea*) cultivated in Southern Italy revealed by SSR markers. Tree Genetics & Genomes 9, 961–973.

Martins-Lopes, P., Gomes, S., Santos, E., & Guedes-Pinto, H. (2008) DNA markers for Portuguese olive oil fingerprinting. Journal of Agricultural and Food Chemistry 56, 11786–11791.

Matos, L.C., Cunha, S.C., Amaral, J.S., Pereira, J.A., Andrade, P.B., Seabra, R.M., & Oliveira, B.P.P. (2007) Chemometric characterization of three varietal olive oils (Cvs. Cobranηosa, Madural and Verdeal Transmontana) extracted from olives with different maturation indices. Food Chemistry 102, 406–414.

Montealegre, C., Marina Alegre, M., & Garciμa-Ruiz, C. (2009) Traceability markers to the botanical origin in olive oils. Journal of Agricultural and Food Chemistry 58, 28–38.

Montemurro, C., Miazzi, M.M., Pasqualone, A., Fanelli, V., Sabetta, W., & di Rienzo, V. (2015) Traceability of PDO olive oil "Terra di Bari" using high resolution melting. Journal of Chemistry 2015,

Montemurro, C., Pasqualone, A., Simeone, R., Sabetta, W., & Blanco, A. (2008) AFLP molecular markers to identify virgin olive oils from single Italian cultivars. European Food Research and Technology 226, 1439–1444.

Mookerjee, S., Guerin, J., Collins, G., Ford, C., & Sedgley, M. (2005) Paternity analysis using microsatellite markers to identify pollen donors in an olive grove. Theoretical and Applied Genetics 111, 1174–1182.

Muleo, R., Cavallini, A., Perrotta, G., Baldoni, L., Morgante, M., & Velasco, R. (2012) Olive tree genomics. INTECH Open Access Publisher, Rijeka, Croatia.

Muñoz-Mérida, A., Gonzalez-Plaza, J.J., Blanco, A.M., del Carmen Garcia-Lopez, M., Rodriguez, J.M., Pedrola, L., Sicardo, M.D., Hernández, M.L., De la Rosa, R., & Belaj, A. (2013) De novo assembly and functional annotation of the olive (*Olea europaea*) transcriptome. DNA Research, dss036.

Muzzalupo, I., Pellegrino, M., & Perri, E. (2007) Detection of DNA in virgin olive oils extracted from destoned fruits. European Food Research and Technology 224, 469–475.

Muzzalupo, I., & Perri, E. (2002) Recovery and characterisation of DNA from virgin olive oil. European Food Research and Technology 214, 528–531.

Muzzalupo, I., Vendramin, G.G., & Chiappetta, A. (2014) Genetic biodiversity of Italian olives (*Olea europaea*) germplasm analyzed by SSR markers. The Scientific World Journal 2014, art. 296590.

Noormohammadi, Z., Trujillo, I., Belaj, A., Ataei, S., & Hosseini-Mazinan, M. (2014) Genetic structure of Iranian olive cultivars and their relationship with Mediterranean's cultivars revealed by SSR markers. Scientia Horticulturae 178, 175–183.

Osathanunkul, M., Madesis, P., & de Boer, H. (2015) Bar-HRM for authentication of plant-based medicines: evaluation of three medicinal products derived from Acanthaceae species. PLoS ONE 10(5), e0128476. doi:10.1371/journal.pone.0128476

Ozdemir Ozgenturk, N., Oruη, F., Sezerman, U., Kuηukural, A., Vural Korkut, S., Toksoz, F., & Un, C. (2010) Generation and analysis of expressed sequence tags from *Olea europaea* L. Comparative and Functional Genomics 2010, art. 757512.

Pafundo, S., Agrimonti, C., & Marmiroli, N. (2005) Traceability of plant contribution in olive oil by amplified fragment length polymorphisms. Journal of Agricultural and Food Chemistry 53, 6995–7002.

Pafundo, S., Busconi, M., Agrimonti, C., Fogher, C., & Marmiroli, N. (2010) Storage-time effects on olive oil DNA assessed by amplified fragments length polymorphisms. Food Chemistry 123, 787–793.

Palmieri, L., Doveri, S., Marmiroli, N., & Donini, P. (2004) Molecular marker characterisation of olive oil variety composition and SNP development in olive. In: Proceedings of XII International Plant and Animal Genome Conference, San Diego, CA, 305.

Pasqualone, A., Caponio, F., & Blanco, A. (2001) Inter-simple sequence repeat DNA markers for identification of drupes from different *Olea europaea* L. cultivars. European Food Research and Technology 213, 240–243.

Pasqualone, A., Montemurro, C., Caponio, F., Blanco, A. (2004) Identification of virgin olive oil from different cultivars by analysis of DNA microsatellites. Journal of Agricultural and Food Chemistry 52, 1068–1071.

Pasqualone, A., Montemurro, C., Summo, C., Sabetta, W., Caponio, F., & Blanco, A. (2007) Effectiveness of microsatellite DNA markers in checking the identity of protected designation of origin extra virgin olive oil. Journal of Agricultural and Food Chemistry 55, 3857–3862.

Pasqualone, A., Rienzo, V.D., Miazzi, M.M., Fanelli, V., Caponio, F., & Montemurro, C. (2015) High resolution melting analysis of DNA microsatellites in olive pastes and virgin olive oils obtained by talc addition. European Journal of Lipid Science and Technology 17(2), 2044–2048.

Poghosyan, Z.P., Haralampidis, K., Martsinkovskaya, A.I., Murphy, D.J., & Hatzopoulos, P. (1999) Developmental regulation and spatial expression of a plastidial fatty acid desaturase from *Olea europaea*. Plant Physiology and Biochemistry 37, 109–119.

Rasmussen, R.S., & Morrissey, M.T. (2008) DNA-Based methods for the identification of commercial fish and seafood species. Comprehensive Reviews in Food Science and Food Safety 7, 280–295.

Reale, S., Angiolillo, A., Baldoni, L., D'Andrea, M., Lima, G., & Scarano, M.T. (2006) Olive authochthonous germplasm of Molise: molecular characterization by means of SSRs and SNPs. Olivebioteq 2006, 5–10.

Reddy, K.J., Jayathilakan, K., & Pandey, M.C. (2015) Olive oil as functional component in meat and meat products: a review. Journal of Food Science and Technology, 1–9.

Rossi, S., Calabretta, A., Tedeschi, T., Sforza, S., Arcioni, S., Baldoni, L., Corradini, R., & Marchelli, R. (2012) Selective recognition of DNA from olive leaves and olive oil by PNA and modified-PNA microarrays. Artificial DNA: PNA & XNA 3, 63–72.

Saimaru, H., Orihara, Y., Tansakul, P., Kang, Y-H., Shibuya, M., & Ebizuka, Y. (2007) Production of triterpene acids by cell suspension cultures of *Olea europaea*. Chemical and Pharmaceutical Bulletin 55, 784–788.

Sanz-Cortís, F., Parfitt, D.E., Romero, C., Struss, D., Llacer, G., & Badenes, M.L. (2003) Intraspecific olive diversity assessed with AFLP. Plant Breeding 122, 173–177.

Sefc, K.M., Lopes, M.S., Mendonηa, D., Santos, M.R.D., Machado, L.M., & Machado, A.D.C. (2000) Identification of microsatellite loci in olive(*Olea europaea*) and their characterization in Italian and Iberian olive trees. Molecular Ecology 9, 1171–1173.

Shibuya, M., Zhang, H., Endo, A., Shishikura, K., Kushiro, T., & Ebizuka, Y. (1999) Two branches of the lupeol synthase gene in the molecular evolution of plant oxidosqualene cyclases. European Journal of Biochemistry 266, 302–307.

Singtonat, S., & Osathanunkul, M. (2015) Fast and reliable detection of toxic *Crotalaria spectabilis* Roth. in *Thunbergia laurifolia* Lindl. herbal products using DNA barcoding coupled with HRM analysis. BMC Complementary and Alternative Medicine 15, 162.

Spaniolas, S., Bazakos, C., Awad, M., & Kalaitzis, P. (2008a) Exploitation of the chloroplast trn L (UAA) intron polymorphisms for the authentication of plant oils by means of a lab-on-a-chip capillary electrophoresis system. Journal of Agricultural and Food Chemistry 56, 6886–6891.

Spaniolas, S., Bazakos, C., Bihmidine, S., Georgousakis, A., & Kalaitzis, P. (2008b) Use of lambda DNA as a marker to assess DNA stability in olive oil during storage. European Food Research and Technology 227, 175–179.

Spaniolas, S., Bazakos, C., Spano, T., Zoghby, C., & Kalaitzis, P. (2010) The potential of plastid *trnL* (UAA) intron polymorphisms for the identification of the botanical origin of plant oils. Food Chemistry 122, 850–856.

Tedeschi, T., Calabretta, A., Bencivenni, M., Manicardi, A., Corrado, G., Caramante, M., Corradini, R., Rao, R., Sforza, S., & Marchelli, R. (2011) A PNA microarray for tomato genotyping. Molecular BioSystems 7, 1902–1907.

Terés, S., Barcelo-Coblijn, G., Benet, M., Alvarez, R., Bressani, R., Halver, J.E., & Escriba, P.V. (2008) Oleic acid content is responsible for the reduction in blood pressure induced by olive oil. Proceedings of the National Academy of Sciences 105, 13811–13816.

Testolin, R., & Lain, O. (2005) DNA extraction from olive oil and PCR amplification of microsatellite markers. Journal of Food Science 70, C108–C112.
Trujillo, I., Ojeda, M.A., Urdiroz, N.M., Potter, D., Barranco, D., Rallo, L., & Diez, C.M. (2014) Identification of the Worldwide Olive Germplasm Bank of Córdoba (Spain) using SSR and morphological markers. Tree Genetics & Genomes 10, 141–155.
Uncu, A.T., Frary, A., & Doganlar, S. (2015) Cultivar origin and admixture detection in Turkish olive oils by SNP-based CAPS assays. Journal of Agricultural and Food Chemistry 63, 2284–2295.
Vietina, M., Agrimonti, C., & Marmiroli, N. (2013) Detection of plant oil DNA using high resolution melting (HRM) post PCR analysis: a tool for disclosure of olive oil adulteration. Food Chemistry 141, 3820–3826.
Visioli, F., Poli, A., & Gall, C. (2002) Antioxidant and other biological activities of phenols from olives and olive oil. Medicinal Research Reviews 22, 65–75.
Wu, Y., Zhang, H., Han, J., Wang, W., Ju, X., & Chen, Y. (2011) PCR-CE-SSCP applied to detect cheap oil blended in olive oil. European Food Research and Technology 233, 313–324.
Xanthopoulou, A., Ganopoulos, I., Koubouris, G., Tsaftaris, A., Sergendani, C., Kalivas, A., & Madesis, P. (2014) Microsatellite high-resolution melting (SSR-HRM) analysis for genotyping and molecular characterization of an *Olea europaea* germplasm collection. Plant Genetic Resources 12, 273–277.
Zohary, D., & Spiegel-Roy, P. (1975) Beginnings of fruit growing in the Old World. Science 187, 319–327.

33 Sensory properties and evaluation of virgin olive oils

Emmanuel Salivaras

33.1 Introduction

Unique sensory characteristics (color, odor, taste, chemaesthetic, and tactile attributes) along with health benefits are the key factors that have placed extra virgin olive oil (EVOO) on the top among all edible fats and oils (Mensink & Katan, 1987; Kiritsakis, 1998; Trichopoulou *et al.*, 2003; Aguilera *et al.*, 2004; Boskou *et al.*, 2006; Karkoula *et al.*, 2012). Sensory attributes of virgin olive oil (VOO) have always been a concern for all involved, from growers and producers to traders and consumers. They were not always evaluated by specific means. However, in 1987 after some years of research and development, the International Olive Council (IOC or COI), based in Madrid, introduced a method entitled "Sensory analysis of olive oil: method for the organoleptic assessment of virgin olive oil" (IOC, 1987). After a number of revisions of the method, apart from the negative attributes (defects) that must be absent in order for an oil to be classified as extra virgin, specific positive attributes (namely, fruity, bitter, and pungent) are evaluated.

However, compounds responsible for fruitiness and molecules responsible for bitterness and pungency have multiple roles. They not only contribute to the global flavor of VOOs but also act beneficially to human health and well-being. Here, throughout the chapter, the term "oil" refers to virgin olive oil.

33.2 Description and review of methodology

The olive oil industry used to rely on the opinion of individual olive oil experts (mainly individuals from the quality control and/or purchasing departments) for the sensory evaluation of olive oils many years before the appearance of the IOC method, known as the "panel test." In this method, the individual has been replaced by a team of 8–12 tasters guided by a panel leader.

Even today, many industries, traders, brokers, olive oil mill owners, and growers assign the evaluation of some of their samples to individual expert tasters, mostly for the following reasons:

- Faster response
- More organoleptic details
- Consulting on correlation of defects with possible false practices.

On the other hand, an individual taster cannot be objective and cannot represent a set of people.

Today, the IOC panel test is the only official practice for the sensory evaluation of olive oil. Olive oil is the first food whose quality is legally determined also from its sensory properties (Mueller, 2012).

In 1991, the European Union (EU) adopted an organoleptic assessment method based on the IOC panel test method of 1987 (EU, 1991). In this very first method, a group of 8–12 tasters (the panel), guided by a leader, was asked to evaluate samples of olive oil by using a profile sheet (Figure 33.1). This early profile

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Virgin olive oil

Profile sheet
Olfactory-gustatory-tactile notes (2)

	0	1	2	3	4	5
Olive fruity (ripe and green) (1)						
Apple..						
Other ripe fruit..						
Green (leaves, grass)................................						
Bitter..						
Pungent..						
Sweet..						
Other allowable attribute(s).........................						
(Specify..						
....)						
Sour/winery/vinegary/acid (1)........................						
Rough..						
Metallic...						
Mustiness/humidity (1)..............................						
Muddy sentiment.....................................						
Fusty ("Atrojado")....................................						
Rancid...						
Other allowable attribute(s).........................						
(Specify..						
....)						

(1) Delete where not applicable.
(2) Perception:
0: (3)
1: barely perceptible,
2: slight,
3: average,
4: great,
5: extreme.

Grading table

Defects	Characteristics	Overall mark: points
None	Olive fruity Olive fruity and fruitiness of other fresh fruit	9 8 7
Slight and barely perceptible	Weak fruitiness of any type	6
Perceptible	Rather imperfect fruitiness, anomalous odours and tastes	5
Considerable, on the border of acceptability	Clearly imperfect, unpleasant odours and tastes	4
Great and/or serious, clearly perceptible	Totally inadmissible odours and tastes for consumption	3 2 1

Remarks: ....
....
Name of taster: ....
....
Legend of sample: ....
....
Date: ....

Figure 33.1 Panel test method profile sheet in EU regulation 2568 of 1991.

sheet consisted of two tables. Both tables had prefixed scales. In the first part of the first table, the taster scored for specific positive attributes, like apples and a few other aromas, but also for bitter, pungent, and sweet characteristics. Other positive attributes could also be scored. On the second part of the first table, seven specific defects were scored, and likewise, other negative attributes could be considered. The scale ranged between 0 (for no perceptible irritation) and 5 (for extreme perceptible irritation). Finally, based on scores from the first table, the taster was called to give a final total mark, on a 1 to 9 scale. From this final total mark, the sample was categorized according to the grades that existed on this first EU regulation (Table 33.1).

That early method also contained the following parts, most of them still in force today *per se* or with some modifications:

- *General basic vocabulary (IOC, 2007a)*: Basic organoleptic terms like sense, taster, taste, sensitivity, acceptance, harmony, compensation, synergy, irritation, smell, body, objective, subjective, kinesthesis, threshold, and so on are described.

Table 33.1 Panel test limits in EU Regulation 2568/1991.

Type	Panel test
Extra virgin	≥6.5
Virgin	≥5.5
Ordinary	≥3.5
Virgin lampante	<3.5

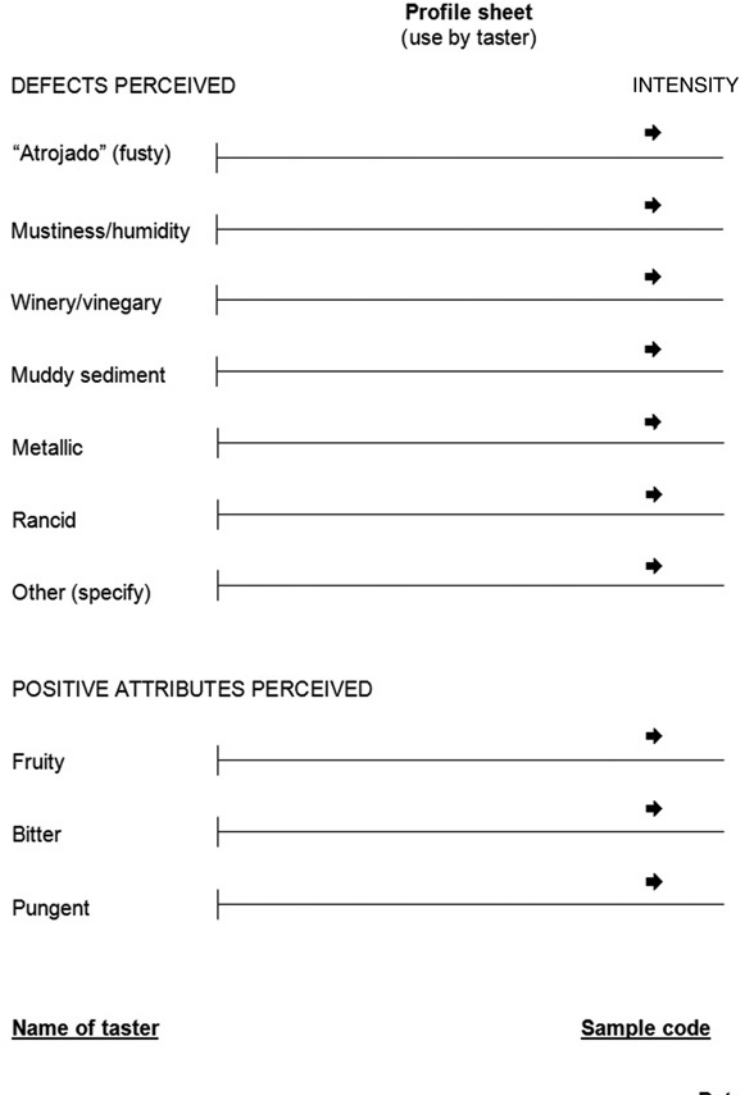
- *Specific vocabulary for olive oil*: Description of defects, a few aromas, and basic positive attributes like fruity, ripe fruity, bitter, and pungent.
- *Glass for oil tasting (IOC, 2007b)*: Description of the specifications of the glass used in olive oil tasting. In addition, it describes the heating unit needed to reach and maintain the right temperature for this analysis. This temperature (28 °C) is considered the optimal temperature for olive oil tasting. Lower temperature results in poor volatility of aromatic compounds, whereas higher temperature can result in off-odors characteristic of heated (or even cooked) oils.
- *Guide for the installation of a test room (IOC, 2007c)*: Description of the basic conditions that have to be met when installing a test room – characteristics and dimensions of the individual tasting booths.
- *Guide for the selection of tasters (IOC, 2013a)*: Methodology for selecting tasters by the intensity rating method (based on a work by Gutierrez Rosales *et al.*, 1984).
- *Determination of panel detection thresholds*: Particularly for fusty, winey, rancid, and bitter characteristics.
- *Training of tasters*: This is in order to recognize, identify, and qualify the sensory attributes, so the final evaluation can be precise and consistent.
- *Panel performance evaluation*: Continuous training and use of reference samples to verify the reliability of the results and for comparison with other panels.
- *Technique for tasting olive oil*: Guidance on how to perform the sensory evaluation of VOO samples.
- Collection of profile sheets by the panel leader and statistical analysis of the results.

Since 1991, a number of amendments to Regulation 2568 (EU, 1991) took place in the EU. Some of them even appeared with new codification, like Regulation 2472 of 1997 (EU, 1997), 796 of 2002 (EU, 2002), 1989 of 2003 (EU, 2003), 640 of 2008 (EU, 2008), 61 of 2011 (EU, 2011), and 1348 of 2013 (EU, 2013), and they came to incorporate new scientific data in the evolution of olive oil quality control in both chemical and sensory parameters. The main change in the sensory evaluation method came in 2002 via Regulation 796 (EU, 2002) with the revision of the profile sheet. The new profile sheet (Figure 33.2) is still in force today with a few changes, mainly in defects terminology. It is much simpler compared to the old sheet, as it considers only the defects and the three main positive attributes of the oil.

Moreover, there is no prefixed scale but rather a 10-cm-long line for each attribute. The tasters evaluate by ticking the lines. The panel leader then collects all sheets and converts ticking marks to numbers (to 1 decimal place) by using a ruler.

Finally, after a statistical process, the median, instead of the mean (like in the early method), is given for the fruitiness and the defects perceived with the greatest intensity. Results for bitter and/or pungent should be reported only if they exceed a score of 5.0. Finally, results are valid only if the coefficient of variation is ≤20%, for both the fruity attribute and the predominant defect. Otherwise, the panel leader calls for the tasters with the most significant deviation to repeat the test. Regulation 1989 of 2003 (EU, 2003) merged ordinary and lampante olive oil to one category, namely, lampante. Later, Regulation 640 of 2008 (EU, 2008) increased the defect limit of the virgin category to 3.5, combined fusty and muddy sediment defects, allowed specific sensorial terminology on product labels, and introduced the terms “green” and “ripe” for fruity. Table 33.2 shows the evolution of the specifications for classification after the merging of categories and the revision of the defect median.

At this point, it is prudent to summarize both positive and negative attributes, as given in the last revisions to both the IOC and EU methods.



Profile sheet
(use by taster)

DEFECTS PERCEIVED — INTENSITY

"Atrojado" (fusty)

Mustiness/humidity

Winery/vinegary

Muddy sediment

Metallic

Rancid

Other (specify)

POSITIVE ATTRIBUTES PERCEIVED

Fruity

Bitter

Pungent

Name of taster

Sample code

Date

Figure 33.2 Panel test method profile sheet in EU Regulation 796 of 2002.

33.2.1 Positive attributes

Positive attributes included in the latest revision of the panel test method from the IOC (2015) are described as follows:

- *Fruity – green or ripe*: Set of olfactory sensations characteristic of the oil, which depends on the cultivar and comes from sound, fresh olives, either ripe or unripe. It is perceived directly (orthonasally) and/or through the back of the nose (retronasally).
- *Bitter*: Characteristic primary taste of oil obtained from green olives or olives turning color. It is perceived in the circumvallate papillae on the "V" region of the tongue.
- *Pungent*: Biting tactile sensation characteristic of oils produced at the start of the crop year, primarily from olives that are still unripe. It can be perceived throughout the whole of the mouth cavity, particularly in the throat.

Table 33.2 Panel test limits in EU Regulations. (Data from EU Regulations 796/2002, 1989/2003 and 640/2008).

Regulation	**Organoleptic assessment (796/2002)**		**Organoleptic assessment (1989/2003)**		**Organoleptic assessment (640/2008)**	
Category	**Median of fruity**	**Median of defect**	**Median of fruity**	**Median of defect**	**Median of fruity**	**Median of defect**
Extra virgin	>0	=0	>0	=0	>0	=0
Virgin	>0	≤2.5	>0	≤2.5	>0	≤3.5
Ordinary	–	≤6	c.a.	c.a.	c.a.	c.a.
Virgin lampante	–	>6	–	>2.5[a]	–	>3.5[a]

Note: c.a.: Category abolition.
[a] Or if the median of defect is less than or equal to 2.5/3.5 and the median of fruity is 0.

The term "fruity" encloses all pleasant odors that are perceived through orthonasal and retronasal senses. Such pleasant odors, due to volatile compounds produced during olive oil extraction, come from healthy olives, either unripe (green), ripe (green-yellow with purple spots), or overripe (black). Odors reminiscent of green and/or red apple, artichoke, almond, cut grass, tomato stem, flowers, as well as red and exotic fruit, among others, could be found in high-quality oils. However, it has been found that the total content of volatile compounds decreases with ripeness (Morales *et al.*, 1996). A class of compounds responsible for the pleasant odor of olive oils reaches their maximum concentration when olives turn their color from green to purple (Marco Gomez *et al.*, 2012).

Pleasant smells that are not combined with olive oil are not included in the fruity term, or are even considered as defects. Examples are the stewed-fruit and/or vanilla smell in some olive oils produced by frostbitten olives, or the caramel-like smell of deodorized oils.

Bitter is the only one of the five basic tastes (sweet, salty, sour, bitter, and umami) that is perceived when olive oil is in the mouth (Tuorila & Recchia, 2013). Bitter taste is perceived on the tongue due to phenolic compounds that exist in higher amounts in unripe olives and can pass to the oil under certain technological conditions during the olive oil production.

Pungent is a chemaesthetic sensation, mainly produced by the chemical irritation of the trigeminal nerve. It is that burning peppery-like sensation when oil flows to the throat after swallowing. However, that burning sensation spreads through the mouth cavity in the case of some olive oils. Again, unripe olives, as in the case of bitterness, can produce oils with a higher pungent sensation, depending on the cultivar and the production technological parameters during olive oil extraction.

The evaluation of aromatic intensity by smelling (orthonasal perception) does not suffer from carryover effect. Therefore, in that way, panelists could test a large number of samples. It is recommended to first evaluate samples by smelling and keeping records, before proceeding to the mouth test. During the nose test, it is important for tasters to keep in mind that they evaluate oils for both positive and negative attributes.

Unlike orthonasal sensation, bringing the oil into the mouth gives rise to a complex mixture of sensations that suffer from severe carryover effects. Retronasal olfactory odors, taste (bitterness), chemaesthesis (pungency), and tactile (viscosity, astringency, and oiliness) perceptions all participate in the oral evaluation.

Bitterness, pungency, and astringency (even if the latter is not evaluated by the method), depending on their intensities, can persist for a long time after olive oil is no longer in the mouth (Tuorila & Recchia, 2013). Bitterness maximum intensity appears after 16 –20 sec, and pungency after 26–29 sec (and along with astringency can persist for more than 60 sec) (Tuorila & Recchia, 2013). Dinnela *et al.* (2012) showed that the emergence of the above sensations pursues the following order: bitterness, pungency, and astringency. The long-lasting effect of these attributes (Esti *et al.*, 2009) is the reason for the "rest period" needed between samples and the fact that only four samples per session are evaluated every time by panelists. Two sessions per day are recommended, interrupted by a one-hour intermission (IOC, 2015). Apart from this method, other techniques for profiling EVOOs have also been described in order to minimize carryover effects (Monteleone, 2013),

Repeated exposures to bitterness and pungency can lead to adaptation phenomena. Furthermore, dietary habits can result in chronic desensitization to the above stimuli, and can affect the preference to oils with different levels of bitterness and pungency (Tuorila & Recchia, 2013). Caporale *et al.* (2004) also showed that the presence of cut grass odorant (due to alcohol cis-3-hexen-1-ol) enhances the bitterness perception. Such a mechanism, which is not fully understood, may also work with other odorants on suppression or enhancement of taste, or have no effect on taste.

Fruity, bitter, and pungent are the three basic positive attributes that the panel test method takes into account. Such a simplified approach is inevitable in an effort to have a robust method with repeatable results from panels all over the world.

However, there are substantial quality differences among EVOOs with similar results in fruity, bitter, and pungent (Figure 33.3). These differences focus on odor profile and may arise from the number and kind of aromas (complexity) and/or from positive or negative synergistic effects (Angerosa *et al.*, 2004). They may also arise from low-intensity defects that are hidden behind the positive odors, especially during the first months after production. They are affected by several factors, including environmental conditions, cultivar, agronomic practices, harvesting time and manner, technological practices during production, and olive oil storage conditions (Angerosa *et al.*, 2001; Servili *et al.*, 2002, 2003, 2007; Morales *et al.*, 2005).

During EVOO competitions, it is very important for panelists to reveal such differences and therefore evaluate samples in such a manner as to highlight details leading to the selection of the exceptional EVOOs. Thus, in most competitions, the profile sheet of the IOC panel method has been substituted by more complicated ones, like the one used in the Mario Solinas Quality Award organized every year by the IOC (Figure 33.4).

Moreover, researchers, in an effort to discriminate olive oils within the extra virgin category, have used perceptual maps (biplot of attributes) (Delgado & Guinard, 2011a) and the more simplified spider plots or radar charts. Delgado and Guinard (2011b) used 22 sensory descriptors to evaluate local and imported EVOOs in California. They pointed out that the IOC method was useful for fast classification (separation of nondefective from defective oils) but, compared to their method, did not allow for a full characterization of the oils. In general, descriptive analysis has been employed not only for screening exceptional olive oils within the extra virgin category, but also to evaluate consumer preferences (Caporale *et al.*, 2006; Dinnela *et al.*, 2012).

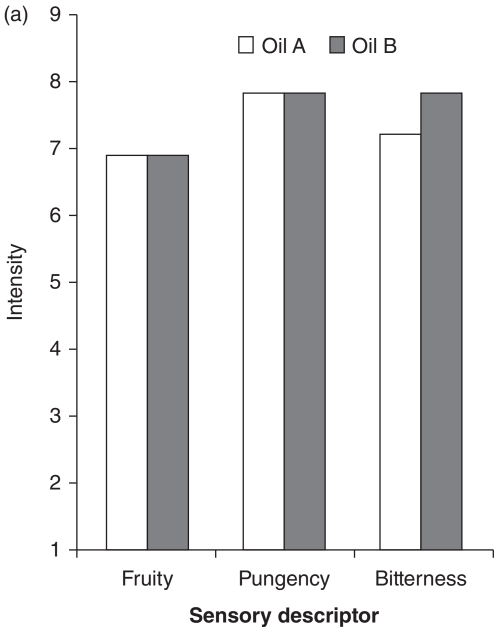
Bongartz and Oberg (2011) introduced the descriptor "harmony" as an additional quality factor. The factor was incorporated to a new sheet called the "advanced profile sheet" under the basic positive attributes of fruity, bitter, and pungent, with or without aroma descriptions. This new method is objective and discriminates different qualities within the EVOO category.

However, in this work, the term "harmony" is somehow linked to the term "complexity" and becomes rather confusing. "Harmony" is a Greek word meaning "the normal relationship of parts to the whole, the correct ratio or their arrangement" (Tegopoulos, 2007), a definition better conveyed by the IOC definition "Well balanced or balanced attributes" (see the fifth item in the list in Section 33.2.3).

33.2.2 Negative attributes

The presence of defects is the key factor for grading VOOs. Total absence is prerequisite for an oil if it is to be characterized as extra virgin. In the EU regulation, the two other categories, virgin and lampante, tolerate defect scores up to 3.5 and greater than 3.5 (on a 0 to 10 scale), respectively. The main defects that have been identified in VOOs, as described in the latest revision of the panel test method from the IOC (2015), are:

- *Fusty/muddy sediment*: Characteristic flavor of oil obtained from olives piled or stored in such conditions as to have undergone an advanced stage of anaerobic fermentation, or of oil that has been left in contact with the sediment that settles in underground tanks and vats and has also undergone a process of anaerobic fermentation.
- *Musty-humid-earthy*: Characteristic flavor of oils obtained from fruit in which large numbers of fungi and yeasts have developed as a result of them being stored in humid conditions for several days, or of oils obtained from olives that have been collected with earth or mud on them and have not been washed.



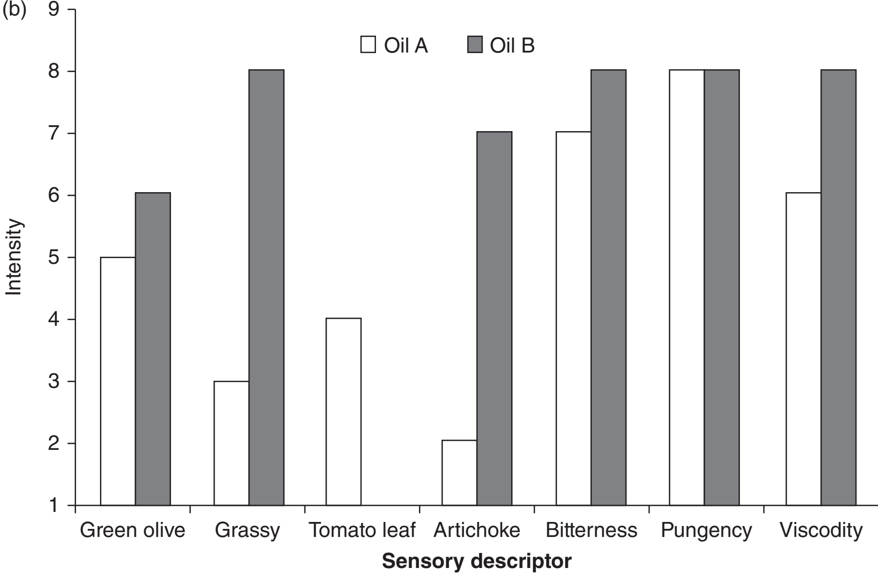


Figure 33.3 Sensory profile of two extra virgin olive oils as described by (a) the IOC method and (b) conventional descriptive analysis. The bars in (a) indicate that the two oils are very similar, whereas those in (b) clearly illustrate the differences between the oils. *Source*: Monteleone (2013). Reproduced with permission of John Wiley & Sons.

- *Winey-vinegary-acid-sour*: Characteristic flavor of certain oils reminiscent of wine or vinegar. This flavor is mainly due to a process of aerobic fermentation in the olives or in olive paste left on pressing mats that have not been properly cleaned, and it leads to the formation of acetic acid, ethyl acetate, and ethanol.
- *Rancid*: Flavor of oils that have undergone an intense process of oxidation.
- *Frostbitten olives (wet wood)*: Characteristic flavor of oils extracted from olives that have been injured by frost while on the tree.

MARIO SOLINAS QUALITY AWARD
INTERNATIONAL COMPETITION FOR EXTRA VIRGIN OLIVE OILS

SENSORY ASSESSMENT SHEET

Sample code:
Taster identification no.:
Date:

Olfactory sensations (maximum 35 points)

Olive fruitiness (0 – 7) ....
Other fruits (0-3) ....
Green (grass/leaves) (0 – 2) ....
Other positive sensations (0 - 3) ....
Harmony (1) (0 – 20) ....
*Partial score*____

Gustatory-retronasal sensations (maximum 45 points)

Olive fruitiness (0 – 10) ....
Sweet (0 – 4) ....
Bitter (0 – 3) ....
Pungent (0 –3) ....
Green (grass/leaves) (0 – 2) ....
Other positive sensations (0 - 3) ....
Harmony (1) (0 – 20) ....
*Partial score*____

Final olfactory-gustatory sensation (maximum 20 points)

Complexity (2) (0 – 10) ....
Persistence (0 – 10) ....
*Partial score*____

Total score..

(1) **Harmony** increases when the attributes are balanced.
(2) **Complexity** increases with the number and intensity of aromas and flavours.

Figure 33.4 Mario Solinas Quality award profile sheet. *Source*: International Olive Council (2003).

- *Heated or burnt*: Characteristic flavor of oils, caused by excessive and/or prolonged heating during processing, particularly when the paste is thermally mixed and if this is done under unsuitable thermal conditions.
- *Hay-wood*: Characteristic flavor of certain oils produced from olives that have dried out.
- *Vegetable water*: Flavor acquired by the oil as a result of prolonged contact with vegetable water that has undergone fermentation processes.
- *Metallic*: Flavor that is reminiscent of metals. It is characteristic of oil that has been in prolonged contact with metallic surfaces during crushing, mixing, pressing, or storage.
- *Cucumber*: Flavor produced when an oil is hermetically packed for too long, particularly in tin containers, and which is attributed to the formation of 2,6-nonadienal.

Tasters may also encounter other noncommon defects, which are not included in the above list. These are difficult to identify (synergy and/or antagonism effects aggravate the problem) and quantify due to the lack of references (unknown range of intensity). For example:

- The odor of rubber, with the most possible cause being the transportation or transfusion of oil through brand-new, unwashed plastic pipes.
- Unknown odors acquired by storage in inappropriate containers or facilities.

Finally, assessors should be very cautious with some typical defects that tend to hide under the intense odor of positive attributes. High temperature and long malaxation time during oil production can lead to formation of unpleasant odors known as heated defect (Angerosa *et al.*, 2001; Angerosa, 2002), which could be unnoticeable during the first days or even months of the oil's life. Intense positive attributes, like green cut grass, have the ability to cover such negative characteristics. Eventually, with the passing of time, the positive odors subside and the negative ones predominate.

33.2.3 Sensorial terms for labeling purposes

Lately, specific sensorial terminology has been allowed (since EU Reg. 640/2008, which was based on the IOC revision of the organoleptic method in 2007) on product labels. Producers may ask panels that evaluate their oil sample to provide them with descriptions in order to use them in product labeling. According to the method (IOC, 2015), the following can be used:

- The term "intense" may be used when the median of the positive attribute (fruity, bitter, or pungent) is greater than 6.
- The term "medium" may be used when the median of the positive attribute is between 3 and 6.
- The term "light" may be used when the median of the positive attribute is less than 3.
- The attributes in question may be used without the adjectives given in the above points when the median of the attribute is 3 or more.
- The oil can be characterized as well balanced when it does not display a lack of balance. A lack of balance occurs when the median of the bitter and/or pungent attributes is two points higher than the median of its fruitiness.
- The term "mild oil" may be used when the medians of the bitter and pungent attributes are 2 or less.

Such an approach must be considered as a first step of providing useful information to consumers for choosing their preferred oil. However, according to Bertuccioli and Monteleone (2014), the above attributes do not give any information about odor and flavor sensations, which are the most characterizing attributes in the consumer's perception and choice.

33.2.4 Organoleptic assessment of extra virgin olive oil applying to use a designation of origin (DO)

The last years, in an effort for added value in agricultural products, the term "designation of origin" has been introduced. Knowing an olive oil's origin is considered as one of the main motivators for purchasing (Caporale *et al.*, 2006). Olive oils seeking such a property must undergo a more detailed sensory evaluation, as described in IOC method COI/T.20/Doc.no.22 (IOC, 2005), issued in November 2005.

The profile sheet for the evaluation of candidate oils is enriched with a maximum of 10 positive descriptors like:

- Almond, apple, artichoke, citrus fruit, exotic fruit, flowers, grass, herbs, tomato leaves, and so on from orthonasal and/or retronasal olfactory sensations
- Bitter and/or sweet from gustatory sensations
- Retronasal persistence (length of time that retronasal sensations persist after the sip of oil is no longer in the mouth) from retronasal olfactory sensations
- Fluidity and pungency from tactile or kinesthetic sensations.

For checking the eligibility of a sample to obtain a specific DO, limits for the descriptors have been established. Possible natural variations that may occur in the oils of a particular origin from one crop year to the next should also be taken into account during formation of these limits.

33.3 Chemistry, functionality, and technology behind senses

Different compounds belonging to different chemical classes have been found to contribute to the organoleptic properties of VOO. Some of them also participate in chemical and/or biological processes with a positive impact on product preservation and human health. Simultaneously, chronic incorrect agronomic and technological practices during production have led to misperceptions about the potentiality of many olive varieties.

33.3.1 Saponifiable matter

Triacylglycerols, diacylglycerols, monoacylglycerols, and free fatty acids (saponifiable matter) account for up to 99% of olive oil constituents. From sensory and functional points of view, triacylglycerols may be detected by the tactile properties they contribute (oiliness and viscosity), but it can also act as carrying agents of flavor compounds (Mattes, 2009). Oleic acid is found in almost 60% of olive oil triacyglycerols (IOC, 2013b). The high content in this monounsaturated fatty acid (up to 83% of the total fatty acids) present in the glycerol fraction is one of the main reasons for the unparalleled nutritional value of olive oil (Kiritsakis, 1991, 1998; Angerosa, 2002; Aguilera *et al.*, 2004; Harper *et al.*, 2006). The unique characteristics of oleic acid and its beneficial role in a number of biological functions are given here (Mariotti & Peri, 2014):

- A melting point lower than the human body temperature is an essential attribute for preventing accumulation on artery walls and for guaranteeing cell membrane fluidity.
- Great resistance to oxidation is an essential attribute for preventing oxidative damage to critical cell structures.
- Lowering blood pressure
- Reducing the clogging and hardening of arteries
- Lowering low-density lipoprotein (LDL)
- Increasing high density lipoprotein (HDL)
- Strengthening of cell membrane integrity and helping to repair cells and damaged tissues
- Anticancer agent
- Relieving symptoms of asthma
- Moisturizing skin.

On the other hand, free fatty acids have a negative impact. Free fatty acid content is an index of low quality in VOOs. Their accumulation starts at the tree from the infestation of olive drupes by the olive fly insect and/or olives' prolonged preservation in poor conditions prior to processing (i.e., stored in plastic bags, high temperatures, and increased humidity) (Vichi *et al.*, 2009; Peri, 2013). According to Mattes (2009), free fatty acids are detectable by the olfactory pathway, and their exposure in the mouth serves as a warning signal to discourage intake and influences lipid metabolism.

33.3.2 Unsaponifiable matter

The unique and delicate flavor of olive oil comes from a large number of constituents that are present at extremely low concentrations (Kiritsakis, 1998), and they are included in the nonglycerol fraction (unsaponifiable matter) that represents approximately 0.5–3% of the oil (Kiritsakis, 1991; Peri, 2013).

33.3.2.1 Compounds responsible for olfactory attributes of virgin olive oil

Volatile compounds are responsible for the aroma of olive oil. Almost 200 molecules have been separated from VOOs by gas chromatography–mass spectrometry (GC-MS) techniques. They belong to different

chemical classes, including aldehydes, ketones, alcohols, esters, acids, aliphatic and aromatic hydrocarbons, and furan and thiophene derivatives (Kiritsakis, 1998; Angerosa *et al.*, 2004). Despite their differences, they exhibit some common properties that are very important for their perception through sensory organs, like low molecular weight, high volatility, good hydrosolubility to diffuse into the mucus of the olfactory cells, good lipophilicity to dissolve in membrane lipids, and chemical features to bond specific proteins and receptors (Angerosa, 2002).

Paradoxically, most of them are not endogenous of the olive fruit. They are produced from the activity of olive enzymes during the extraction of the oil through a proposed pathway known as the lipoxygenase pathway. During the critical period of ripening, an increase in proteins and enzyme activities occurs. These enzymes are released during the olive crushing due to disruption of the olive fruit tissues and continue to act during the malaxation process.

Kalua *et al.* (2007) summarized the pathway leading to volatile compounds formation based on the presence and action of different enzymes. First, lipolytic acyl hydrolase (AH) releases free fatty acids from triacylglycerols and phospholipids. Then, lipoxygenase (LOX) oxidizes both linoleic and linolenic acids, yielding a high percentage up to 90% of hydroperoxides. Hydroperoxide lyase (HPL) produces C6 aldehydes and C12 ω-oxoacids from the 13-hydroperoxides of linolenic or linoleic acid or C9 aldehydes and C9 ω-oxoacids from the 9-hydroperoxides of linolenic or linoleic acid, whereas cis-3-trans-2-enal isomerase converts cis-unsaturated aldehydes to the corresponding trans isomers. Afterward, alcohol dehydrogenase (ADH) reduces aliphatic aldehydes to alcohols. Finally, alcohol acetyl transferase (AAT) catalyzes the formation of acetate esters and other esters of alcohols. From the above sequence, the most important compounds formed that contribute to the aroma are C6 and C5 saturated and unsaturated aldehydes, alcohols and esters responsible for the cut grass and floral notes of VOOs (Servili *et al.*, 2003; Angerosa *et al.*, 2004). On the contrary, C7–C11 monounsaturated aldehydes, C6–C10 dienals, C5 branched aldehydes and alcohols, and C8 ketones are related to organoleptic defects (Angerosa *et al.*, 2004).

The descriptors used in sensory profiles, their definitions, and their relationship to volatile compounds are given in Table 33.3.

This diversity of positive aromatic sensations grants complexity and excellence, making VOO a unique natural product that contributes to the well-being of humans through pleasure and positively influences lipid and food metabolism (Kiritsakis, 1991). In most positive aromatic descriptors (Table 33.3), C6 and C5 compounds (aldehydes) mainly prevail. This is characteristic of the high-quality VOOs. C6 esters like cis-3-hexenyl acetate and hexyl acetate and some C6 alcohols like cis-3-hexen-1-ol also contribute to the final delicate and unique aroma of these exceptional oils. Nevertheless, it is not the concentration of particular compounds that will determine the overall and final aroma of the oil. The odor threshold (OT) value (i.e., the minimum concentration of a compound that is perceived by the olfactory sense) plays a very important role in sensory evaluation. For example, cis-3-hexenal has an odor threshold value of 3 μg/kg oil, whereas trans-2-hexenal ranges from 420 to 1125 μg/kg oil (Kalua *et al.*, 2007). Kalua *et al.* (2007) also reported that the presence of carbohydrates and proteins can decrease the aroma intensity, thus increasing OT values. Chain length, stereochemistry, and matrix effects can influence the interaction of a volatile molecule with olfactory receptors and consequently its OT value. But the final perception of the overall aroma of a VOO is formed through positive and/or negative synergistic effects between particular compounds (Kiritsakis, 1998). Thus, analytical determination of the concentration of individual compounds works only complementarily and cannot substitute the sensory evaluation of the aroma of VOOs by tasters (Angerosa *et al.*, 2004).

The formation of aromatic compounds with positive attributes in VOO is generated through the LOX pathway. The amount of the enzymes involved in this pathway depends on the olive cultivar. However, their activity depends on technological factors during production. In general, enzymatic activity reaches a maximum when olive fruit color turns from green-yellow to purple (Taticchi *et al.*, 2013). When the fruit becomes overripe, the activity of the enzymes is decreased, and therefore a decrease of volatile compounds with pleasant odor is expected (Morales *et al.*, 1996; Angerosa *et al.*, 2004). Under ideal conditions of climate, cultivation, ripeness, harvesting, transportation, storage, and extraction of the olives, the LOX pathway prevails. Differently, other pathways involving enzymes from microbial growth will rise with subsequent production of off-flavors. In fly-infested olives, olives picked from the ground (a harvesting method that is unfortunately still in use today in some areas), or olives stored in inappropriate conditions (sacks and large piles, prolonged length of storage, and unsuitable temperature and humidity levels), the development of different bacteria species, yeast, and molds will result in the production of volatile compounds related to the

Table 33.3 Aromatic olfactory sensations and correlation with volatile compounds (Data from: Kiritsakis, 1998; Morales and Aparicio, 1999; Angerosa *et al.*, 2000; Angerosa *et al.*, 2001; Angerosa *et al.*, 2004; IOC, 2005; Morales *et al.*, 2005; Kalua *et al.*, 2007; Vichi *et al.*, 2009; IOC, 2015).

Aromatic olfactory sensations	Description	Volatile compounds
Almond	Olfactory sensation reminiscent of fresh almonds	Trans-2-hexenal, benzaldehyde, hexanal, cis-2-penten-1-ol, cis-3-hexenyl acetate, cis-2-hexenal, trans-2-pentenal
Apple	Olfactory sensation reminiscent of the odor of fresh apples	Hexanal, trans-2-hexenal, hexan-1-ol, cis-3-hexenyl acetate, ethyl propanoate, trans-2-pentenal, 2-methyl-2-butenal, cis-3-hexenal, ethanol, ethyl propanoate
Artichoke	Olfactory sensation of artichokes	Cis-3-hexenyl acetate, trans-2-pentenal, 1-penten-3-one, pentene dimmers, trans-3-hexenal
Chamomile	Olfactory sensation reminiscent of that of chamomile flowers	Trans-2-octenal
Citrus fruit	Olfactory sensation reminiscent of that of citrus fruit (lemon, orange, bergamot, mandarin, and grapefruit)	Octanal, nonanal, decanal
Eucalyptus	Olfactory sensation typical of *Eucalyptus* leaves	–
Exotic fruit	Olfactory sensation reminiscent of the characteristic odors of exotic fruit (pineapple, banana, passion fruit, mango, papaya, etc.)	Hexan-1-ol, cis-3-hexen-1-ol, hexanal, cis-3-hexenyl acetate, trans-2-penten-1-ol, 1-penten-3-ol, 3-methylbutyl acetate, cis-2-penten-1-ol, propyl butanoate
Fig leaf	Olfactory sensation typical of fig leaves	–
Flowers	Complex olfactory sensation generally reminiscent of the odor of flowers; also known as "floral"	Trans-2-hexen-1-ol, trans-2-hexenal, cis-3-hexenyl-acetate, cis-3-hexen-1-ol, cis-1,5-octadiene-3-one, acetaldehyde, propanal, trans-2-pentenal, trans-3-hexenal, ethanol, hexyl acetate, methyl nonanoate
Grass	Olfactory sensation typical of freshly mown grass	Trans-2-hexenal, cis-3-hexenal, cis-3-hexen-1-ol, trans-2-hexen-1-ol, hexan-1-ol, cis-3-hexenyl acetate, 1-penten-3-one, hexanal, 2,4-hexadienal
Green	Complex olfactory sensation reminiscent of the typical odor of fruit before it ripens	Hexanal, cis-3-hexenal, trans-2-hexenal, trans-3-hexen-1-ol, trans-2-hexen-1-ol, cis-2-nonenal, cis-3-hexenol, butyl acetate, hexyl acetate, cis-3-hexenyl acetate, 1-penten-3-one, 6-methyl-5-hepten-2-one, octan-2-one, trans-2-pentenal, cis-2-pentenal, cis-2-hexenal, trans-3-hexenal
Green fruity	Olfactory sensation typical of oils obtained from olives that have been harvested before or during color change	Cis-2-penten-1-ol, hexanal, trans-2-hexenal, cis-3-hexenyl acetate, cis-2-hexenal, hexyl acetate, 6-methyl-5-hepten-2-one, nonan-2-one, hexan-1-ol, pentanol, ethyl propanoate, ethyl butanoate, ethyl isobutyrate, ethyl-2-methylbutyrate, ethyl-3-methylbutyrate, ethyl cyclohexylcarboxylate, butan-2-one, trans-3-hexen-1-ol, cis-3-hexen-1-ol, trans-2-hexen-1-ol, cis-2-hexen-1-ol

Table 33.3 (*Continued*)

Aromatic olfactory sensations	Description	Volatile compounds
Green pepper	Olfactory sensation of green peppercorns	–
Herbs	Olfactory sensation reminiscent of that of herbs	Trans-2-octenal, 6-methyl-5-hepten-2-one
Olive leaf	Olfactory sensation reminiscent of the odor of fresh olive leaves	1-penten-3-one, cis-3-hexenyl acetate, hexan-1-ol, cis-3-hexenal, cis-3-hexen-1-ol
Pear	Olfactory sensation typical of fresh pears	–
Pine kernel	Olfactory sensation reminiscent of the odor of fresh pine kernels	–
Ripe fruity	Olfactory sensation typical of oils obtained from olives that have been harvested when fully ripe	Heptan-2-one, hexyl acetate, ethanol
Soft fruit	Olfactory sensation typical of soft fruit: blackberries, raspberries, bilberries, blackcurrants, and redcurrants	Hexan-1-ol, ethyl propanoate, 1-penten-3-one
Sweet pepper	Olfactory sensation reminiscent of fresh sweet red or green peppers	–
Tomato	Olfactory sensation typical of tomato leaves	Hexan-1-ol, 1-penten-3-one, cis-2-penten-1-ol
Vanilla	Olfactory sensation of natural dried vanilla powder or pods; different from the sensation of vanillin	–
Walnut	Olfactory sensation typical of shelled walnuts	Cis-3-hexenyl acetate, trans-2-pentenal, hexyl acetate, pentene dimmers, trans-2-hexen-1-ol
Cucumber	Flavor produced when oil is hermetically packed for too long	2,6-nonadienal
Fusty/muddy sediment	Characteristic flavor of oil obtained from olives piled or stored in such conditions as to have undergone an advanced stage of anaerobic fermentation, or of oil that has been left in contact with the sediment that settles in underground tanks and vats and that has also undergone a process of anaerobic fermentation	2-methylpropyl butanoate, butan-2-ol, 3-methyl butan 1-ol, pentan-1-ol, acetic acid, propanoic acid, butanoic acid, pentanoic acid, hexanoic acid
Musty-humid earthy	Characteristic flavor of oils obtained from fruit in which large numbers of fungi and yeasts have developed as a result of its being stored in humid conditions for several days or of oil obtained from olives that have been collected with earth or mud on them and that have not been washed	3-methyl-butan-1-ol, 1-octen-3-ol, heptan-2-ol, propanoic acid, butanoic acid, pentanoic acid, octan-2-ol, guaiacol
Rancid	Flavor of oils that have undergone an intense process of oxidation	Trans-2-heptenal, octanal, 2,4-heptadienal, trans-2-decanal, butanoic acid, hexanoic acid, heptanoic acid, 4-vinylphenol
Winey-vinegary acid-sour	Characteristic flavor of certain oils reminiscent of wine or vinegar. This flavor is mainly due to a process of aerobic fermentation in the olives or in olive paste left on pressing mats that have not been properly cleaned, and it leads to the formation of acetic acid, ethyl acetate, and ethanol.	Ethanol, ethyl acetate, butan-2-ol, 2-methyl butan-1-ol, 3-methyl butan-1-ol, pentan-1-ol, octan-2-one, acetic acid, propanoic acid, butanoic acid

Table 33.4 Minimum and maximum temperatures at Heraklion, Crete, Greece, from September to December (Data from Meteo.gr, 2015)[a].

Month	September		October		November		December	
Year	Min	Max	Min	Max	Min	Max	Min	Max
2006	15.5	30.8	11.0	27.9	5.1	23.4	5.0	20.0
2007	15.2	35.9	13.3	27.4	9.8	28.9	6.9	20.3
2008	15.5	32.2	13.4	31.5	10.8	29.7	6.1	24.8
2009	17.2	30.9	14.5	30.9	9.6	24.3	8.2	26.6
2010	17.8	36.4	12.7	32.1	11.4	29.3	3.8	28.3
2011	17.9	31.5	11.0	28.4	8.1	20.6	5.8	21.0
2012	17.5	34.5	15.6	31.9	12.4	30.1	7.7	23.9
2013	17.4	32.6	13.5	31.3	–	–	–	–

[a] Data provided to Meteo by National Observatory of Athens.

common olive oil defects (Proietti, 2014) known as fusty (from anaerobic bacteria amino acid conversion pathways), musty and humid (from enzymatic activity of molds), winey (from sugar fermentation by yeasts), and vinegary (from enzymatic activity of bacteria).

The enzymes in the LOX pathway exhibit different optimum temperatures for activity. In general, crushing elevates olive fruit temperature. In recent decades, hammer mills have been used very extensively (in almost all olive oil producing plants in Greece), rising the olive temperature to a level where desirable enzyme activity is reduced, especially for hydroperoxide lyase, which is responsible for the formation of the important aroma aldehyde fraction. This has a negative effect on both quality and quantity of volatile compounds characteristic of the unique VOO aroma. The situation is made worse when atmospheric temperature is high during processing. Harvesting takes place in the winter from October to December (in the northern hemisphere) when the temperature is often lower than 10 °C (Peri, 2014a). But in recent years, in countries like Greece, during that time of the year temperatures have been a lot higher, resulting in excessive heating of the olive paste produced during milling (Table 33.4).

The use of other crushers, like blade or disc mills, can help overcome this problem, and are found to produce oils with higher concentrations of volatile compounds and overall improved organoleptic quality due to an increase of the cut-grass (from increased concentration of aldehydes such as 1-hexanal and trans-2-hexenal) and floral (from increased concentration of esters like hexyl acetate and 3-hexenyl acetate) sensory notes (Servili *et al.*, 2002).

Similarly, elevated temperature during malaxation is responsible for sensory flattening of oils; decrease of concentration of C6 esters and cis-3-hexen-1-ol, which impart delicate and pleasant green notes; and increase of alcohols hexan-1-ol and trans-2-hexen-1-ol, which are accused of less attractive perceptions (Angerosa *et al.*, 2001).

High temperatures in the malaxation process also trigger the pathway of amino acid conversion, ending with the production of undesirable compounds like 2-methyl-butanal and 3-methyl-butanal (Kalua *et al.*, 2007). Malaxation time must always be considered as temperature dependent (Tamborrino, 2014). Long malaxation time results in lipid oxidation due to the exposure of oil droplets to the atmospheric oxygen. Servili *et al.* (2003) demonstrated that the ideal temperature and time of exposure of olive pastes to air contact are cultivar dependent. For example, optimal combinations were found to be 30 min at 22 °C and 0 min at 26 °C for the 'Frantoio' and 'Moraiolo' varieties, respectively. Optimal malaxation temperature and time for the formation of hexanal (described as green, apple, or green fruity), cis-3-hexenol (described as grass or banana), and cis-3-hexenyl acetate (described as fruity or green leaves) are found to be 25 °C and 45 min; for hexanol (described as fruity, banana, or soft) and hexyl acetate (fruity or sweet), 25 °C and 15 min; and for cis-3-hexenal (described as green or cut grass), 25 °C and 30 min. Longer time and higher temperature favor the formation of trans-2-hexenal (described as green, fruity, or almonds) at 35 °C for 60 min and trans-2-hexenol (described as green, grassy, or sweet) at 35 °C for 90 min (Morales & Aparicio, 1999). However, in later studies, other scientists have found that temperatures above 28 °C and time greater than 45 min can lead to deactivation of enzymes responsible for production of volatile compounds with positive attributes (Kalua *et al.*, 2007). There is no doubt that cultivar and desirable aromatic profile influence the optimization of malaxation conditions.

The use of water during the final steps of separation in horizontal centrifuges (decanters with separation of oil from solids and water) and vertical centrifuges (separation of oil from remaining water) decreases volatile compounds due to partition phenomena between the oil and water phases (Gomez da Silva *et al.*, 2012). The use of two-phase decanters (no need for water addition) or a reduced amount of water in three-phase decanters and vertical centrifuges can minimize the problem.

Finally, during storage of VOO, the gradual loss of aromatic compounds formed through the LOX pathway is inevitable. Light, pigments (especially when exposed to light), temperatures above 14 °C, oxygen, time, metals (Cu and Fe), polyunsaturated fatty acid content, antioxidants present, and containers used are all factors affecting positively or negatively the oxidation of lipids. On the other hand, sediment formation promotes enzymatic degradation. The result of oxidation of fatty acids is the formation of volatile compounds that have low odor threshold values and are thus very easily perceptible. 2,6-nonadienal is an unsaturated aldehyde, the main contributor to the cucumber defect that develops when a VOO, completely sealed in containers, is stored for a long time period. Unsaturated aldehydes like 2-octenal, 2-heptenal, and 2-decenal; saturated aldehydes like pentanal, hexanal, heptanal, octanal, and nonanal; and short-chain fatty acids like acetic, butanoic, and hexanoic are the main contributors to the rancid defect (Morales *et al.*, 2005). For controlling the above factors that promote or affect lipid oxidation, the following points must be considered:

- Use of dark conditions and dark containers
- Low storage temperatures between 12 and 14 °C
- Exclusion of oxygen by using inert gases like nitrogen
- Exclusion of metal catalysts (water used for olive washing often contains iron and copper; new processing plants or production lines used for the first time every new crop year can contaminate VOOs with metals)
- Enhancing the presence of natural antioxidants through agronomic and technological practices.

Fermentation of sediment in VOOs gives the well-known defect called "muddy sediment." It is produced from the development of anaerobic bacteria, which ferment remaining sugars and proteins found in sediments (Bendini *et al.*, 2012). (This is the reason why the panel test method has combined this defect with the fusty one. Both defects emerge from anaerobic fermentations. Fusty comes from anaerobic fermentations during olive fruit storage. Muddy comes from anaerobic fermentation in olive oil sediment.) The main volatile compounds responsible are butyrates and 2-ethyl butyrates (Angerosa *et al.*, 2004). To overcome this problem, filtration of VOOs after final vertical centrifugation is an essential condition prior to good oil storage (Peri, 2014b).

33.3.2.2 Compounds responsible for taste, chemaesthetic and tactile attributes of virgin olive oil

The main contributors to these attributes are phenolic compounds. Some volatile compounds have also been found to exhibit or to enhance such sensations.

From the hundreds of phenolic compounds found in nature, at least 36 have been identified in VOO (Cicerale *et al.*, 2008). They come directly from the olive fruit or derive from the more polar phenolic compounds that are found in the olive fruit through enzymatic modification. According to Servili *et al.* (2004), the main phenolic compounds found in olive fruit are:

- *Phenolic acids*: chlorogenic acid, caffeic acid, *p*-hydroxybenzoic acid, protocatechuic acid, vanillic acid, syringic acid, *p*-coumaric acid, o-coumaric acid, ferulic acid, sinapic acid, benzoic acid, cinnamic acid, and gallic acid
- *Phenolic alcohols*: 3,4-dihydroxyphenyl ethyl alcohol (3,4-DHPEA) and p-hydroxyphenyl ethyl alcohol (*p*-HPEA)
- *Hydroxycinnamic acid derivatives*: verbascoside
- *Anthocyanins*: cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3-caffeyglucoside, cyanidin-3-caffeylaitinoside, and delphinidin-3-rhamosylglucoside-7-xyloside
- *Flavonols*: quercetin-3-rutinoside

- *Flavones*: luteolin-7-glucoside, luteolin-5-glucoside, and apigenin-7-glucoside
- *Secoiridoids*: oleuropein, demethyloleuropein, ligstroside, and nuzhenide.

Servili *et al.* (2004) also cite the phenolic compounds of VOO:

- *Phenolic acids and derivatives*: caffeic acid, *p*-hydroxybenzoic acid, protocatechuic acid, vanillic acid, syringic acid, *p*-coumaric acid, *o*-coumaric acid, ferulic acid, benzoic acid, cinnamic acid, gallic acid, 4-(acetoxyethyl)-1,2-dihydroxybenzene, and hydroxyisocromans
- *Phenolic alcohols*: 3,4-dihydroxyphenyl ethyl alcohol (3,4-DHPEA), p-hydroxyphenyl ethyl alcohol (*p*-HPEA), and 3,4-dihydroxyphenyl ethyl alcohol glucoside
- *Flavones*: apigenin and luteolin
- *Lignans*: 1-acetoxypinoresinol and pinoresinol
- *Secoiridoids*: dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-dihydroxyphenyl ethyl alcohol (3,4-DHPEA-EDA), dialdehydic form of decarboxymethyl elenolic acid linked to *p*-hydroxyphenyl ethyl alcohol (*p*-HPEA-EDA), oleuropein aglycon (3,4-DHPEA-EA), ligstroside aglycon (*p*-HPEA-EA), oleuropein, *p*-HPEA-derivative, dialdehydic form of oleuropein aglycon, and dialdehydic form of ligstroside aglycon.

The olive fruit has a very high content in phenolic compounds, up to 3% of the fresh pulp weight. Phenolic acids, phenolic alcohols, and flavonoids are common in many fruits and vegetables. However, the botanical family Oleaceae, including *Olea europaea* (olive tree), are the only plants that contain secoiridoids (Servili *et al.*, 2004). Therefore, olives and olive oil are the main foods in human nutrition that provide these unique compounds. Oleuropein, demethyloleuropein, and ligstroside are the main secoiridoids found in olive fruit, but due to their structure (glycosides), they are almost insoluble in oil; hence, they are present in it only in a few mg/kg. They are very important compounds as they offer resistance to insect infestations and diseases to the olive fruit (Zanoni, 2014). Because of their polar nature and good solubility in water, they are present in high concentrations in olive mill wastewater, resulting in waste disposal problems due to their toxicity to plants (Zanoni, 2014). During the oil extraction process, the endogenous enzyme β-glucosidase catalyzes the hydrolysis of oleuropein, demethyloleuropein, and ligstroside, leading to the formation of the aglycon derivatives 3,4-DHPEA-EDA from oleuropein and demethyloleuropein and *p*-HPEA-EDA from ligstroside (Servili *et al.*, 2004; Taticchi *et al.*, 2013). This is a very critical point during extraction as the aglycon molecules change polarity and become almost insoluble in water but moderately soluble in oil. Thus, especially during malaxation, part of these newly formed compounds are transferred to the oil. In fact, aglycons of oleuropein and ligstroside and their derivatives (aldehydic, dialdehydic, and decarboxymethyl) are the main phenolic compounds (quantitatively) in VOO. As is discussed later in this chapter, they are the most important of the phenolic compounds in terms of functionality in oil sensory quality, oil stability, and human health promotion.

Two more endogenous enzymes, polyphenol oxidase (PPO) present in olive fruit mesocarp and peroxidase (POX) present in the seed, negatively affect the final phenolic profile of VOO. They both oxidize secoiridoid compounds, mainly hydroxytyrosol derivatives 3,4-DHPEA-EA and 3,4-DHPEA EDA, and such loss results in VOO with lower total phenolic content (Garcia-Rodriguez *et al.*, 2011). Tyrosol (*p*-HPEA) and hydroxytyrosol (3,4-DHPEA) are present in VOO in small quantities due to their high polarity. Increased content in the above compounds is an indication of the final step of phenolic compounds transformation through the hydrolysis of the ester bond linking tyrosol or hydroxytyrosol to elenoic acid. The reaction occurs during storage of VOO in the presence of water. However, water also promotes lipolysis and microbial growth. Therefore, despite their antioxidant power, increased tyrosol and hydroxytyrosol content is an indication of oil degradation (Zanoni, 2014).

Numerous studies have shown the positive correlation between phenolic compounds and flavor attributes of VOO. Bitter sensation is generated from polar molecules interacting with the lipid portion of taste papillae membrane. Pungent sensation comes from the stimulation of the free endings of the trigeminal nerve from polar molecules (Angerosa *et al.*, 2000). The same authors found a positive correlation of phenolic compounds with bitter ($r^2 = 0.6$) and pungent ($r^2 = 0.57$). They concluded that other compounds were also involved in the perception of these sensory attributes. Leaf sensation and 1-penten-3-one had a positive correlation with bitter and pungent. Cis-3-hexen-1-ol correlated with bitter perception, confirmed later by

Caporale *et al.* (2004). In general, tyrosol, hydroxytyrosol, and their derivatives are considered to be responsible for the bitter and pungent sensory attributes. Garcia-Rodriguez *et al.* (2011) found a good correlation between oil bitterness and hydroxytyrosol derivatives content. The relationship of oil bitterness with the derivatives of oleuropein and dimethyl oleuropein – 3,4-DHPEA-EA and 3,4-DHPEA-EDA – has been suggested by several authors (Kiritsakis, 1998; Garcia *et al.*, 2001). Strong correlation between bitter and the content of 3,4-DHPEA-EDA ($r = 0.9819$), p-HPEA-EDA ($r = 0.9830$), and 3,4-DHPEA-EA ($r = 0.7929$) has also been reported (Servili *et al.*, 2009). 3,4-DHPEA-EDA has been found to produce a burning/pungent sensation as well, but it is perceived mostly on the tongue. On the other hand, *p*-HPEA-EDA (oleocanthal) is the main contributor to the burning/pungent sensation perceived on the throat (Beauchamp *et al.*, 2005; Servili *et al.*, 2009). Pungent perception on the throat is often accompanied by coughing. The specificity of oleocanthal for a single sensory receptor and the anatomical restriction of this receptor to the pharynx are responsible for the unusual pungency of VOO (Taticchi *et al.*, 2013).

Esti *et al.* (2009) concluded that tyrosol and hydroxytyrosol, oleuropein, and ligstroside derivatives were relevant predictors of bitterness and pungency, whereas *p*-HPEA-EDA (oleocanthal) was only a predictor of pungency. Taste, chemaesthetic, and tactile sensations and their correlation with phenolic and volatile compounds are summarized in Table 33.5.

Gawel and Rogers (2009) reported in a study that expert tasters classed all oils with low total phenol content (<80 mg/kg) as mild and all oils with high total phenol content (>440 mg/kg) as robust. But there is no doubt, as in olfactory-stimulating compounds, that synergy and threshold values of the individual phenolic compounds play an important role for the final sensory perception.

Table 33.5 Taste, chemaesthetic, and tactile sensations, and correlation with phenolic and volatile compounds (Data from: Kiritsakis, 1998; Angerosa *et al.*, 2000; Angerosa *et al.*, 2001; Servili *et al.*, 2004; Kalua *et al.*, 2007; Esti *et al.*, 2009; Servili *et al.*, 2009; Taticchi *et al.*, 2013; Tsimidou, 2013; IOC, 2015).

Taste, chemaesthetic, and tactile sensations	Description	Phenolics[a] and related volatile compounds
Bitter	Characteristic primary taste of oil obtained from green olives or olives turning color. It is perceived in the circumvallate papillae on the "V" region of the tongue.	3,4-DHPEA-EA; 3,4-DHPEA-EDA; p-HPEA-EA; 3,4-DHPEA; *p*-HPEA; 1-penten-3-one; cis-3-hexen-1-ol
Sweet	Complex gustatory-kinesthetic sensation characteristic of oil obtained from olives that have reached full maturity	Hexanal; trans-2-hexen-1-ol; cis-3-hexen-1-ol; trans-2-penten-1-ol; 1-penten-3-ol; ethylfuran; 3-pentanone; 4-methylpentane-2-one; ethyl acetate; 3-methylbutyric acid; octane
Pungent	Biting tactile sensation characteristic of oils produced at the start of the crop year, primarily from olives that are still unripe. It can be perceived throughout the whole of the mouth cavity, particularly in the throat.	p-HPEA-EDA; 3,4-DHPEA-EDA; p-HPEA-EA; 3,4-DHPEA; *p*-HPEA; hexyl acetate; cis-2-penten-1-ol; 1-penten-3-one;
Astringent	Complex tactile sensation involving dryness of the oral surface due to loss of saliva lubricating properties from the interaction of tannins with lubricating salivary proteins	3,4-DHPEA-EDA; p-HPEA-EDA; *p*-HPEA-EA; 3,4-DHPEA-EA; *p*-HPEA

[a] 3,4-DHPEA-EA: 3,4-dihydroxyphenethyl alcohol-elenolic acid or oleuropein aglycone; 3,4-DHPEA-EDA: 3,4-dihydroxyphenethyl alcohol-elenolic dialdehydic acid or oleacin; *p*-HPEA-EA: *p*-hydroxyphenethyl alcohol-elenolic acid or ligstroside aglycone; *p*-HPEA-EDA: *p*-hydroxyphenethyl alcohol-elenolic dialdehydic acid or oleocanthal; *p*-HPEA: *p*-hydroxyphenethyl alcohol or tyrosol; 3,4-DHPEA: 3,4-dihydroxyphenethyl alcohol or hydroxytyrosol.

VOOs with similar levels of total phenols but different phenolic profiled (qualitatively and quantitatively) are perceived differently by tasters (Tsimidu, 2013). Instrumental analysis cannot substitute the sensory evaluation but certainly can work supplementarily. Different analytical techniques have been proposed with high-performance liquid chromatography (HPLC) (IOC, 2009) and Vis-spectroscopy (Gutfinger, 1981) to gain ground over more sophisticated but very expensive techniques like nuclear magnetic resonance (NMR) spectroscopy (Christophoridou and Dais, 2009). Today, the HPLC method (IOOC, 2009) has been simplified, as far as the sample preparation is concerned, but still suffers from difficulties in the identification of individual compounds due to complexity and lack of standards. Recently, the method is under modification by the IOC. On the other hand, the colorimetric measurement of total phenols (Vis-spectroscopy) is a simple method and can be used in the routine analysis of VOO (Psomiadou *et al.*, 2003). As early as 1989, Gutierrez *et al.* (1989) related bitterness with the area of specific peaks eluted from HPLC analysis of VOO phenolics. An automatic method using ultraviolet (UV) absorption spectra at 225 nm has been proposed as an objective method to measure bitterness (Kiritsakis, 1998). But later it was indicated that nonbitter phenolic compounds also absorb at 225 nm, and therefore the method cannot be correctly applied to compare olive oils from varieties with different phenolic profiles (Boskou *et al.*, 2006). An improved method using reverse-phase HPLC (RP-HPLC) with diode-array UV detection introduced by Mateos *et al.* (2004) revealed a better correlation of the aldehydic form of oleuropein aglycon (3,4-DHPEA-EA) with bitterness.

Phenolic compounds are responsible for bitter, pungent, and astringent attributes when VOO is in the oral cavity. But their role does not end there. Many studies have proved the antioxidant activity of phenolic compounds. A direct relationship between total phenolic content and shelf life of VOO has been established. Hydroxytyrosol (3,4-DHPEA) and its mainly bitter derivatives 3,4-DHPEA-EDA and 3,4-DHPEA-EA (oleuropein aglycon) are stronger antioxidants than tyrosol (*p*-HPEA), its derivatives, and α-tocopherol, probably due to the presence of two hydroxyl groups in the former's molecules (Servili *et al.*, 2009). During heating, hydroxytyrosol and its derivatives decrease rapidly, confirming their protective action. On the contrary, p-HPEA, p-HPEA-EDA, *p*-HPEA-EA, and lignans exhibit high stability, confirming their lower effect on oil protection from oxidation (Servili *et al.*, 2004). Phenolic compounds protect α-tocopherol during olive oil heating, contributing to the nutritional value of cooked foods (Servili *et al.*, 2009).

The antioxidant activity of the phenolic compounds found in VOO has received much attention in recent years because of its relationship to protection from chronic diseases like aging-related diseases, coronary heart diseases, and tumor formation. Chemically reactive molecules containing oxygen are called reactive oxygen species (ROS), for example oxygen ions and peroxides. When ROS levels increase drastically, under stressful conditions, significant damage to cell structures may occur. This situation is known as oxidative stress, resulting in cell damage. Damage in cells spreads to many other components, including lipids, proteins, and DNA. Oxidative stress has been accused of being involved in a number of health problems like atherosclerosis and cardiovascular diseases, cancer, aging, and Parkinson's and Alzheimer's diseases.

Servili *et al.* (2009) summarized the health-related properties of VOO compounds:

- Phenolics intake through VOO consumption is associated with reduced risk of cancer in breast, prostate, lung, larynx, ovary, and colon.
- Hydroxytyrosol (3,4-DHPEA) inhibits platelet aggregation that leads to thrombus formation and LDL oxidation, and it can prevent DNA strand breaks and DNA base modification.
- 3,4-DHPEA, 3,4-DHPEA-EDA (the bitter and less pungent, mainly in tongue, compound of VOO), and *p*-HPEA-EDA (oleocanthal – the main throat-burning and pungent constituent of VOO) can induce apoptosis in different tumor cells.
- *p*-HPEA-EDA (oleocanthal) inhibits the activity of both of the cyclooxygenase enzymes COX-1 and COX-2, which catalyze the formation of prostaglandins, molecules that are derived from arachidonic acid and involved in mediating inflammation pathways.

Cicerale *et al.* (2008) emphasized the high bioavailability of phenolics when consumed naturally as constituents of VOO. They reviewed the biological activities of VOO phenolics that may be important in the prevention of chronic diseases, as follows:

- VOO rich in phenolic compounds can decrease the levels of LDL cholesterol, increase HDL cholesterol, and decrease total cholesterol and triacylglycerols, thus reducing risk of cardiovascular incidents.

- Olive oil phenolics lower the levels of oxidized LDL, which is considered the main cause of atherosclerosis.
- Consumption of VOO phenolics reduces DNA oxidation, which is considered to be a crucial step to human carcinogenesis.
- Olive oil phenolics exhibit beneficial effects on decreasing oxidative stress.
- Olive oil phenolics have anti-inflammatory effects as they decrease proinflammatory and inflammatory agents. In particular, oleocanthal (p-HPEA-EDA, the main throat-burning and pungent constituent of virgin olive oil) has pharmacological properties like the anti-inflammatory drug ibuprofen.
- Olive oil phenolics and particular hydroxytyrosol (3,4-DHPEA) inhibit platelet aggregation responsible for atherosclerosis and cardiovascular diseases.
- Olive oil phenolic extract and oleuropein aglycon (3,4-DHPEA-EA, the most bitter compound of VOO) have beneficial effects on cellular function. They inhibit cell proliferation of tumors and reduce expression of oncogenes involved in malignant transformation, tumorigenesis, and metastasis.
- 3,4-DHPEA-EDA, p-HPEA-EDA, 3,4-DHPEA, and p-HPEA have bactericidal action against many microorganisms *in vitro*.

Finally, binding of metal ions that induce radical formation, and increased immune activity, anti-allergic activity, and skin protection are a few more functional properties of VOO phenolic compounds (Mariotti & Peri, 2014).

The importance of VOO phenolic compounds in human health is indisputable. Since in most studies their effect is dose dependent, and since they are “consumed” for the protection of the oil during storage (an antioxidant property with technological importance), a high content in VOOs is desirable. To ensure such content, the main factors influencing their presence in VOO must be considered. These factors are: cultivar, ripeness, irrigation, processing, and storage conditions.

Olive cultivar is a key factor for the phenolic content and profile of the olive fruit. Oleuropein is present in all cultivars, whereas demethyloleuropein and verbascocide are only in some olive cultivars (Servili *et al.*, 2004). This is not the case with VOOs, where the cultivar can determine the concentration of the phenolic compounds but the phenolic profile is almost the same (Taticchi *et al.*, 2013). It is understood that cultivars with high phenolic content like ‘Picual’, ‘Coratina’, and ‘Koroneiki’ have the potential to produce VOOs with total phenol content above 500 mg/kg (under suitable processing conditions that will be discussed later in this chapter). On the other hand, cultivars with low phenolic content like ‘Verdial de Velez’ and ‘Leccino’ produce VOOs with lower total phenol content, below 200 mg/kg (Servili *et al.*, 2004; Boskou *et al.*, 2006).

Maturity drastically affects the content of phenolic compounds of the olive drupes and consequently of the oil produced. During olive fruit ripening, tyrosol and hydroxytyrosol (due to breakdown of their higher molecular weight derivatives) increase, whereas oleuropein constantly decreases (Cicerale *et al.*, 2008). The lowest content of total phenolics occurs in overripened olives. Even a one-month delay in harvesting (from December to January) reduced total phenol content by almost 30% (Gambacorta *et al.*, 2010).

Irrigation has an impact on concentration of phenolic compounds in olive fruit. Servili *et al.* (2007) studied three different irrigation systems (i.e., full, deficit, and severe deficit). They concluded that olive fruits from severe deficit irrigation had the highest concentration in almost all individual phenolic components except tyrosol (*p*-HPEA). Lignans were the only compounds not affected by the irrigation system. However, the VOOs obtained from the deficient irrigation system exhibit a very interesting combination of both high phenol constituents and volatile compounds responsible for positive olfactory attributes.

During olive storage prior to extraction, the endogenous enzymes polyphenoloxidase and peroxidase catalyze phenol degradation (Taticchi *et al.*, 2013). Therefore, there is a decrease in phenolic compounds with storage time (Kiritsakis, 1998).

The extent of fragmentation of olive tissues is crucial to the extraction of phenolic compounds. Intense milling equipment like the hammer crushers release more phenolic compounds available to endogenous β-glucosidases for the subsequent production of secoiridoid aglycons. Hammer crushers should be used for varieties that are poor in phenolic substances to enhance the total phenol content in the extracted oil. But the simultaneous violent crushing of the seed releases the endogenous peroxidase enzymes, responsible for phenolic oxidation and losses. Even the rotation rate and sieve diameter of the hammer crusher affect the final phenolic concentration in the oil. Changes like 700 more rpm in the rotation rate result in approximately a 40% increase in the total antioxidant power (Boskou *et al.*, 2006). This is also illustrated in Table 33.6.

Table 33.6 Effect of crushing conditions on some quality indices and phenolic content of virgin olive oil from a mixture of 'Megaritiki' and 'Kothreiki' ('Manaki') cultivars.

Olive mill[a]	Acidity%	Fruity	Bitter	Pungent	Total phenols (mg/kg)[b]
A	0,29	4,2	1,2	3,0	256
B	0,31	4,5	4,5	5,5	602

[a] A: Two-phase decanter: low-rotation-speed, big-sieve diameter of hammer crusher. B: Two-phase decanter: high-rotation-speed, small-sieve diameter of hammer crusher.
[b] Determination by the colorimetric Folin-Ciocalteu method.

Stone mills are more gentle crushers, producing oils with less bitterness and pungency. To overcome hammer crusher disadvantage (they increase the temperature of the olive paste) but retain good total phenolic content (unlike stone mills), new mills like the blade and the teeth crusher are recommended. In a comparative study of hammer and blade crushers, the concentration of phenolic compounds was not significantly affected. However, oils obtained using the blade crusher had higher concentrations of volatile compounds, resulting in increased cut-grass and floral sensory attributes (Servili *et al.*, 2002). Olive destoning prior to crushing limits the release of peroxidases, thus increasing individual and total phenols in the oil (Gambacorta *et al.*, 2010). It also removes the woody smell of the pit, preventing the formation of an undesirable odor in the oil.

Increased malaxation temperature (35 °C) and time (above 45 min) result in weakening of bitterness and pungency due to reduction of secoiridoid compounds and their enhancer, 1-penten-3-one, by activation of endogenous oxidoreductase enzymes, which have high optimal temperatures of activity (Angerosa *et al.*, 2001). For phenolic substances, Servili *et al.* (2003) demonstrated that optimal temperature and time of exposure of olive pastes to air contact are cultivar dependent, similar to the case of volatile compounds. Better operative conditions for optimum concentration of *o*-diphenol, 3,4-DHPEA-EDA, and *p*-HPEA-EDA were found to be 30 min at 22 °C and 0 min at 26 °C for the 'Frantoio' and 'Moraiolo' varieties, respectively. In malaxation under nitrogen, losses of phenols are eliminated since O_2 is a prerequisite for oxidoreductase enzymes activity (Servili *et al.*, 2008).

The separation of the oil from the olive paste is achieved either by pressing or by centrifugation in three- or two-phase decanters. The use of presses is limited nowadays. Although they can produce oils with high phenolic content, since there is no water addition to the olive paste during pressing, they suffer from fermentation phenomena (development of defects like winey-vinegary) of the leftover paste on the filtering units. On the other hand, decanters are continuous systems with high efficiency. Thus, their use is now widespread. Two-phase decanters, which do not require the addition of water for the separation of oil, have been found to produce oils with higher phenolic content. In a study that compared the two-phase with the three-phase decanters, the resulting oils had 673 and 585 mg/kg total phenolic content, respectively, for a cultivar with high phenolic content. In the same study using a cultivar with lower phenolic content, the resulting oils had 304 and 263 mg/kg, respectively (De Stefano *et al.*, 1999). Today, however, this supremacy of the two-phase decanters is arguable since modern three-phase decanters require only a small addition of water (20–30 kg per 100 kg olives). Moreover, it is believed that the migration of phenolic compounds from the water to the oil phase is basically achieved through the change of their solubility by enzymatic reactions during malaxation (Baccioni & Peri, 2014). Partitioning equilibria of the phenolic compounds between water and oil phases exist during decantation but are less important. Furthermore, the washing-out effect of water on the oil phenolic compounds takes place more on the final vertical centrifugation step (clarification of the oil from very small solids and droplets of water that decanters are unable to separate), which is common after the use of both decanter types. Finally, filtration will lessen the phenolic content, but it will keep it more stable through oil storage.

Part of the phenolic compounds and especially *o*-diphenols (mainly the bitter compounds of VOO) will be "sacrificed" during storage of VOO, protecting triacylglycerols from oxidation. This could explain the decrease of bitterness during storage time and the parallel insistence of pungency. Romani *et al.*, (2007) examined the changes in phenolic compounds in two oils with different initial content, 350 and 300 mg/kg. Samples were stored for 18 months in special glass bottles, which screened from 99.99% of UV light radiation; capped with nitrogen; and kept in the dark at 18 °C. The oils lost 22 and 48% of their

initial concentration, respectively. Good storage conditions (see points at the end of Section 33.3.2.1) will minimize such losses and will allow more phenolics to play their unique health-beneficial role.

33.4 Positive sensory attributes of virgin olive oil and its consumption

Positive attributes of olive oil add sensorial superiority and health properties to it. Expert tasters characterize EVOO as excellent when diversity of intense pleasant odors along with medium to robust bitterness, pungency, and astringency, all in balance, occur. Unfortunately, only a small part of well-informed consumers appreciate this category of olive oils. And this is the case not only in emergent markets but also for many consumers in traditional olive oil producing Mediterranean countries. In recent years, consumption of olive oil has increased exponentially in countries like the USA (IOC, 2008), mainly for health benefit and flavor reasons. But in recent studies, there is a disconnection between expert taster evaluations and consumer preferences. Consumers perceive bitterness and pungency (two of the main attributes reflecting health benefits) as negative attributes, while they recognize rancidity and other defects as positive characteristics (Delgado & Guinard, 2011a). Surprisingly, the same consumers rank health benefits and flavor among the top motivators for consumption of olive oil. In a survey for consumer attitudes on olive oil, less than 25% responded correctly to statements about the different grades of olive oil (Wang *et al.*, 2013). The same survey suggested that producers should avoid commonly accepted "freshness" terms like fruity, peppery, and grassy since these terms are not yet considered by consumers as descriptors of tasty oil.

More recently, a study about consumer acceptance of 140 olive oil samples characterized by expert tasters as high-quality EVOOs was conducted (Valli *et al.*, 2014). During the International Olive Oil Award at a popular trade fair in Zurich, consumers who were considered to be accustomed to gourmet foods more than average were asked to evaluate the olive oils that participated in the competition. The consumers liked only 19% of all samples, while the panel tasters characterized all samples as high quality without defects. Moreover, consumers appreciated the ripe fruity and sweet oils and were opposed to the positive attributes bitter, pungent, green fruity, fresh-cut grass, green tomato, harmony, and persistency. Consequently, consumers do not have the appropriate information and opportunity to experience high-quality EVOOs. Incorrect agronomic and technological practices during production have led not only to misperceptions about the potentiality of many olive cultivars but also to consumer adaptation to low-quality EVOOs without aromatic complexity, while bitterness and pungency are either absent or present in a low degree. Adulteration with inferior categories of olive oil or inexpensive seed oils (Salivaras & McCurdy, 1992) and use of intensely defective virgin olive oils (lampante virgin olive oil), illegally labeled as extra virgin, have aggravated the conditions mentioned above. Unfortunately, consumers appreciate and tend to choose what is familiar and generate expectations. For example, information about the origin of oil creates positive hedonic expectations in a consumer familiar with this origin (Caporale *et al.*, 2006).

In fact, bitterness, pungency, and astringency are initially disliked during humans' early life stage, since they could be considered as warning signals for the presence of toxic substances (Tuorila & Recchia, 2013). The same authors state that later in life, these sensory attributes are not only accepted as part of many food and drink products but also become sources of sensory enjoyment. Consumers reject many vegetables and fruits due to bitterness and astringency, although they are aware of their health benefits (Lesschaeve & Noble, 2005).

Not all individuals perceive these sensory attributes to the same extent due to genetic reasons. For example, variations in salivary flow rates affect the perception of astringency in people (Lesschaeve & Noble, 2005). Therefore, limited information like "bitter and pungent olive oils contain nutritional components and have long shelf lives" (Tsimidou, 1998) is not enough to encourage people to consume excellent-quality EVOOs.

On the other hand, there are authors claiming that evaluation of olive oils should take into account the real-life conditions in which consumers experience them (i.e., as a condiment mixed with other foods) (Dinnella *et al.*, 2012). In their study, the use of different oils with foods modified the sensory properties of food pairings. Characteristic attributes of an oil may not be experienced in regular use with certain food pairing. Cerretani *et al.* (2007) introduced the terms non-harmonic, harmonic-enough, and harmonic pairing. When fruitiness and pungency of the olive oil are too light or too strong compared to the food saltiness, spiciness,

and aroma, then non-harmonic pairing occurs. When fruitiness and pungency of the olive oil balance the food saltiness, spiciness, and aroma, then we have harmonic-enough pairing. Finally, when the sweetness and ripeness of the olive oil balance the sweetness and fatness of the food, then we end with harmonic pairing. In later articles, the effect of EVOO on the sensory properties of a dish is considered too wide to be fairly expressed by the above terms (Dinnella, 2013). Interactions among the different ingredients of a dish may be extensive, leading to the final overall flavor. When a food is eaten, these interactions belong to three categories: chemical, physiological, and cognitive interactions (Dinnella, 2013).

To increase consumption of high-quality EVOOs, consumers must learn to appreciate their diversity in aroma, bitterness, pungency, and astringency attributes that offer countless culinary choices and specific pharmacological properties.

A successful learning process for consumers in order to appreciate the positive attributes of high-quality EVOOs consists of: (a) repeated exposures (which lead to adaptation), (b) social interactions involving contact with oils and learning, (c) conditioning and a shift in liking through momentary situational and social reinforcement, (d) pairing with favorite foods, (e) satiety effect, and (f) pharmacological effects of compounds simultaneously responsible for the positive sensory attributes of olive oil (Tuorila & Recchia, 2013).

Producers must be committed to production of high-quality EVOO. But in order to interpret the style of their oil, they should consider the opinion of expert tasters and use analytical results correlated to bitter and pungent attributes. They will be able then to reliably communicate to their consumers the best use of their oil (Gawel & Rogers, 2009). The intensity of bitterness, pungency, and astringency could be modified by the use of additives (Lesschaeve & Noble, 2005). In any case, this is not allowed in VOOs. Besides, consumers can learn to like foods that initially they had rejected. A different approach might be the blending of carefully selected oils in a way to increase shelf life (Caporale *et al.*, 2004) and health benefits without increasing the intensity of bitterness and/or pungency. This approach is based on the fact that volatile compounds of VOO affect (by enhancing or depressing) the perception of attributes with antioxidant properties like bitterness and/or pungency.

Chefs, salespeople in gourmet food shops, journalists for both print media and the internet, and educators in culinary classes and on television are all involved in consumer education and can change eating and cooking habits (Tardi, 2014). But they in turn should have the proper information to deliver. Olive oil experts should pass this information to producers, journalists, and educators. Finally, producers must inform importers, and importers could inform retailers and chefs. It is obvious that this is an interactive process.

There is no doubt that a lot is left to be done in the correlation of known and yet-unknown VOO compounds with specific sensory stimuli and biological processes important in the prevention of chronic diseases. However, today there is already enough evidence for this correlation. Organoleptic-related compounds contribute to the sensory diversity of EVOO, but especially those compounds responsible for bitter, pungent, and astringent, depending on their intensity, can even indicate directly the degree of its healthy value. This last property is amazing for a food product. Adequate and organized information will allow more consumers to experience and enjoy the different sensory styles and countless culinary uses, and take advantage of the functional compounds of the high-quality EVOOs.

References

Aguilera, C.M., Mesa, M.D., Ramirez-Tortosa, M.C., Nestares, M.T., Ros, E., & Gil, A. (2004) Sunflower oil does not project against LDL oxidation as virgin olive oil does in patients with peripherals vascular disease. Clinical Nutrition 23, 673–681.

Angerosa, F. (2002) Influence of volatile compounds on virgin olive oil quality evaluated by analytical approaches and sensor panels. European Journal of Lipid Science and Technology 104, 639–660.

Angerosa, F., Mostallino, R., Basti, C., & Vito, R. (2000) Virgin olive oil odour notes: their relationships with volatile compounds from the lipoxygenase pathway and secoiridoid compounds. Food Chemistry 68, 283–287.

Angerosa, F., Mostallino, R., Basti, C., & Vito, R. (2001) Influence of malaxation temperature and time on the quality of virgin olive oils. Food Chemistry 72, 19–28.

Angerosa, F., Servili, M., Selvaggini, R., Taticchi, A., Esposto, S., & Montedoro, G. (2004) Volatile compounds in virgin olive oil: occurrence and their relationship with the quality. Journal of Chromatography A 1054, 17–31.

Baccioni, L., & Peri, C. (2014) Centrifugal separation. In: The extra-virgin olive oil handbook, ed. C. Peri. John Wiley & Sons, Ltd, Chichester, 139–153.
Beauchamp, G.K., Keast, R.S.J., & Morel, D. (2005) Ibuprofen-like activity in extra-virgin olive oil. Nature 437, 45–46.
Bendini, A., Valli, E., Barbieri, S., & Toschi, T.G. (2012) Sensory analysis of virgin olive oil. In: Olive oil: constituents, quality, health properties and bioconversions, ed. D. Boskou. Intech, Rijeka, Croatia, 110–130.
Bertuccioli, M., & Monteleone, E. (2014) The sensory quality of extra-virgin olive oil. In: The extra-virgin olive oil handbook, ed. C. Peri. John Wiley & Sons, Ltd, Chichester, 35–58.
Bongartz, A., & Oberg, D.G. (2011) Sensory evaluation of extra virgin olive oil (EVOO) extended to include the quality factor "harmony." Journal of Agricultural Science and Technology A 1, 422–435.
Boskou, D., Tsimidou, M., & Blekas, G. (2006) Polar phenolic compounds. In: Olive oil chemistry and technology, 2nd ed., ed. D. Boskou. American Oil Chemists' Society Press, Champaign, IL, 73–92.
Caporale, G., Policastro, S., Carlucci, A., & Monteleone, E. (2006) Consumer expectations for sensory properties in virgin olive oils. Food Quality and Preference 17, 116–125.
Caporale, G., Policastro, S., & Monteleone, E. (2004) Bitterness enhancement induced by cut grass odorant (cis-3-hexen-1-ol) in a model olive oil. Food Quality and Preference 15, 219–277.
Cerretani, L., Biasini, G., Bonoli-Carbognin, M., & Bendini, A. (2007) Harmony of virgin olive oil and food pairing: a methodological proposal. Journal of Sensory Studies 22, 403–416.
Christophoridou, S., & Dais, P. (2009) Detection and Quantification of Phenolic Compounds in Olive Oil by High Resolution 1H Nuclear Magnetic Resonance Spectroscopy. Anal. Chim. Acta 633, 283–292.
Cicerale, S., Conlan, X.A., Sinclair, A.J., & Keast, R.S.J. (2008) Chemistry and health of olive oil phenolics. Critical Reviews in Food Science and Nutrition 49(3), 218–236.
Delgado, C., & Guinard, J-X. (2011a) How do consumer hedonic ratings for extra virgin olive oil relate to quality ratings by experts and descriptive analysis rating? Food Quality and Preference 22, 213–225.
Delgado, C., & Guinard, J.-X. (2011b) Sensory properties of Californian and imported extra virgin olive oils. Journal of Food Science 76, Nr. 3, S170–S176.
De Stefano, G., Piacquadio, P., Servili, M., Di Giovacchino, L., & Sciancalepore, V. (1999) Effect of extraction systems on the phenolic composition of virgin olive oils. Fett/Lipid 101(9), 328–332.
Dinnella, C. (2013) Sensory functionality of extra virgin olive oil. In: Olive oil sensory science, ed. E. Monteleone & S. Langstaff. John Wiley & Sons, Ltd, Chichester, 171–194.
Dinnella, C., Masi, C., Zoboli, G., & Monteleone, E. (2012) Sensory functionality of extra-virgin olive oil in vegetable foods assessed by temporal dominance of sensations and descriptive analysis. Food Quality and Preference 26, 141–150.
Esti, M., Contini, M., Moneta, E., & Sinesio, F. (2009) Phenolic compounds and temporal perception of bitterness and pungency in extra-virgin olive oils: changes occurring throughout storage. Food Chemistry 113, 1095–1100.
European Union (EU). (1991) The characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Regulation 2568. Brussels, EU.
European Union (EU). (1997) Amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Regulation 2472. Brussels, EU.
European Union (EU). (2002) Amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Regulation 296. Brussels, EU.
European Union (EU). (2003) Amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Regulation 1989. Brussels, EU.
European Union (EU). (2008) Amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Regulation 640. Brussels, EU.
European Union (EU). (2011) Amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Regulation 61. Brussels, EU.
European Union (EU). (2013) Amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Regulation 1348. Brussels, EU.
Gambacorta, G., Faccia, M., Previtali, M.A., Pati, S., La Notte, E., & Baiano, A. (2010) Effects of olive maturation and stoning on quality indices and antioxidant content of extra virgin oils (cv. Coratina) during storage. Journal of Food Science 75(3), 229–235.
Garcia, J.M., Yousfi, K., & Mateos, R. (2001) Reduction of bitterness by heating of olive oil (*Olea europaea*). Journal of Agricultural and Food Chemistry 49, 4231–4235.
Garcia-Rodriguez, R., Romero-Segura, C., Sanz, C., Sanchez-Ortiz, A., & Perez, A.G. (2011) Role of polyphenol oxidase and peroxidase in shaping the phenolic profile of virgin olive oil. Food Research International 44, 629–635.

Gawel, R., & Rogers, D.A.G. (2009) The relationship between total phenol concentration and the perceived style of extra virgin olive oil. Grasas y Aceites 60(2), 134–138.
Gomez da Silva, M.D.R., Costa Freitas, A.M., Cabrita, M.J.B., & Garcia, R. (2012) Olive oil composition: volatile compounds. In: Olive oil: constituents, quality, health properties and bioconversions, ed. D. Boskou. Intech, Rijeka, Croatia, 17–46.
Gutfinger, T. (1981) Polyphenols in olive oil. Journal of the American Oils Chemists' Society 58, 966–968.
Gutierrez, F., Albi, M.A., Palma, R., Rios, J.J., & Olias, J.M. (1989) Bitter taste of virgin olive oil: correlation of sensory evaluation and instrumental HPLC analysis. Journal of Food Science 54(1), 68–70.
Gutierrez-Rosales, F., Risco, M.A., & Gutierrez-Gonzales-Quijano, R. (1984) Selección de catadores mediante el metodo de clasificacion por intensidad. Grasas y Aceites 35, 310–314.
Harper, C.R., Edwards, M.C., & Jacobson, T.A. (2006) Flaxseed supplementation does not affect plasma lipoproteín concentration or particle size in human subjects. Journal of Nutrition 136, 2844–2848.
International Olive Council (IOC). (1987) T.20/Doc.no.3. Sensory analysis of olive oil: method for the organoleptic assessment of virgin olive oil. IOC, Madrid, Spain.
International Olive Council (IOC). (2003) T.30/Doc.no.6. Mario Solinas Quality award: rules of the international competition for extra virgin olive oils. IOC, Madrid, Spain.
International Olive Council (IOC). (2005) T.20/Doc.no.22. Organoleptic assessment of DO extra virgin olive oil. IOC, Madrid, Spain.
International Olive Council (IOC). (2007a) T.20/Doc.no.4/Rev.1. General basic vocabulary. IOC, Madrid, Spain.
International Olive Council (IOC). (2007b) T.20/Doc.no.5/Rev.1. Glass for oil tasting. IOC, Madrid, Spain.
International Olive Council (IOC). (2007c) T.20/Doc.no.6/Rev.1. Guide for the Installation of a test room. IOC, Madrid, Spain.
International Olive Council (IOC). (2008) Olive products market report summary. Market Commentary. IOC, Madrid, Spain.
International Olive Council (IOC). (2009) T.20/Doc.no.29. Determination of biophenols in olive oils by HPLC. IOC, Madrid, Spain.
International Olive Council (IOC). (2013a) T.20/Doc.no.14. Guide for the selection of tasters. IOC, Madrid, Spain.
International Olive Council (IOC). (2013b) T.20/Doc.no.25/rev.1. Global method for the detection of extraneous oils in olive oils. IOC, Madrid, Spain.
International Olive Council (IOC). (2015) T.20/Doc.no.15/Rev.7. Method for the organoleptic assessment of virgin olive oil. IOC, Madrid, Spain.
Kalua, C.M., Allen, M.S., Bedgood, D.R., Bishop, A.G., Prenzler, P.D., & Robards, K. (2007) Olive oil volatile compounds, flavour development and quality: a critical review. Food Chemistry 100, 273–286.
Karkoula, E., Skantzari, A., Melliou, E., & Magiatis, P. (2012) Direct measurement of oleocanthal and oleacein levels in olive oil by quantitative ^{1}H NMR: establishment of a new index for the characterization of extra virgin olive oils. Journal of Agricultural and Food Chemistry 60, 11696–11703.
Kiritsakis, A. (1991). Olive oil. American Oil Chemists' Society, Champaign, IL.
Kiritsakis, A.K. (1998), Flavor components of olive oil: a review. Journal of the American Oils Chemists' Society 75(6), 673–681.
Lesschaeve, I., & Noble, A.C. (2005) Polyphenols: factors influencing their sensory properties and their effects on food and beverage preferences. The American Journal of Clinical Nutrition 81(suppl.), 330S–5S.
Mariotti, M., & Peri, C. (2014) The composition and nutritional properties of extra-virgin olive oil. In: The extra-virgin olive oil handbook, ed. C. Peri. John Wiley & Sons, Ltd, Chichester, 21–34.
Mateos, R., Cert, A., Perez-Camino, M.C., & Garcia, J.M. (2004) Evaluation of virgin olive oil bitterness by quantification of secoiridoid derivatives. Journal of the American Oils Chemists' Society 81, 71–75.
Mattes, R.D. (2009) Is there a fatty acid taste? Annual Review of Nutrition 29, 305–327.
Mensink, R.P & Katan, M.B (1987) Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men and women. Lancet 1, 122–125.
Monteleone, E. (2013) Sensory methods for optimizing and adding value to extra virgin olive oil. In: Olive oil sensory science, ed. E. Monteleone & S. Langstaff. John Wiley & Sons, Ltd, Chichester, 109–140.
Morales, M.T., & Aparicio, R. (1999) Effect of extraction conditions on sensory quality of virgin olive oil. Journal of the American Oils Chemists' Society 76, 295–300.
Morales, M.T., Aparicio, R., & Calvente, J.J. (1996) Influence of olive ripeness on the concentration of green aroma compounds in virgin olive oil. Flavour and Fragnance Journal 11, 171–178.
Morales, M.T., Luna, G., & Aparicio, R. (2005) Comparative study of virgin olive oil sensory defects. Food Chemistry 91, 293–301.
Mueller, T. (2012) Extra virginity: the sublime and the scandalous world of olive oil. W.W. Norton, New York.
Peri, C. (2013) Quality excellence in extra virgin olive oils. In: Olive oil sensory science, ed. E. Monteleone & S. Langstaff. John Wiley & Sons Ltd, Chichester, 3–32.

Peri, C. (2014a) Olive cleaning. In: The extra-virgin olive oil handbook, ed. C. Peri. John Wiley & Sons, Ltd, Chichester, 113–116.

Peri, C. (2014b) Extra-virgin olive oil storage and handling. In: The extra-virgin olive oil handbook, ed. C. Peri. John Wiley & Sons, Ltd, Chichester, 165–178.

Proietti, P. (2014) Olive handling, storage and transportation. In: The extra-virgin olive oil handbook, ed. C. Peri. John Wiley & Sons, Ltd, Chichester, 107–112.

Psomiadou, E., Karakostas, K.X., Blekas, G., Tsimidou, M.Z., & Boskou, D. (2003) Proposed parameters for monitoring quality of virgin olive oil (Koroneiki cv). European Journal of Lipid Science and Technology 105, 403–408.

Romani, A., Lapucci, C., Cantini, C., Ieri, F., Mulinacci, D., & Visioli, F. (2007) Evolution of minor polar compounds and antioxidant capacity Turing storage of bottled extra virgin olive oil. Journal of Agricultural and Food Chemistry 55, 1315–1320.

Salivaras, E., & McCurdy, R.A. (1992) Detection of olive oil adulteration with canola oil from triacylglycerol analysis by reversed-phase high-performance liquid chromatography. Journal of the American Oils Chemists' Society 69, 935–938.

Servili, M., Esposto S., Fabiani R., Urbani S., Taticchi A, Mariucci F., Selvaggini R., & Montedoro G.F. (2009) Phenolic compounds in olive oil: antioxidant, heath and organoleptic activities according to their Chemical structure. Inflammopharmacology, 17, 76–84.

Servili, M., Esposto S., Lodolini E., Selvaggini R., Taticchi A., Urbani S., Montedoro G., Serravalle M., & Gucci R. (2007) Irrigation effects on quality, phenolic composition, and selected volatiles of virgin olive oils cv. Leccino. Journal of Agricultural and Food Chemistry 55, 6609–6618.

Servili, M., Piacquadio P., De Stefano G., Taticchi A., & Sciancalepore V. (2002) Influence of a new crushing technique on the composition of the volatile compounds and related sensory quality of virgin olive oil. European Journal of Lipid Science and Technology 104, 483–489.

Servili, M., Selvaggini R., Esposto S., Taticchi A., Montedoro G., & Morozzi G. (2004) Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. Journal of Chromatography A 1054, 113–127.

Servili, M., Taticchi A., Esposto S., Urbani S., Selvaggini R., & Montedoro G.F. (2008) Influence of the decrease in oxygen during malaxation of olive paste on the composition of volatiles and phenolic compounds in virgin olive oil. Journal of Agricultural and Food Chemistry 56, 10048–10055.

Servili, M., Selvaggini R., Taticchi A., Esposto S., & Montedoro G. (2003) Volatile compounds and phenolic composition of virgin olive oil: optimization of temperature and time of exposure of olive pastes to air contact during the mechanical extraction process. Journal of Agricultural and Food Chemistry 51, 7980–7988.

Tamborrino, A. (2014) Olive paste malaxation. In: The extra-virgin olive oil handbook, ed. C. Peri. John Wiley & Sons, Ltd, Chichester, 107–112.

Tardi, A. (2014) The culinary uses of extra-virgin olive oil. In: The extra-virgin olive oil handbook, ed. C. Peri. John Wiley & Sons, Ltd, Chichester, 321–337.

Taticchi, A., Esposto S., & Servili M. (2013) The basis of the sensory properties of virgin olive oil. In: Olive oil sensory science, ed. E. Monteleone & S. Langstaff. John Wiley & Sons, Ltd, Chichester, 33–54.

Tegopoulos, F. (2007) Major Greek dictionary, 6th ed. Armonia Publications Inc., Athens.

Trichopoulou, A., Costacou T., Bamia C., & Trichopoulos D. (2003) Adherence to a Mediterranean diet and survival in a Greek population. New England Journal of Medicine, 348, 2599–2608.

Tsimidou, M. (1998) Polyphenols and quality of virgin olive oil in retrospect. Italian Journal of Food Science 10, 99–116.

Tsimidou, M.Z. (2013) Analytical Methodologies: Phenolic Compounds related to Olive Oil taste issues. Handbook of Olive Oil: Analysis and properties (eds Aparicio- Ruiz R., Larwood J.), Springer, New York, 311–334.

Tuorila, H., & Recchia, A. (2013) Sensory perception and other factors affecting consumer choice of olive oil. In: Olive oil sensory science, ed. E. Monteleone & S. Langstaff. John Wiley & Sons, Ltd, Chichester, 55–80.

Valli, E., Bendini, A., Popp, M., & Bongartz, A. (2014) Sensory analysis and consumer acceptance of 140 high-quality extra virgin olive oils. Journal of Science Food Agriculture, 94, 2124–2132.

Vichi, S., Romero, A., Gallardo-Chacon, J., Tous, J., Lopez-Tamames, E., & Buxaderas, S. (2009) Volatile phenols in virgin olive oils: influence of olive variety on their formation during fruits storage. Food Chemistry 116, 651–656.

Wang, S., Moscatello, B., & Flynn, D. (2013) Survey – consumer attitudes on olive oil. Available from http://olivecenter.ucdavis.edu/research/files/surveyfinal052913reduced.pdf

Zanoni, B. (2014) The role of oxygen and water in the extra-virgin olive oil process. In: The extra-virgin olive oil handbook, ed. C. Peri. John Wiley & Sons, Ltd, Chichester, 69–74.

34 International standards and legislative issues concerning olive oil and table olives and the nutritional, functional, and health claims related

Stylianos Koulouris

34.1 Introduction

During the last decade, along with the globalization of trade, an ever-growing recognition of the nutritional importance of olive oil has been witnessed along with an increasing consumption worldwide. These facts are raising the need to establish new and improve the existing international standards and trading rules for the relevant products and to harmonize the various legislation frameworks for olive oil and the nutritional and health claims related to it.

34.2 The international perspective

In most cases, the international body under the United Nations administration that tries to set global standards on the characteristics and categorization of food is the Codex Alimentarius. The Codex Alimentarius international food standards, guidelines, and codes of practice contribute to the safety, quality, and fairness of the international food trade and are coordinated by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations (WHO/FAO, 2015). For olive oil, the standards are described in Codex Stan 33-1981. Seven categories of olive oil are described (see Codex Alimentarius, 1981, revisions 1989, 2003). Furthermore, Codex Stan 33-1981 describes all the other essential characteristics such as the organoleptic characteristics, the fatty acid composition, the sterol and trierpene dialcohol composition, and others (Codex Alimentarius, 1981, revisions 1989, 2003). Regarding labeling, Codex Stan 33-1981 foresees that olive oil products should be labeled according to the General Standard for Labelling of Pre-packaged Foods (Codex Stan 1-1985) (Codex Alimentarius, 1981, revisions 1989, 2003). There are also no specific requirements for nutrition and health claims regarding olive oil, but the relevant products should follow the guidelines as described in the "General Guidelines on Claims" (Cac/GL1-1979 Rev.1_1991) (Hawkes, 2004).

For table olives, the standards are described in Codex Stan 66-1981 (Codex Alimentarius, revisions 1987, 2013). According to these, table olives is the product:

1. prepared from the sound fruits of varieties of the cultivated olive tree (*Olea europaea* L.), having reached an appropriate degree of development for processing, that are chosen for production of olives and whose volume, shape, flesh-to-stone ratio, fine flesh, taste, firmness, and ease of detachment from the stone make them particularly suitable for processing;

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2. treated to remove its bitterness and preserved by natural fermentation, and/or by heat treatment, and/or by other means so as to prevent spoilage and to ensure product stability in appropriate storage conditions with or without the addition of preservatives; and
3. packed with or without a suitable liquid packing medium.

Further provisions are included in the standard regarding the quality, the size, the allowed ingredients, and the packaging, among others (Codex Alimentarius, 1981, Revisions 1987, 2013). Especially for products related to olives, the United Nations went one step further and the International Olive Council (IOC) was created in Madrid, Spain, in 1959 under its auspices. The main role of the Council is to contribute to the sustainable and responsible development of olive growing and to serve as a world forum for discussing policy-making issues and tackling present and future challenges (IOC, 2015c). It does so by performing these functions:

1. Encouraging international technical cooperation on research and development projects, training, and the transfer of technology
2. Encouraging the expansion of international trade in olive oil and table olives, drawing up and updating product trade standards, and improving quality
3. Enhancing the environmental impact of olive growing and the olive industry
4. Promoting world consumption of olive oil and table olives through innovative campaigns and action plans
5. Supplying clear, accurate information and statistics on the world olive and olive oil market
6. Enabling government representatives and experts to meet regularly to discuss problems and concerns and to fix priorities for IOC action
7. Working in close partnership with the private sector.

Its current membership includes the leading international producers and exporters of olive oil and table olives. IOC producer members account for 98% of world olive production, located primarily in the Mediterranean region. Figures 34.1 and 34.2 show the current members of the Council.

The latest agreement of the IOC is the 2005 Agreement, officially known as the International Agreement on Olive Oil and Table Olives, 2005 (IOC, 2005). In article 21 of the Agreement, and also more detailed in its Annex B, the seven main categories of the Codex Alimentarius are retained, but the categorization system has been slightly altered in order to describe some additional subcategories; the designations of the different categories of olive oils and olive-pomace oils are given here with the definition corresponding to each designation:

Virgin olive oils: Oils that are obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to deterioration of the oil; and that have not undergone any treatment other than washing, decantation, centrifugation, and filtration. Virgin olive oils shall be classified and designated as follows:

Extra virgin olive oil: Virgin olive oil that has a free acidity, expressed as oleic acid, of no more than 0.8 g per 100 g and the other characteristics that correspond to those laid down for this category.

Virgin olive oil: Virgin olive oil has a free acidity, expressed as oleic acid, of not more than 2.0 g per 100 g and the other characteristics that correspond to those laid down for this category.

Ordinary virgin olive oil: Virgin olive oil that has a free acidity, expressed as oleic acid, of not more than 3.3 g per 100 g and the other characteristics that correspond to those laid down for this category.

Lampante virgin olive oil: Virgin olive oil that has a free acidity, expressed as oleic acid, of more than 3.3 g per 100 g and/or the organoleptic characteristics and other characteristics that correspond to those laid down for this category.

Refined olive oil: Olive oil obtained by refining virgin olive oils. It has a free acidity, expressed as oleic acid, of no more than 0.3 g per 100 g, and its other characteristics correspond to those laid down for this category.

	Albania	13.02.2009
	Algeria	29.06.1963
	Argentina	08.05.2009
	European Union	Members are included in Figure 34.2
	Egypt	21.05.1964
	Iran	06.01.2004
	Iraq	26.03.2008
	Israel	Founding Member since 10.09.1958
	Jordan	02.12.2002
	Lebanon	10.11.1973
	Libya	Founding Member on 14.02.1956; rejoined 28.01.2003
	Montenegro	13.11.2007
	Morocco	Founding Member since 11.08.1958
	Syria	29.12.1997
	Tunisia	Founding Member since 14.02.1956
	Turkey	21.02.2010
	Uruguay	30.07.2013

Figure 34.1 Members and date of membership (last update: December 2013). *Source*: International Olive Council (2015a, 2015b).

Olive oil: Oil consisting of a blend of refined olive oil and virgin olive oils fit for consumption as they are. It has a free acidity, expressed as oleic acid, of no more than 1 g per 100 g, and its other characteristics correspond to those laid down for this category.

Olive-pomace oil: Oil obtained by treating olive pomace with solvents or other physical treatments, to the exclusion of oils obtained by re-esterification processes and of any mixture with oils of other kinds. It is classified as follows:

Crude olive-pomace oil: Olive-pomace oil whose characteristics are those laid down for this category. It is intended for refining for use for human consumption, or it is intended for technical use.

Refined olive-pomace oil: Oil obtained by refining crude olive-pomace oil. It has a free acidity, expressed as oleic acid, of no more than 0.3 g per 100 g, and its other characteristics correspond to those laid down for this category.

Figure 34.2 Current European Union members.

Olive-pomace oil: Oil consisting of a blend of refined olive-pomace oil and virgin olive oils fit for consumption as they are. It has a free acidity, expressed as oleic acid, of not more than 1 g per 100 g, and its other characteristics correspond to those laid down for this category. In no case will this blend of oil be called "olive oil" (IOC, 2005).

Regarding table olives, the IOC describes only three basic classification types:

Green olives: Fruits harvested during the ripening period, prior to coloring, and when they have reached normal size. They may vary in color from green to straw yellow.
Olives turning color: Fruits harvested before the stage of complete ripeness is attained, at color change. They may vary in color from rose to wine rose or brown.
Black olives: Fruits harvested when fully ripe or slightly before full ripeness is reached. They may vary in color from reddish black to violet black, deep violet, greenish black, or deep chestnut (IOC, 2005).

Most of the individual countries, including the USA and the countries of the European Union, don't have special legislation referring to table olives, or the legislations they have are outdated (the "United States standards for grades of green olives" are from 1967). The provisions for table olives are included in the general food legislation regarding the allowed food additives, the quality standards, the packaging material, the labeling, and so on. The basic classification of the Codex is mostly followed (green olives, black olives, and olives turning color), but even for that classification there is no direct reference in most national legislation or in the EU legislation (European Council, 2004).

34.3 Legislative approach by various countries

Although one of the objectives of Codex Alimentarius and the IOC is to set global rules regarding the specifications, labeling, and marketing of olive oils, the fact is that the Codex guides are not compulsory but only recommendations for voluntary application. As for the IOC, many countries that consume and trade considerable volumes of olive oils and olives are not members (e.g., the USA, Russia, and China), and these countries are not bound by the IOC's agreements. This leads to variation regarding the legislation of the categorization, specifications, and mostly labeling (including the nutritional and health claims) between

different markets and countries. As it is not possible to present all the different legislative frameworks, some characteristic cases will be presented as examples of the general situation.

34.3.1 The USA and Canada

The USA recognizes eight categories of relevant products, five of them concerning olive oils and three for grades of olive-pomace oil. The categories are US Extra Virgin Oil, US Virgin Olive Oil, Lampante Virgin Olive Oil, US Refined Olive Oil, US Olive Oil, US Olive-Pomace Oil, US Refined Olive Pomace Oil, and US Crude Olive-Pomace Oil, with very specific criteria for each category (Table 34.1).

34.3.1.1 Claims in the USA

1. *Health claims*: Health claims describe a relationship between a food substance (a food, food component, or dietary supplement ingredient) and reduced risk of a disease or health-related condition. To make such a claim, an "approval" is needed from the US Food and Drug Administration (FDA). The simpler way is for the food business operator to submit a petition to FDA presenting the "scientific evidence" for the statement under question (US Department of Health and Human Services, 2013).

   There is a list of the relevant claims approved by FDA, and it includes one related to olive oil, specifically to the qualified claims about cardiovascular disease risk. The claim is based on the relation between monounsaturated fatty acids from olive oil and coronary health disease, and the actual approved statement is as follows:

   Limited and not conclusive scientific evidence suggests that eating about 2 tablespoons (23 g) of olive oil daily may reduce the risk of coronary heart disease due to the monounsaturated fat in olive oil. To achieve this possible benefit, olive oil is to replace a similar amount of saturated fat and not increase the total number of calories you eat in a day. (U.S. Department of Health and Human Services, 2014)

2. *Nutrient content claims*: The Nutrition Labelling and Education Act (NLEA, 1990) permits the use of label claims that characterize the level of a nutrient in a food (i.e., nutrient content claims) if they have been authorized by the FDA and are made in accordance with the FDA's authorizing regulations. Nutrient content claims describe the level of a nutrient in the product, using terms such as "free," "high," and "low," or they compare the level of a nutrient in a food to that of another food (US Department of Health and Human Services, 2013).

   Nutrient content claims that are relevant and used in olive oils include the "sodium free" claim, "salt free" claim, and "cholesterol free" and "non-cholesterol" claims (North American Olive Oil Association, 2006).
3. *Structure and function claims, and related dietary supplement claims*: Structure–function claims have historically appeared on the labels of conventional foods and dietary supplements as well as drugs. The Dietary Supplement Health and Education Act of 1994 (DSHEA) established some special regulatory requirements and procedures for using structure–function claims and two related types of dietary supplement labeling claims: claims of general well-being and claims related to a nutrient deficiency disease. Structure–function claims may describe the role of a nutrient or dietary ingredient intended to affect the normal structure or function of the human body, for example "Calcium builds strong bones." In addition, they may characterize the means by which a nutrient or dietary ingredient acts to maintain such structure or function, for example "Fiber maintains bowel regularity," or "Antioxidants maintain cell integrity." General well-being claims describe general well-being from consumption of a nutrient or dietary ingredient. Nutrient deficiency disease claims describe a benefit related to a nutrient deficiency disease (like vitamin C and scurvy). These three types of claims are not preapproved by the FDA, but the manufacturer must have substantiation that the claim is truthful and not misleading and must submit a notification with the text of the claim to the FDA no later than 30 days after marketing the dietary supplement with the claim. (US Department of Health and Human Services, 2013). There are no specific structure–function claims related in particular with olive oils.

Table 34.1 The requirements of each grade of olive oil.

Quality criteria	US extra virgin olive oil	US virgin olive oil	Lampante virgin olive oil[1]	US refined olive oil	US olive oil	US olive-pomace oil	US refined olive-pomace oil	US crude olive-pomace oil
a. Organoleptic characteristics	Excellent	Good	Poor	Acceptable	Good	Good	Acceptable	N/A
– Odor and flavor	Md = 0	0 < Md ≤2.5	Md >2.5[2]	N/A	N/A	N/A	N/A	N/A
– Odor and flavor (on a continuous scale):	Mf >0	Mf >0	N/A	N/A	N/A	N/A	N/A	N/A
● Median of defect (Md) ● Median of the fruity (Mf) ● Color	Yellow to green	Yellow to green	Yellow to green	Light yellow	Light yellow to green	Light yellow to green	Light yellow to brownish yellow	Dark green, brown, or black
b. Free fatty acid content, and m/m expressed as oleic acid	≤0.8	≤2.0	>2.0	≤0.3	≤1.0	≤1.0	≤0.3	No limit
c. Peroxide value, in milleq. peroxide oxygen per kg/oil	≤20	≤20	No limit	≤5	≤15	≤15	≤5	No limit
d. Absorbency in ultraviolet (UV) (K% 1 cm)								
– 270 nm	≤0.22	≤0.25	N/A	≤1.10	≤0.90	≤1.70	≤2.00	N/A
– ΔK	≤0.01	≤0.01	N/A	≤0.16	≤0.15	≤0.18	≤0.20	N/A
– 232 nm	≤2.50[6]	≤2.60[6]	N/A	N/A	N/A	N/A	N/A	N/A
Purity Criteria								
e. Fatty acid composition as determined by gas chromatography (% m/m methyl esters)	– Arachidic acid (C20:0) ≤0.6 – Behenic acid (C22:0) ≤0.2[3] – Gadoleic acid (eicosenoic) (C20:1) ≤0.4 – Heptadecanoic acid (C17:0) ≤0.3 – Heptadecenoic acid (C17:1) ≤0.2 – Linoleic acid (C18:2) 3.5–21.0				– Linoleic acid (C18:3) ≤1.5[4] – Myristic acid (C14:0) ≤0.05 – Oleic acid (C18:1) 55.0–83.0 – Palmitoleic acid (C16:1) 0.3–3.5 – Palmitic acid (C16:0) 7.5–20.0 – Stearic acid (C18:0) 0.5–5.0			

f. Trans fatty acid (T) content (%) C18:1 T[5]	≤0.05	≤0.05	≤0.10	≤0.20	≤0.20	≤0.40	≤0.40	≤0.20
g. Trans fatty acid content (%) C18:2 T + C18:3 T	≤0.05	≤0.05	≤0.10	≤0.30	≤0.30	≤0.35	≤0.30	≤0.10
h. Desmethylsterol composition (% total sterol)		– Brassicasterol ≤0.1[7] – Campesterol ≤4.5[8] – Cholesterol ≤0.5 – Delta – 7 stigmasterol ≤0.5 – Stigmasterol < campesterol in edible oils Clerosterol + sitostanol + beta-sitosterol + Delta 5-24-stigmastadienol + Delta-5-23-stigmastadienol + Delta-5-avenasterol ≥93.0						
i. Total sterol content (mg/kg)	≥1000	≥1000	≥1000	≥1000	≥1000	≥1600	≥1800	≥2500
j. Stigmastadiene content (mg/kg)	≤0.15	≤0.15	≤0.50	N/A	N/A	N/A	N/A	N/A

1. *For lampante oil only*: the criteria in (a), (b), and (c) are not required to be concurrent; one is sufficient.
2. Or when the median of the defect attribute is ≤2.5 and the median of the fruity attribute is equal to 0.
3. Limit raised to ≤0.3 for olive-pomace oils.
4. Linoleic acid values between 1.0 and 1.5 percent would be subject to further testing.
5. Fatty acid with 18 carbon atoms (C) and one *trans* isomer (T).
6. Commercial partners in the country of retail sale may require compliance.
7. Limit raised to ≤0.2 for olive-pomace oils.
8. Campesterol values between 4.0 and 4.5 would be subject to further testing.

Source: US Department of Agriculture (2010).

34.3.1.2 Claims in Canada

The situation regarding the different grades of olive oils in Canada is similar to the one in the USA. The only important difference is that Canada tolerates a wider use of different nutrition claims. For example, the term "light" may be tolerated in an olive oil as it is considered to refer to the taste or color of the product and certainly not to the fat content (North American Olive Oil Association, 2001).

34.3.2 Australia and New Zealand

Australia is following in general the standards of the IOC as these have been incorporated into "Australian Standard AS5264-2011." The problem is that AS5264-2011 is a voluntary standard developed by Standards Australia, and as such it is not compulsory for companies to follow (Australian Olive Association, 2013). Regarding nutrition and health claims, Australia has adopted a new standard, and companies should comply from January 18, 2016, onward. According to the new standard, nutrition content claims are claims about the content of certain nutrients or substances in a food, such as "low in fat" or "good source of calcium." These claims will need to meet certain criteria set out in the standard. For example, with a "good source of calcium" claim, the food will need to contain more than the amount of calcium specified in the standard. "Health claims" refer to a relationship between a certain food and health rather than a statement of content. There are two types of health claims:

- General-level health claims refer to a nutrient or substance in a food and its effect on a health function. They must not refer to a serious disease or to a biomarker of a serious disease. For example, "Calcium is good for bones and teeth."
- High-level health claims refer to a nutrient or substance in a food and its relationship to a serious disease or to a biomarker of a serious disease. For example, "Diets high in calcium may reduce the risk of osteoporosis in people 65 years and over." An example of a biomarker health claim is "Phytosterols may reduce blood cholesterol."

Food businesses wanting to make general-level health claims will be able to base their claims on one of the more than 200 preapproved food–health relationships in the standard or self-substantiate a food–health relationship in accordance with detailed requirements set out in the standard. High-level health claims must be based on a food–health relationship preapproved by Food Standards Australia New Zealand (FSANZ). There are currently 13 preapproved food–health relationships for high-level health claims listed in the standard. All health claims are required to be supported by scientific evidence to the same degree of certainty, whether they are preapproved by FSANZ or self-substantiated by food businesses. Food–health relationships derived from health claims approved in the European Union, Canada, and the USA have been considered for inclusion in the standard (FSANZ, 2015). Of the approved 13 high-level health claims, none is relevant to olive oils (Australian Government ComLaw, 2014).

There are many different legislative approaches by different countries. Due to the globalization of trade, most other countries are trying to follow the Codex Alimentarius standards, or, depending on their main trade interests, they follow either the US legislation (e.g., Canada) or the EU legislation (EU candidates, pre-accession countries, and European Neighbourhood countries).

34.4 The European Union perspective

The European Union is one of the members of the IOC, and as such the European legislation is following the standards and suggestions of the Council. The appropriate descriptions and definitions of olive oil and olive pomace oils are described in Annex XVI and Article 118 of Council Regulation (EC) No. 1234/2007 (European Union, 2007).

The quality criteria and the analysis process applicable in order to determine the name and description of olive oils and residues are described in Annex I of Commission Regulation (EEC) No. 2568/1991 as it has

been amended by Commission Implementing Regulation (EU) No. 1348/2013, and they include: acidity, peroxide value, ultraviolet absorbance, and sensory evaluation (European Union, 2015). These methods are described in other chapters of this volume.

Further information regarding the marketing standards for olive oils is described in Article 3 of the Commission Implementing Regulation (EU) No. 29/2012 (as it has been amended by regulation No. 1335/2013) and Council Regulation (EC) No. 865/2004. The regulation (EC) No. 1019/2012 has been repealed.

34.4.1 Packaging

Olive oil intended for sale to the final consumer must be sold in containers of maximum capacity of 5 L with a closing system where the seal is broken after the first use. However, in the case of oils intended for consumption in restaurants, hospitals, canteens, and other similar collective establishments, EU Member States may set a maximum capacity over 5 L for packaging depending on the type of establishment concerned.

34.4.2 Labeling

34.4.2.1 Indications on the label

For mandatory indications, the labeling of these products must include the name and descriptions of the various categories of olive oils and olive-pomace oils:

- Extra virgin oil
- Virgin olive oil
- Olive oil composed of refined olive oils and virgin olive oils
- Olive-pomace oil.

In addition, but not necessarily close to it, the labeling should bear the following information on the category of oil:

- *Extra virgin olive oil*: "superior category olive oil obtained directly from olives and solely by mechanical means";
- *Virgin olive oil*: "olive oil obtained directly from olives and solely by mechanical means";
- *Olive oil composed of refined olive oils and virgin olive oils*: "oil comprising exclusively olive oils that have undergone refining and oils obtained directly from olives"; and
- *Olive-pomace oil*: "oil comprising exclusively oils obtained by treating the product obtained after the extraction of olive oil and oils obtained directly from olives", or "oil comprising exclusively oils obtained by processing olive pomace oil and oils obtained directly from olives."

Optional indications related to quality standards may appear on the labeling of olive oils:

- *The indication "first cold pressing"*: This may be used only for virgin or extra virgin olive oils obtained at a temperature below 27 °C by means of an extraction system using hydraulic presses.
- *The indication "cold extraction"*: This may be used only for virgin or extra virgin olive oils obtained at a temperature below 27 °C by percolation or centrifugation of the olive paste. According to some experts, however, the proper term to be established should be "cold processing" (A.K. Kiritsakis, personal communication).
- *Indications of organoleptic properties*: Indications referring to taste and/or smell may appear only for extra virgin and virgin olive oils; the terms referred to in Annex XII to Commission Regulation (EEC) No. 2568/91 may appear only if they are based on the results of a method of analysis stated in Commission Regulation (EEC) No. 2568/91 (organoleptic assessment).
- *Indication of the acidity or maximum acidity*: This may appear only when it is accompanied by an indication, in lettering of the same size and in the same visual field, of the peroxide value, the wax

content, and the ultraviolet absorption, determined in accordance with the methods of analysis stated in Commission Regulation (EEC) No. 2568/91.

34.4.3 Blends of olive oil

The percentage of olive oil must be declared for blends of olive oil with other vegetable oils, when the presence of olive oil is highlighted on the labeling elsewhere than in the list of ingredients using words, images, or graphics. Regulation prohibits highlighting the presence of olive oil on the label by images or graphics unless olive oil represents a minimum of 50% of the blend concerned. EU Member States may not prohibit the marketing on their territory of blends of olive oil and other vegetable oils coming from other countries, and they may not prohibit the production on their territory of such blends for marketing in another Member State or for exportation. With the exception of tuna in olive oil and sardines in olive oil, where the presence of olive oils is highlighted on the labeling elsewhere than in the list of ingredients, using words, images, or graphics, the blend concerned must bear the following trade description: "Blend of vegetable oils (or the specific names of the vegetable oils concerned) and olive oil," directly followed by the percentage of olive oil in the blend.

The percentage of added olive oil relative to the total net weight of the foodstuff may be replaced by the percentage of added olive oil relative to the total weight of fats, adding the words "percentage of fats."

34.4.4 Designation of origin

Only extra virgin olive oil and virgin olive oil shall bear a designation of origin on the labeling. Therefore, olive oil composed of refined olive oils and virgin olive oils and olive-pomace oil shall not bear such designation. The "designation of origin" shall appear on the packaging or on the label attached to the packaging.

For virgin and extra virgin olive oils obtained from olive fruits processed in a Member State or a third country where the olives were harvested, the designation of origin shall contain the following wording: "(extra) virgin olive oil obtained in (the Union or the name of the Member State concerned) from olives harvested in (the Union or the name of the Member State or third country concerned)" (European Commission, 2013).

34.5 Nutrition and health claims related to olive oils

Food nutritional and health claims in the European Union have to be in compliance with Regulation (EC) No. 1924/2006 and are included in the list of Commission Regulation No. 432/2012. Especially for health, the food business operator should make an application to the European Food Safety Authority (EFSA), including all the supportive scientific evidence for the claims; the EFSA issues an opinion, and finally the commission will authorize or not authorize the proposed claim (European Union, 2006). All the information regarding approved and rejected nutrition and health claims made on food can be found in the relevant EU Register (European Commission, 2015). Regarding olives and olive oils, 16 claims have been evaluated and only one authorized. Table 34.2 shows the nutrition and health claims regarding olives and olive oils.

The only authorized claim is related to olive oil polyphenols in relation with oxidative stress, and the exact claim is "Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress." The claim may be used only for olive oils that contain at least 5 mg of hydroxytyrosol and its derivatives (e.g., oleuropain complex and tyrosol) per 20 g of olive oil. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 20 g of olive oil.

Be aware that for the claims that have been "not authorized," it doesn't mean that they are permanently rejected. It only means that the applicant didn't submit enough scientific evidence to establish a clear cause–effect relationship between the food or ingredient and the suggested health function. In the future, the same or another company may apply for the same or a similar claim with additional scientific data, and at that time the claim may be authorized.

Table 34.2 Nutrition and health claims regarding olives and olive oils.

Claim type	Nutrient, substance, food, or food category	Claim	Conditions of use of the claim, restrictions of use, and/or reasons for non-authorization	Health relationship	EFSA opinion reference	Commission regulation	Status	Entry ID
Art.13(1)	Huile d'olive	"régule le niveau de sucre dans le sang"	Noncompliance with the regulation because, on the basis of the scientific evidence assessed, this claimed effect for this food has not been substantiated.	Maintenance of normal blood glucose concentrations	2011;9(4):2044		Non-authorized	4244
Art.13(1)	Polyphenols (general and from grape, olive, and cacao in particular)	Polyphenols contained in this product: – ensure antioxidant action – help prevent tissue oxidation – helps guard against oxidation caused by free radicals – have an antioxidant effect – help mop up free radicals in cells/antioxidants.	Noncompliance with the regulation because, on the basis of the scientific evidence assessed, this food is not sufficiently characterized for a scientific assessment of this claimed effect, and the claim could not therefore be substantiated.	Not validated	2011;9(4):2082		Non-authorized	1637
Art.13(1)	Olive oil and/or olive pomace oil.	1. Olive oil consumption helps to maintain the health of the cardiovascular system.	Noncompliance with the regulation because, on the basis of the scientific evidence assessed, this claimed effect for this food has not been substantiated.	Maintenance of normal (fasting) blood concentrations of triglycerides	2011;9(4):2044		Non-authorized	1316
Art.13(1)	Olive oil and/or olive pomace oil.	1. Olive oil consumption helps to maintain the health of the cardiovascular system.	Noncompliance with the regulation because, on the basis of the scientific evidence assessed, this claimed effect for this food has not been substantiated.	Maintenance of normal blood HDL-cholesterol concentrations	2011;9(4):2044		Non-authorized	1316

(*continued*)

Table 34.2 (*Continued*)

Claim type	Nutrient, substance, food, or food category	Claim	Conditions of use of the claim, restrictions of use, and/or reasons for non-authorization	Health relationship	EFSA opinion reference	Commission regulation	Status	Entry ID
Art.13(1)	Olive oil and/or olive pomace oil.	1. Olive oil consumption helps to maintain the health of the cardiovascular system	Noncompliance with the regulation because, on the basis of the scientific evidence assessed, this claimed effect for this food has not been substantiated.	Maintenance of normal blood LDL-cholesterol concentrations	2011;9(4):2044		Non-authorized	1316
Art.13(1)	Name of food product: Olive biophenols Description of food in terms of food legislation categories: Food supplement Was food on Irish market before 1 July 2007: No	A potent source of biophenols with strong antibacterial properties Olive biophenols are effective in combating bacterial infections.	Noncompliance with the regulation because, on the basis of the scientific evidence assessed, this claimed effect for this food has not been substantiated.	Decreasing potentially pathogenic intestinal microorganisms	2009;7(9):1245		Non-authorized	1877
Art.13(1)	Name of Food product: Olive Biophenols Description of food in terms of food legislation categories: Food supplement Was food on Irish market before 1 July 2007: No	Exact wording of claim as it appears on product: "A potent source of olive biophenols with anti-inflammatory properties" Examples of any alternative wording that may be used in relation to claim: "Olive biophenols can reduce inflammation related to osteoarthritis and rheumatoid arthritis" Is claim a picture: No	Noncompliance with the regulation because, on the basis of the scientific evidence assessed, this claimed effect attributes to this food the property of preventing, treating or curing a human disease, or refers to such properties that is prohibited for foods.	"Anti-inflammatory properties"	2011;9(4):2033		Non-authorized	1882

Art.13(1)	Name of Food product: Olive Biophenols Description of food in terms of food legislation categories: Food supplement Was food on Irish market before 1 July 2007: No	Exact wording of claim as it appears on product: "A potent source of antioxidant biophenols for strengthening and balancing of the immune system from free radicals" Examples of any alternative wording that may be used in relation to claim: "Olive biophenols are important for a balanced immune system." Antioxidant activity of olive biophenols for a healthy and balanced immune system Is claim a picture: No	Noncompliance with the regulation because, on the basis of the scientific evidence assessed, this claimed effect for this food has not been substantiated.	Protection of DNA, proteins, and lipids from oxidative damage	2010;8(2):1489	Non-authorized	1367
Art.13(1)	Name of food product: Olive biophenols Description of food in terms of food legislation categories: Food supplement Was food on Irish market before 1 July 2007: No	Exact wording of claim as it appears on product: "A potent source of olive biophenols that have anti-UV damage properties" Examples of any alternative wording that may be used in relation to claim: "Olive biophenols can help in repairing skin damage due to sun burn and UV rays" Is claim a picture: No	Noncompliance with the regulation because, on the basis of the scientific evidence assessed, this claimed effect for this food has not been substantiated.	Protection of DNA, proteins, and lipids from oxidative damage	2010;8(2):1489	Non-authorized	1878

(*continued*)

Table 34.2 (*Continued*)

Claim type	Nutrient, substance, food, or food category	Claim	Conditions of use of the claim, restrictions of use, and/or reasons for non-authorization	Health relationship	EFSA opinion reference	Commission regulation	Status	Entry ID
Art.13(1)	Omega 6 (linolenic acid from olive oil)	Nutrient for the skin	Noncompliance with the regulation because, on the basis of the scientific evidence assessed, this claimed effect for this food has not been substantiated.	Protection of the skin from UV-induced damage	2011;9(6):2235		Non-authorized	3659
Art.13(1)	Olive oil	Olive oil promotes your heart health	Noncompliance with the regulation because, on the basis of the scientific evidence assessed, this claimed effect for this food has not been substantiated.	Maintenance of normal (fasting) blood concentrations of triglycerides	2011;9(4):2044		Non-authorized	1332
Art.13(1)	Olive Oil	Olive oil promotes your heart health	Noncompliance with the regulation because, on the basis of the scientific evidence assessed, this claimed effect for this food has not been substantiated.	Maintenance of normal blood HDL-cholesterol concentrations	2011;9(4):2044		Non-authorized	1332
Art.13(1)	Olive Oil	Olive oil promotes your heart health	Noncompliance with the regulation because, on the basis of the scientific evidence assessed, this claimed effect for this food has not been substantiated.	Maintenance of normal blood LDL-cholesterol concentrations	2011;9(4):2044		Non-authorized	1332
Art.13(1)	Olive oil polyphenols	Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress	The claim may be used only for olive oil that contains at least 5 mg of hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) per 20 g of olive oil. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 20 g of olive oil.	Protection of LDL particles from oxidative damage	2011;9(4):2033	Commission Regulation (EU) 432/2012 of 16/05/2012	Authorized	1333, 1638, 1639, 1696, 2865

Art.13(1)	Polyphenols from olive (olive fruit, olive mild wastewaters, or olive oil)	Polyphenols are absorbed from olive oil in the intestine and metabolized there or in the liver, and have been shown to be able to bind LDL *in vivo*. They have demonstrated scavenging properties *in vitro* that ensure olive oil stability and explain their ability to protect LDL against oxidation. Contributes to good HDL cholesterol level. Polyphenols from olive have an antioxidant activity that may help protect LDL cholesterol and lipid oxidation.	Noncompliance with the regulation because, on the basis of the scientific evidence assessed, the evidence provided is insufficient to substantiate this claimed effect for this food.	Maintenance of normal blood HDL-cholesterol concentrations	012;10(8):2848, 2011;9(4):2033	Non-authorized (expiry of transitional period 02/01/2014) […]	1639
Art.13(1)	Beta-glucan + olive leaf extract	Supports the body's own defense mechanism/immunity Maintains natural defense mechanism/immunity Helps strengthen natural immunity	Noncompliance with the regulation because, on the basis of the scientific evidence assessed, this claimed effect for this food is not sufficiently defined to be able to be assessed and the claim could not therefore be substantiated.	"Immune function/ immune system"	2011;9(4):2061	Non-authorized	1793

34.6 Conclusion

Most of the countries are following in general the recommendations of Codex Alimentarius and the IOC, and, depending on who their main trade partners are, their food legislation is similar to that of the USA or that of the European Union.

Regarding the harmonization of the classification of olive oils, very important steps have been made, and most of the countries are using the same descriptions. The main problem continues to be with other labeling details and mostly with the nutrition and health claims related to olive oils. Even the presentation of standard labeling elements, like the nutritional label, has to follow different rules in the presentation and the information it contains in Europe and in the USA (European Food Information Council, 2013).

Especially for nutrition- and health-related claims, the main problem is that due to overuse of such claims in the past, both the industry and the related authorities have lost to a large degree the customers' trust. To regain this trust, a very thorough, objective, transparent, and independent procedure should exist for the authorization of such claims. The only such procedure is currently the one followed in the European Union. The EFSA is the independent scientific body evaluating the proposed claims, and by publishing its opinions the EFSA guarantees the objectivity of the authorization process, where the European Commission with the publishing of both authorized and non-authorized claims in a public access Internet database is contributing to the transparency. For this reason, the procedure for the approval of nutrition and health claims, as described in the Regulation (EC) No. 1924/2006 and included in the list of Commission Regulation No. 432/2012, is regarded today globally as "best practice" and is used as a reference by many other countries.

References

Australian Government ComLaw. (2014) Australia New Zealand Food Standards Code – Standard 1.2.7 – Nutrition, Health and Related Claims. Available from https://www.comlaw.gov.au/Details/F2014C01191

Australian Olive Association. (2013) Consumers are being conned. Why Australia needs to mandate the Australian Olive Oil Standard. Australia. Available from https://www.australianolives.com.au/assets/files/pdfs/aoa-forms

Codex Alimentarius. (1981, revisions 1987, 2013) Codex Standard for Table Olives (Codex Stan 66-1981).

Codex Alimentarius. (1981, revisions 1989, 2003, Amendment in 2009 and 2013) Codex Standard for Olive Oils and Olive Pomace Oils (Codex Stan 33-1981). Available from http://www.codexalimentarius.org/input/download/standards/88/CXS-033e.pdf

European Commission. (2013) Marketing standards for olive oil. Trade – Export Helpdesk, Brussels.

European Commission. (2015) EU register of nutrition and health claims made on foods. Available from http://ec.europa.eu/nuhclaims

European Council. (2004) Council Regulation (EC) No. 865/2004. Available from http://eur-lex.europa.eu/legal-content

European Food Information Council. (2013) Global update on nutrition labelling. EUFIC, Brussels.

European Union. (2006) Regulation (EC) No. 1924/2006 of the European Parliament and of the council on nutrition and health claims made on foods. EC, Brussels.

European Union. (2007) Council Regulation (EC) No. 1234/2007. Available from http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:299:0001:0149

European Union. (2015) Commission Regulation (EEC) 2568/91. EUR-Lex. Available from http://eur-lex.europa.eu/legal-content

Food Standards Australia New Zealand (FSANZ). (2015) Nutrition content claims and health claims. Available from http://www.foodstandards.gov.au/consumer/labelling/nutrition/pages/default.aspx.

Hawkes, C. (2004) Nutrition labels and health claims: the global regulatory environment. World Health Organization (WHO), Geneva. Available from http://whqlibdoc.who.int/publications

International Olive Council (IOC). (2005) International Agreement on Olive Oil and Table Olives, 2005. United Nations Conference on Trade and Development, Geneva, United Nations.

International Olive Council (IOC). (2015a) Current European Union members. Available from http://www.internationaloliveoil.org/estaticos/view/74-miembros-actuales-de-la-union

International Olive Council (IOC). (2015b) List of IOC members. Available from International Olive Council: http://www.internationaloliveoil.org/estaticos/view/103-list-of-ioc-members

International Olive Council (IOC). (2015c) Mission statement. Available from http://www.internationaloliveoil.org/estaticos/view/100-mission-statement

North American Olive Oil Association. (2001) Brief labeling guide for olive oil: Canadian regulations. North American Olive Oil Association, Neptune, NJ.
North American Olive Oil Association. (2006) Brief labeling guide for olive oil. North American Olive Oil Association, Neptune, NJ.
US Departmant of Agriculture. (2010, October 25) United States standards for grades of olive oil. US Departmant of Agriculture, Washington, DC.
US Department of Health and Human Services. (2013) Label claims for conventional foods and dietary supplements. US Food and Drug Administration, Washington, DC.
US Department of Health and Human Services. (2014) Summary of qualified health claims subject to enforcement discretion. US Food and Drug Administration, Washington, DC.
WHO/FAO. (2015) About Codex. Available from http://www.codexalimentarius.org/about-codex/en

35 The functional olive oil market: marketing prospects and opportunities

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35.1 Introduction

Olive oil, besides being an important element of the Mediterranean diet, constitutes a valuable agricultural crop and processed product offering significant income to farmers, processors, and traders (Owen *et al.*, 2000). Over the last decade, olive oil consumption has experienced a major growth since consumption increased in nonproducing countries, especially among consumers in the USA, Australia, and large parts of Asia (International Olive Council [IOC], n.d.; Santosa & Guinard, 2011). An increase in consumption led to a substantial increase in the worldwide production (Parras, 2013).

However, the increase in the global demand for olive oil has not been followed by an increase in profits for the involved farms and enterprises. Thus, a strategy to strengthen the companies' marketing positions by entering and exploring new markets and new products while maintaining their existing market shares could offer a solution to the problem. Thus, implementation of differentiation strategies offers the possibilities to create new products that can capture new market segments. Olive oil differentiation could aim at managing both the internal and external attributes of olive oil that are highly valued by consumers, like quality, health, natural character, geographical region, environmental aspect, brand, origin, and production method. Therefore, being aware of exact consumer preferences constitutes the first step in defining marketing strategies and related policy choices regarding product, price, communication, and distribution (Pagliuca & Scarpato, 2011).

This chapter aims to provide an overview of the olive oil market, including new trends and marketing strategies, and changes in supply chains and consumption patterns.

The first section introduces the topic and summarizes the content of the next units. The second section offers a general description of the olive oil market worldwide, including the evolution of global olive oil production and consumption, and the evolution of global imports and exports in olive oil, completed by the presentation of new attractive markets for olive oil. In the third section, origin-based certification systems, like protected designation of origin (PDO) and protected geographical indication (PGI), as well as the organic farming (OF) certification, are presented and analyzed through an overview and discussion of the influence of these certifications on olive oil's quality and the consumers' willingness to pay for olive oil with geographical indication (GI) labels. In the fourth section, a case study on consumers' knowledge and awareness of the quality certification systems for olive oil is presented, and the chapter finally concludes with the main points summarizing the proposed strategies.

35.2 The olive oil market

35.2.1 World production

Global olive oil production and consumption are mainly concentrated in countries surrounding the Mediterranean Basin, although trade and consumption nowadays include the whole world. The Mediterranean

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Table 35.1 Olive oil world production, marketing years 1990–1991, 2000–2001, 2010–2011, 2012–2013, 2013–2014, and 2014–2015 (1.000 tons).

Country	1990–1991	2000–2001	2010–2011	2011–2012	2012–2013	2013–2014[a]	2014–2015[b]
Spain	639.4	973.7	1.391,90	1,615.0	618.2	1,775.8	825.7
Italy	163.3	509.0	440.0	399.2	415.5	461.2	302.5
Greece	170.0	430.0	301.0	294.6	357.9	131.9	300.0
Other EU countries	21.0	27.8	76.2	86.4	70.1	107.7	104.0
Total EU	**993.7**	**1940.5**	**2209.10**	**2395.20**	**1461.7**	**2476.6**	**1532.2**
Tunisia	175.0	130.0	120.0	182.0	220.0	70.0	260.0
Turkey	80.0	175.0	160.0	191.0	195.0	190.0	190.0
Morocco	36.0	35.0	130.0	120.0	100.0	120.0	110.0
Chile	0.0	0.0	16.0	21.5	15.0	15.0	24.0
Australia	0.0	1.0	18.0	15.5	9.5	18.0	12.0
Argentina	8.0	4.0	20.0	32.0	17.0	30.0	6.0
USA	1.0	1.0	4.0	4.0	4.0	5.0	40.0
All others	159.0	279.5	397.9	306.3	386.3	345.5	254.5
Total	**1453.0**	**2565.50**	**2209.10**	**3321.0**	**2401.50**	**3270.0**	**2393.0**

[a]Provisional.
[b]Projected.
Source: IOC (2014).

climate, with its long, hot, dry summers and mild winters, provides ideal growing conditions for olive trees, which have been cultivated in the region for several millennia (Lynch & Rozema, 2013).

During the period from 1990–1991 to 2013–2014, the global production of olive oil doubled, surpassing 3 million tons (Table 35.1). The European Union (EU) is the major olive oil producer. Specifically, in 1990–1991, EU countries produced 68.4% of global olive oil production; in 2000–2001, the EU olive oil production amounted to 75.6% of global production, also remaining at the same levels in 2013–2014.

Among the EU producers, Spain, Italy, and Greece are the major suppliers, producing about 54, 20, and 20% of European olive oil production in 2014–2015, respectively (Table 35.1). Almost one-quarter of the global olive oil production was produced in non-EU countries, led by Tunisia, Turkey, and Morocco.

Non-Mediterranean countries, most notably Argentina, Chile, Australia, and the USA, accounted for a very small share of world production, although their share is growing rapidly (Table 35.1). These countries benefit from intensive and efficient production methods, counterseasonal production to supply the world's major markets in the Northern Hemisphere, and the ability of exporters to use previously established marketing channels for other products, such as wine, to get their olive oil into high-value gourmet food markets (Lynch & Rozema, 2013).

35.2.2 World consumption

Over the last 15 years, consumption grew steadily, mainly due to dietary changes and expansion of countries that consume olive oil (USA, Canada, Japan, and Australia). The world consumption of olive oil was 1.6 million tons in 1990–1991, almost doubled by 2013–2014 to 3.0 tons, and was estimated to be about 2.8 million tons in 2014–2015 (Table 35.2).

Historically, the EU was the main driver of global consumption growth since two-thirds of growth occurred in the EU (Table 35.2). Italy is the country with the highest consumption of olive oil, followed by Spain and Greece. The rise in EU consumption could be attributed to EU-financed promotion programs that highlighted the health and quality characteristics of olive oil, and the historical and cultural ties with the product as a food staple, leading to an increase in consumers as well as an increase in purchasing frequency by those who already consume it (Anania & Pupo D'Andrea, 2011). However, a downward trend in EU olive oil consumption was noticed from 2010–2011 until today, due to the decline in consumption in both Italy and

Table 35.2 Olive oil world consumption, marketing years 1990–1991, 2000–2001, 2010–2011, 2012–2013, 2013–2014, and 2014–2015 (1.000 tons).

Country	1990–1991	2000–2001	2010–2011	2011–2012	2012–2013	2013–2014[a]	2014–2015[b]
Spain	394.1	580.8	554.2	574.0	486.9	530.4	515.0
Italy	540.0	729.0	660.0	610.0	550.0	620.0	520.0
Greece	204.0	270.0	227.5	200.0	180.0	171.0	160.0
Portugal	27.0	60.5	82.0	78.0	74.0	74.0	74.0
France	28.0	92.0	112.8	112.0	113.1	94.7	99.5
Germany	10.3	36.3	58.8	61.0	60.5	59.8	58.7
Other EU countries	76.0	66.2	171.2	155.3	156.9	167.2	164.5
Total EU	**1214.6**	**1835.1**	**1866.5**	**1790.3**	**1621.4**	**1717.1**	**1591.7**
Turkey	55.0	72.5	131.0	150.0	150.0	160.0	130.0
Morocco	37.0	45.0	100.0	122.0	129.0	132.0	120.0
Canada	10.0	24.5	40.0	39.5	37.0	40.5	37.5
United States	88.0	194.5	275.0	300.0	287.0	301.5	290.0
Japan	4.0	30.0	35.5	43.0	51.0	54.0	51.0
Australia	13.5	31.0	44.0	40.0	37.0	44.0	37.0
All others	243.5	368.0	569.0	600.7	676.6	580.9	566.3
Total	**1665.5**	**2590.5**	**3061.0**	**3085.5**	**2989.0**	**3030.0**	**2823.5**

[a]Provisional.
[b]Projected.
Source: IOC (2014).

Greece. The persistence of economic weakness in these two countries seems to be the reason for a decrease in olive oil consumption.

On the other hand, an increase in olive oil consumption was noticed in non-Mediterranean countries. Specifically, the USA is the third largest country in olive oil consumption with a consumption increase of 49% between 2000–2001 and 2014–2015. Strong consumption growth is observed in other high-income countries, such as Canada, Japan, and Australia (Bauer, 2011). In most cases, these countries have been targeted, over the years, by EU-financed promotional programs aimed at informing consumers about olive oil quality and nutritional attributes. Most of these promotional campaigns seem to be effective, taking into consideration the growing demand and the rise in consumption in the olive oil sector (Anania & Pupo D'Andrea, 2011).

35.2.3 Global trade

Over the last 15 years, imports have followed an upward trend, mainly due to imports from non-EU countries (USA, Canada, and Japan). The USA is the leading olive oil importing country (Table 35.3). US olive imports grew significantly in the early 1990s, and Italy and Spain have been the major suppliers. However, in the last decade, the US has imported large quantities of olive oil from Tunisia, Morocco, Argentina, and Chile, reducing simultaneously olive oil imports from Italy.

Among the EU countries, Italy leads in imports mainly from EU countries. A significant share of the imported olive oil is blended and bottled in Italy for export, although a part could also be imported for domestic consumption. Italy imports olive oil mainly from Spain, Greece, Tunisia, and Portugal.

France and Germany import olive oil, mostly from Italy and Spain. The upward trend in the olive oil imports stems from increases in domestic consumption, fueled by consumers' awareness of the positive attributes of olive oil (Datamonitor, 2010).

According to the IOC database (n.d.), the countries bordering the Mediterranean Basin are the major exporters of olive oil. Spain and Italy are by far the leading exporting countries and are both accountable for about 90% of EU exports during 2013–2014 and about 68% of the global exports (Table 35.4). In particular,

Table 35.3 Olive oil global imports, marketing years 1990–1991, 2000–2001, 2010–2011, 2012–2013, 2013–2014, and 2014–2015 (1.000 tons).

Country	1990–1991	2000–2001	2010–2011	2011–2012	2012–2013	2013–2014[a]	2014–2015[b]
Spain	26.7	15.8	14.7	14.2	54.7	11.2	30.0
Italy	95.7	110.8	58.0	73.9	79.2	70.0	59.5
Greece	0.0	0.0	0.0	0.1	0.0	0.0	0.0
France	2.7	0.2	6.3	6.7	8.9	6.4	7.0
Germany	0.1	0.1	0.8	0.6	0.4	0.3	0.3
Other EU countries	0.3	0.2	2.5	1.1	10.0	4.1	10.7
Total EU	**125.5**	**127.1**	**82.3**	**96.6**	**153.2**	**92.0**	**107.5**
USA	90.0	200.0	275.0	300.0	288.0	302.5	290.0
Australia	13.5	30.0	32.0	31.5	28.5	28.0	28.0
Canada	10.0	25.5	40.0	39.5	37.0	40.5	37.5
Brazil	13.5	25.0	61.5	68.0	73.0	72.5	72.0
Russia	5.0	4.0	21.0	24.0	27.0	27.0	26.5
Japan	4.0	29.0	35.5	43.0	51.0	54.0	51.0
All others	48.5	76.4	157.2	166.4	195.3	177.5	173.5
Total	**310.0**	**517.0**	**704.5**	**769.0**	**853.0**	**794.0**	**786.0**

[a]Provisional.
[b]Projected.
Source: IOC (2014).

Spain carries out the largest volume of exports to the EU countries, mostly to Italy. Leading destinations for Italian exports are the USA, Germany, France, Canada, the United Kingdom, and China.

Tunisia prevails as an olive oil exporting country since it exports one-fifth of the domestic olive oil production. However, its exports dropped sharply during 2013–2014 because of a poor domestic production. The EU, and specifically Italy, can be characterized consistently as Tunisia's largest overseas market. Other major EU destinations are Spain and France. Also, Tunisia has expanded its exports to non-EU markets,

Table 35.4 Olive oil global exports, marketing years 1990–1991, 2000–2001, 2010–2011, 2012–2013, 2013–2014, and 2014–2015 (1.000 tons).

Country	1990–1991	2000–2001	2010–2011	2011–2012	2012–2013	2013–2014[a]	2014–2015[b]
Spain	65.8	88.3	196.2	248.0	197.6	310.0	225.0
Italy	66.5	173.0	223.5	233.2	217.6	245.0	245.0
Greece	6.0	10.0	13.0	15.5	18.0	3.7	11.6
Portugal	6.4	17.3	42.7	51.5	50.5	54.3	53.3
Other EU countries	1.4	2.4	5.9	7.0	7.7	7.9	6.8
Total EU	**146.1**	**291.0**	**481.3**	**555.5**	**491.4**	**620.9**	**541.7**
Tunisia	161.5	95.0	108.0	129.5	170.0	65.0	170.0
Turkey	10.0	92.0	12.0	20.0	92.0	35.0	35.0
Morocco	1.5	0.0	30.5	11.0	10.0	11.0	10.0
Chile	0.0	0.0	6.5	10.0	10.0	10.0	12.0
All others	17.9	24.0	57.2	80.5	77.1	83.1	72.8
Total	**337.0**	**502.0**	**695.5**	**803.0**	**843.0**	**817.5**	**832.5**

[a]Provisional.
[b]Projected.
Source: IOC (2014).

including the USA, China, and some Middle Eastern countries. Spain, Italy, and the USA constitute the main destination for Morocco's olive oil.

35.2.4 New markets for olive oil

Olive oil has been introduced to the dietary patterns of many countries whose foods traditionally have never been based on olive oil consumption. The USA, Canada, Brazil, Australia, Japan, China, and Russia can be characterized as emerging markets, since they continuously increase the imported quantity of olive oil to fulfill the growing demand (Niklis *et al.*, 2014).

It seems that generic promotional campaigns for the health benefits of olive oil persuade consumers to start consuming olive oil (Mili, 2006). Furthermore, consumers' awareness of the health benefits of olive oil and the basics of the Mediterranean diet helped to heighten consumers' interest for olive oil in developing countries (China and elsewhere in Asia) and in other countries like the USA and Russia.

Apart from the health benefits of olive oil, the increase in global olive oil consumption could be attributed to the rising living standards in the developing countries and to the opening of their economies to international markets. Thus, residents of developing countries have the economic power to incorporate new products in their diet, like olive oil, or to change their eating habits, choosing or trying alternative healthy diets.

China is one of the main developing countries where the economy is growing rapidly, simultaneously presenting an economy opening up to international markets. The size of China's population and the opportunity for its inhabitants to travel and adopt new trends make this country a market with huge potential for expanding olive oil consumption. Olive oil is increasingly appreciated by the Chinese due to its nutritional and health properties, and quality and brand are decisive factors to attract wealthy clientele. These consumers who know its virtues are not yet able to distinguish the different qualities of olive oil, requiring regulatory labeling that gives a clear indication of the quality, country, and designation of origin.

The Mediterranean countries Spain (supplying 40% of Chinese imports), Italy (30%), and Greece (20%), followed by Turkey, Tunisia, and France, are the leaders of the Chinese market. However, the Mediterranean countries will need more than a quality product if they want to enter the Chinese mass market, especially since Australia is becoming a serious competitor in the high-end market. In addition, substantial public relations will be necessary to raise awareness of the benefits of Mediterranean olive oil in China, to attract the attention of importers and arouse the curiosity of the Chinese looking for new products (Lazzeri, 2011).

In the USA, olive oil consumption is increasing due to rising interest in its health benefits. Extra virgin olive oil is recognized by many US consumers as the highest quality oil and as a result receives a price premium in the US market (Lynch & Rozema, 2013).

Broadly, two types of olive oil consumers exist in the US market: those who are aware of the product, with the most significant purchase criteria being product attributes; and those who are less aware of the product, with the most significant purchase criterion being price. Research shows that most US retail consumers do not distinguish the various grades of olive oil (Wang *et al.*, 2013), with some olive oil marketers reporting that at product-tasting events hosted at US retail outlets, many consumers are trying olive oil for the first time (Lynch & Rozema, 2013). Other research has suggested that some US consumers actually consider oil defects, such as rancidity and mustiness, to be positive taste attributes (Delgado & Guinard, 2011).

Overall, the US olive oil market can be characterized by a lack of consumer knowledge about the different qualities of olive oil, so that many US consumers who are unable to distinguish quality differences gravitate toward less costly oils, giving an advantage to large bottlers that sell low-cost imported products. At the same time, US consumers are generally unaware of the wide range of choices associated with olive oil as well as its proper use, since they use olive oil for finishing dishes rather than cooking (Lynch & Rozema, 2013).

The lack of consumer awareness suggests that there is considerable room for consumption growth in the extra virgin olive oil grade in the USA, if more consumers were better educated about olive oil quality differences, its health benefits, and its usage.

Last but not least, Russia is another emerging market for olive oil. Vegetables oils are the type of oil mainly consumed by Russian consumers so far, and this displays an opportunity to the olive oil producing countries to enter a new huge market (Niklis *et al.*, 2014).

35.3 The influence of certifications of origin and production methods in olive oil

In recent years, an increasing number of food products have been marketed in a way that shows where they come from. Regional products or origin-based products are local products based on a strong territorial identity and reputation, and/or typical products based on specific modes of production, and their quality, reputation, or any other characteristics are attributable essentially to their geographical origin (Van de Kop *et al.*, 2006).

An origin-based strategy aims at decreasing the asymmetry of information, preserving product reputation, inferring quality signals, strengthening relations between producers and consumers, adding value to farm produce, and preserving local knowledge and culture (Chaaban, 2012).

Because of the growing importance of regional products, the EU has created several certifications through which producers can protect products with regional identity from counterfeit products, ensuring simultaneously the quality of products based on their link with a particular territory. The PDO means that these products are produced, processed, and prepared within a specific geographical area using recognized knowhow. The PGI shows that the processing or preparation of these products occurs within a specific geographical area. The EU's Organic Farming (OF) certification refers to products produced according to the rules applied in organic farming, protecting organic products and organic producers, and simultaneously offering added value to their intrinsic and extrinsic ethical characteristics.

In the olive oil marketing literature, a number of studies attempted to assess consumers' evaluation of the above certifications (PDO, PGI, and OF). In particular, Krystallis and Ness (2005) investigated the influence of organic and PDO labels on consumer preferences for olive oil in Greece. The results indicated that younger individuals and those with higher educational and income levels attached great importance to both organic and PDO labels when purchasing high-quality olive oil. Van der Lans *et al.* (2001) conducted a study to test whether certified denominations of origin, such as the PDO label, are perceived by consumers as an indicator of the oil's quality. They found that consumers linked the PDO labels to quality, therefore affecting consumers' preferences indirectly through this perception.

Research has also showed that there are differences in the evaluation of olive oil attributes by the consumers in the same country. In particular, Scarpa and Del Giudice (2004) found that there is a bias in preferences toward local products, since they revealed that olive oil from the south of Italy is more frequently preferred in the south than in the north of Italy. The same results also emerged in the survey of Vita *et al.* (2013), as consumers of traditional production areas, such as the southern Italian regions, tended to identify their local olive oil as better, while consumers from areas not producing olive oil considered a high price as an indicator of quality. Findings also indicated that Italian consumers, in both producing and nonproducing areas, are positively influenced by a PDO certification since they valued it as an additional factor of perceived quality.

The evaluation and the willingness of the consumers to pay an additional premium in order to purchase PDO or PGI olive oil constituted the subject of several investigations. In particular, Santos and Ribeiro (2005), studying the influence of the region on consumers' willingness, found that consumers are willing to pay up to a 30% price premium for GI labels, such as the PDO label. This means that the region of origin can be used by consumers as a surrogate quality index when they are not able to distinguish products on the basis of the intrinsic quality. Furthermore, Menapace *et al.* (2011) studied whether consumers of extra virgin olive oil recognize and value the informational content of a variety of GI labels as well as the consumers' willingness to pay for GI olive oil in Canada. Their findings showed that Canadian consumers have a greater willingness to pay for GI-labeled than non-GI-labeled extra virgin olive oil, showing a stronger preference for olive oil with a GI label compared to olive oil without certification of origin. However, between these two certifications of origin, PDO and PGI, consumers valued extra virgin olive oil with a PDO label more than olive oil with a PGI label.

The consumers' preference for an olive oil with a PDO label over the PGI label also emerged in the survey of Aprile *et al.* (2012). Their research focused on the evaluation of consumers' preferences and willingness to pay for a set of labels certified by the EU. Derived results indicated that participants are willing to pay the highest premium price for a product with a PDO label, followed by a product with a PGI label. According to researchers, the preference for a PDO label over a PGI label might reflect consumers' attitudes that the

PDO label is a GI certification that strongly guarantees the production, processing, and preparation of the olive oil in a defined geographical area or region.

Concerning organic olive oil, research showed that the demand for organic olive oil is positively affected by the socioeconomic characteristics of consumers, such as food safety and environment (Tsakiridou *et al.*, 2006). However, studies on organic certifications in Italy (Aprile *et al.*, 2012; Vita *et al.*, 2013) indicated differentiation in consumer preference, as they recognize that the organic farming label indicates that the olive oil was produced according to the specific rules of organic production.

35.4 Case study: survey on consumption patterns, labeling, certification, and willingness to pay for olive oil

In this section, different olive oil consumption patterns are presented on the basis of a recent survey in a typical Mediterranean country (Greece). The survey attempts to provide an overview of consumers' quality awareness and their willingness to buy such products. Hence, the effects of food labels during the purchasing process, knowledge of the certification systems, and the importance of quality systems and information presented on olive oil labels are explored. Also, the willingness of the consumers to pay an additional premium to buy a certified olive oil such as organic olive oil, eco-label olive oil, PDO or PGI olive oil, locally produced olive oil, and extra virgin olive oil is also examined. A primary survey of 300 consumers was carried out in northern Greece. The collection of primary data was based on the questionnaire, which was drawn up according to the objectives of this research, and the main results are provided in this section.

35.4.1 Demographic characteristics and olive oil consumption

The descriptive analysis of the consumers who participated in this survey show that most of them are women (63.5%), highly educated (~50% university graduates) and with a middle-level income (56% annual income between 10.000 and 30.000€). The vast majority of consumers (almost 100%) interviewed stated that they consume olive oil, while 72.0% of the respondents are aware of the price variation in olive oil.

Consumers, generally, buy olive oil in various time intervals. In particular, 33.3% of the consumers interviewed buy olive oil every three months, 29% purchase olive oil every two months, whereas 20.3% buys olive oil once a month. The remaining respondents (17.4%) buy olive oil once or twice a year. Most of the consumers (~50%) purchase olive oil in packages larger than three liters, and the rest buy it in bottles of a liter or less.

The vast majority of the respondents (89.0%) stated that they pay attention to food labels. In particular, 32.9% of the consumers declared that the information on the labels somehow affects their decision to buy olive oil, while 26.2% of the respondents stated that the information on labels moderately affects their purchasing decisions. The percentage of the consumers who were quite affected and absolutely affected by the label information reached 20.8 and 8.4%, respectively. Only 11.7% of the respondents stated that the information on labels does not affect their decision.

Regarding the consumers' awareness about the food certification systems, the majority of respondents stated that they have some knowledge of certification systems. Specifically, 52.3% of the participants are aware of the HACCP certification system, 55.0% of the ISO22000 certification system, 74.3% of the ISO9001, and 80.3% of the organic product certification system.

Also, the rates of consumers' knowledge about the certification systems of GIs are quite high. In particular, 58.3% of the participants are aware of the certification of PGI, and 68.3% are aware of the certification of PDO. Furthermore, 70.3% of the respondents have knowledge about the eco-label certification system. Different results are presented for the certification system of EUREGAP, since only 25.7% of the consumers stated that they are aware of it.

According to the results, quality systems such as the organic production of olive oil, the certification of GIs, as well as the reference of "locally produced" are considered of high importance by the respondents during the olive oil purchase decision. Although the eco-label certification is considered important for 51.6% of the respondents, 36% declared that certification has a moderate influence during the purchase decision.

The results also indicate that consumers are aware of the meaning of the olive oil certification systems. Specifically, 58.5% of the respondents argue that in organic olive oil production, the use of pesticides and additives is prohibited, whereas 58.2% believe that organic olive oil is exposed to a lower risk of contamination because of this prohibition. The majority of respondents (60.8%) stated that the organic olive oil production process has a lower impact on the environment compared to other cultivation systems. Furthermore, the vast majority of the respondents (70.2%) believe that the nutritional value of organic olive oil is higher compared to noncertified olive oil because of the specifications followed by the producers during the production process.

Concerning GIs, the majority of participants (58.5%) considered that the PDO and PGI certifications constitute a strong indication about the origin of olives and olive oil. In addition, 56.2% of the respondents believe that eco-labels indicate that the production of this specific category of olive oil has no adverse impact on the environment. The percentage of consumers who do not express a specific opinion about the eco-label reached 36.1%, and a very small group (7.7%) disagree with the eco-label meaning

As regards extra virgin olive oil, 65.9% of the respondents agree that this category of olive oil is more beneficial for health compared to uncertified olive oil. Very few consumers (6.0%) do not accept this differentiation, while 28.1% do not express a specific opinion. Also, information that is listed on olive oil packaging like acidity, expiration date, and price seems to be considered the most important by the participants. On the other hand, the brand, packaging quality, and image of the olive oil have a moderate effect during the purchase decision.

35.4.2 Willingness to pay for certified olive oil

In this subsection, the results about consumers' willingness to pay an additional amount in order to buy organic olive oil, PDO or PGI olive oil, eco-label olive oil, locally produced olive oil, and extra virgin olive oil are presented. The results show that most of the consumers (60.2%) are willing to pay extra money in the case of purchasing organic olive oil, while for the rest of the certification systems, only 30.4 and 23.2% of the consumers are willing to pay extra money in order to obtain olive oil certified according to place of origin and eco-label, respectively. More precisely, 61.7% are willing to pay up to 10% extra on the average price for purchasing organic olive oil, and 30% choose to spend more than 10% on the average price. In the PDO/PGI and eco-label categories, 59.8 and 50%, respectively, of the participants are willing to pay up to 10% extra on the average price of standardized olive oil. Regarding the locally produced category of olive oil, almost 50% of the consumers chose to spend up to 10% extra in order to purchase olive oil produced in a specific area, and 23.2% are willing to pay 10 to 20% above the average price. In the extra virgin olive oil category, 50.8% of the consumers are willing to pay extra money, up to 10% above the average price, to buy it.

35.5 Promotional strategies

As in almost all food product companies, marketing plays an important role in the development of a successful enterprise, with the same holding true in the olive oil market, where there are many food producers in the market offering different qualities of their products as well as many markets worldwide. This leaves enough room for producers and traders to develop different plans of development and promotion for products. Thus, companies can target various objectives, including increasing their market share, growing sales, creating local customers, and strengthening their brand, among others.

Within this complicated system involving a huge number of domestic and international processors, retailers, and olive oil products, firms develop different strategies to conquer and maintain their market share. Those strategies are adjusted and formed according to the size of the firm and the targeted market. Large processors and traders (mainly Italian) can develop long-run strategies to lead and prevail in the market.

Large firms predict the production trends and intervene, buying the production at a lower price; and then when the price, due to poor production, is raised, they supply the product at a very competitive price. Also, consumers become familiar with the trademark of the monopolistic firm and insist on purchasing their products, offering a competitive advantage to the selling firm. In addition, huge firms can offer a broader spectrum of olive oil quality to retailers, satisfying all the income levels.

On the other hand, small and medium-sized enterprises do not have the economic power to create product distribution channels abroad in order to enhance their economic activities. Therefore, small and medium-sized enterprises either may implement a selective strategy focused on targeted market areas, or may opt for a differentiated strategy in order to achieve a wide geographical scope. In this context, e-commerce could open up new horizons by helping them to enter new markets at much lower operating costs, simultaneously minimizing the middleman's role. Furthermore, the e-commerce channel offers the opportunity to small and medium-sized enterprises to create virtual stores providing consumers with original and highly differentiated products that are often difficult to find at offline retail locations, simultaneously expanding the potential market from local to global.

When focusing on developing markets in new countries, even though several educational campaigns have targeted informing consumers about olive oil, the majority of consumers in those markets still do not know the differences between olive oil grades, and they are not aware of the health benefits and the appropriate culinary uses of olive oil. It is obvious, therefore, that informational campaigns should be intensified to supply consumers, both existing and potential, with clarifying information on the characteristics, designations, health benefits, and uses of different olive oils. Product certification could be a crucial aspect in the promotion process declaring that olive oil's quality and production process are certified by an accredited certification body, making the end product more attractive to consumers.

The survey, which took place in Greece, showed that consumers, even though they are aware of many certification systems for olive oil, are not willing to pay extra money in the majority of cases for certified olive oil. Consequently, enterprises that target supplying the market with top-range products (organic olive oil, varietal oils, flavored oils, etc.) and selling them at higher prices because of their specific attributes should guarantee that the product properties, the package design, and the labeling closely meet the specific needs of this consumer group, which is often sophisticated and very demanding.

Especially for the quality labels such as PGI and PDO, it is necessary to provide consumers with relevant, complete, and accurate information in an understandable form. Producers and enterprises of these products should communicate to consumers the sector's enormous experience in the cultivation of olive oil, as well as the quality and safety processes used to obtain the end product, enabling them to make more informed purchases. The aim, therefore, of the communication campaign should focus on building awareness and credibility of the labels, stimulating simultaneously consumers' interest in such products.

Last but not least, real taste experiences are fundamental. Consumers should have the possibility to taste the different categories of certified olive oil, and there should be explanations that mainly concentrate on the differences between these products and corresponding generic products.

Promotion strategies are developed by almost all involved companies, which adjust their marketing strategy according to the company's size and to the consumer groups that constitute the company's target.

35.5.1 Extended summary

Olive oil is one of the most important elements of the Mediterranean diet and a significant agricultural crop for the countries of southern Europe (Owen *et al.*, 2000). During the last decade, olive oil consumption has increased because of the rise in demand in nonproducing countries, like the USA, Australia, and the countries of Asia (Santosa & Guinard, 2011).

Global olive oil production is centered in countries surrounding the Mediterranean Basin, with Spain, Italy, and Greece being the major suppliers, whereas one-quarter of global olive oil production comes from non-EU countries led by Tunisia, Turkey, and Morocco.

The EU remains the main driver of the global consumption of olive oil, even though it presents a downward trend, mainly due to the decline of olive oil consumption in both Italy and Greece (Occhinegro, 2008). On the other hand, an important rise in olive oil consumption has occurred in the USA and in other high-income countries like Canada, Japan, and Australia (Bauer, 2011), because of the effective communicational campaigns aimed at informing consumers about olive oil quality and nutritional attributes (Anania & Pupo D'Andrea, 2011).

The USA is the leading olive oil importing country, with Italy and Spain recognized as the major suppliers in the early 1990s. Nowadays, their exports to the USA have fallen due to the emergence of Tunisia, Morocco, Argentina, and Chile as suppliers to the US market at the expense of Italy. Among the

Mediterranean importing countries, Italy leads in imports supplied mostly by Spain and Greece, followed by France and Germany.

The countries bordering the Mediterranean Basin are the major exporters of olive oil (IOC, n.d.). Spain and Italy hold, by far, the first rank in global exports, followed by Greece and Portugal. Outside the EU, Tunisia is by far the leading olive oil exporting country, while Moroccan exports have been growing strongly from a low base.

Nowadays, the USA, Canada, Brazil, Australia, Japan, China, and Russia can be characterized as emerging markets, since they continuously increase the imported quantity of olive oil to fulfill the growing demand (Niklis *et al.*, 2014). The promotional campaigns focused on health benefits of olive oil, the rise in living standards in the developing countries, and the opening of their economies to international markets can explain the increase in olive oil demand globally. However, there is considerable room for consumption growth in these markets, since research has suggested that consumers are not aware of olive oil's different quality grades, its health benefits, and the appropriate usage of it (Delgado & Guinard, 2011; Wang *et al.*, 2013; Niklis *et al.*, 2014).

Given that olive oil consumption worldwide presents an upward trend, enterprises that want to enter and explore new markets and new products, maintaining at the same time the existing market shares, can take advantage of differentiation strategies to create new products perceived as unique by different market segments. Aspects of differentiation in the olive oil sector aim at managing both internal and external attributes of olive oil that are highly valued by consumers, like quality, health, naturalness, territoriality, environmental respect, brand, origin, and production method. Thus, origin-based products (PDO and PGI) and products produced according to specific rules during the production process (OF products) constitute diversified products through which olive oil enterprises can expand their economic activities, increasing their profits.

In olive oil marketing literature, a number of studies attempted to assess consumers' evaluation of PDO, PGI, and OF certifications. Research results showed that consumers mainly linked the PDO label of olive oil with higher quality, and they are willing to pay a price premium in order to purchase it (Van der Lans *et al.*, 2001; Santos & Ribeiro, 2005; Vita *et al.*, 2013). According to Aprile *et al.* (2012), the preference for the PDO label over the PGI label might reflect consumers' attitude that the PDO label is a GI certification that strongly guarantees the production, processing, and preparation of the olive oil in a defined geographical area. Concerning the organic method of producing olive oil, recent research showed that consumers are positively influenced by an OF label, mainly due to the specific conditions that are followed during the production process (Aprile *et al.*, 2012; Vita *et al.*, 2013).

Knowledge of the certification systems presented in olive oil labeling, as well as the consumers' willingness to pay for purchasing certified olive oil, was the main research question in the survey in which 300 Greek consumers participated.

Results showed that the majority of the respondents are aware of the existing certification systems in the food industry. In the olive oil category, quality systems such as the organic production of olive oil, the certification of geographical indications, and the reference to "locally produced" are considered of high importance by the respondents, whereas olive oil acidity, expiration date, as well as price also influence consumer choices in packaged olive oil.

Regarding consumers' willingness to pay for certified olive oil, the results showed that they are willing to pay extra money in the case of purchasing organic olive oil. For the remaining certification systems for olive oil, the majority of consumers are not willing to pay extra money. In particular, rates for consumers who do not want to pay additional money are especially increased for the eco-label and PDO/PGI olive oil categories.

35.6 Conclusion

As in almost all food products, marketing plays an important role for successful enterprise development. The olive oil sector consists of large and small/medium-sized enterprises, which should follow different marketing strategies for efficient promotion of their products. Large firms, since they have the economic

power, can buy the production at a lower price, and when the price is higher, due to poor production, they can supply markets with olive oil at a very competitive price. On the other hand, small and medium-sized enterprises can take advantage of new technologies by creating a virtual store for selling their products, expanding their economic activities across a wide geographical scope and simultaneously minimizing the operation costs and the middleman's role.

Communication campaigns can constitute a useful tool to inform consumers in developing countries about the health benefits of olive oil, different quality grades, designations of origin, and uses, given that olive oil consumption in nonproducing countries presents an upward trend lately.

Furthermore, enterprises that focus on selling top-range products (organic olive oil, flavored olive oil, varietal olive oil, and origin-based olive oil) should persuade consumers about their high-quality products and safe production processes, and they should also sell these products with different packaging and labels, highlighting their quality superiority and their functional effects.

References

Anania, G., & Pupo D'Andrea, M.R. (2011) Olive oil in the Mediterranean: area, production, consumption, and trade. CIHEAM Watch Letter (16), 1–6.

Aprile, C.M., Caputo. V., & Nayga, R.M. (2012) Consumers' valuation of food quality labels: the case of the European geographic indication and organic farming labels. International Journal of Consumers Studies 36, 158–165.

Bauer, B. (2011) The U.S. import market: an inside view. Olivae 116, 26–31.

Chaaban, J. (2012, December) The efficiency of food labeling as a rural development policy: the case of olive oil in Lebanon. Research, Advocacy and Public Policy-Making, Working Paper Series No. 12. American University of Beirut, Beirut.

Datamonitor. (2010) Study on the promotion of consumption of olive oil and olives in the USA and Canada. Datamonitor Report, 20 January.

Delgado, C., & Guinard, J.X. (2011) How do consumer hedonic ratings for extra virgin olive oil relate to quality ratings by experts and descriptive analysis ratings? Food Quality and Preference 22(2), 213–225.

International Olive Council (IOC). (N.d.) [Database]. Available from http://www.internationalolive oil.org

Krystallis, A., & Ness, M. (2005) Consumer preferences for quality foods from a South European perspective: a conjoint implementation on Greek olive oil. International Food and Agribusiness Management Review 8, 62–91.

Lazzeri, Y. (2011) China: an emerging market with high potential. CIHEAM Watch Letter 16, 8–10.

Lynch, B., & Rozema, A. (2013) Olive oil: Conditions of competition between U.S and major foreign supplier industries. Investigation No. 332-537 USITC Publication 4419.

Menapace, L., Colson, G., Grebitus, C., & Facendola, M. (2011) Consumers' preferences for geographical origin labels: evidence from the Canadian olive oil market. European Review of Agricultural Economics 38, 193–212.

Mili, S. (2006) Olive oil marketing on non-traditional markets: prospects and strategies. New Medit, 5(1), 27–37.

Niklis, D., Baourakis, G., Boubaker, T., & Manthoulis, G. (2014) Trade and logistics: the case of the olive oil sector. Mediterra, 203–226.

Owen, R.W., Giacosa, A., Hull, W.E., Haubner, R., Würtele, G., Spiegelhalder, B., & Bartsch, H. (2000) Olive-oil consumption and health: the possible role of antioxidants. Lancet Oncology 1, 107–112.

Pagliuca, M.M., & Scarpato, D. (2011) Food quality, consumer perception and preferences: an analysis of olive oil. Electronic Journal of Applied Statistical Analysis 4(2), 215–226.

Parras, M. (2013) New global and local marketing strategies: creation of added value through differentiation and high quality products. In: Present and future of the Mediterranean olive sector, ed. N. Arcas, F.N. Arroyo López, J. Caballero, R. D'Andria, M. Fernández, R. Fernandez Escobar, A. Garrido A., J. López-Miranda, M. Msallem, M. Parras, L. Rallo, & R. Zanoli. 87–95.

Santos, F., & Ribeiro, C. (2005, August) Product attribute saliency and region of origin: some empirical evidence from Portugal. Paper presented at the 99th seminar of the European Association of Agricultural Economists (EAAE), Copenhagen.

Santosa, M., & Guinard, J.X. (2011) Means-end chains analysis of extra virgin olive oil purchase and consumption behavior, Food Quality and Preference 22, 304–316.

Scarpa, R., & Del Giudice, T. (2004) Market segmentation via mixed logit: extra virgin olive oil in urban Italy. Journal of Agricultural and Food Industrial Organization, 2, 1–18.

Tsakiridou E., Mattas K., & Tzimitra-Kalogianni I. (2006) The influence of consumer characteristics and attitudes on the demand for organic olive oil. Journal of International Food and Agribusiness Marketing 18(3–4).
Van de Kop, P., Sautier D., & Gerz A. (2006) Origin-based products: lessons for pro-poor market development. Bulletin 372. KIT, Amsterdam, The Netherlands, and CIRAD, Montpellier, France.
Van der Lans, I.A., van Ittersum, K., De Cicco, A., & Loseby, M. (2001) The role of the region of origin in consumer evaluation of food products. European Review of Agricultural Economics, 28, 451–477.
Vita, D.G., Amico, D.M., Via, L.G., & Caniglia, E. (2013) Quality perception of PDO extra virgin olive oil: which attributes most influence Italian consumers? Agricultural Economics Review 14(2), 46–58.
Wang, S., Moscatello, B., & Flynn, D. (2013) Survey: consumer attitudes on olive oil. University of California at Davis Olive Center, Davis, CA.

Future Research Needs

A multilevel research approach is recommended for improvement of the quality of olives and olive oil without adversely affecting their functional compounds. Starting at the production stage, the search should be continuous for olive tree cultivars best suited for each area and with the best functional compounds. It is desirable to consider the most recent genetic engineering techniques in order to attain better productivity and enhance the content of healthful compounds.

Olive fruit harvesting methods must be updated. As farm labor becomes increasingly expensive, mechanical means of fruit collection without damaging the tree and/or the olive fruit must, besides being efficient, be perfected. Improved ways of transporting and storing fruits prior to processing that would prevent oil quality deterioration and loss of functional activity should also be sought. Although significant progress has been made in designing efficient olive-processing equipment, there is always room for improvement. The use of enzymes facilitating the release of oil from the tissue, leaving more phenols in the oil, should be further explored. As natural antioxidants hold promise for prolonging the market shelf life of olive oil and increasing its functionality, additional work is needed on the application of these additives for preservation of olive oil and other fatty substances.

The utilization of by-products of the olive oil industry – olive leaves, olive pomace, and wastewater – should be further explored. Further work on functional compounds existing in olive oil and table olives is needed due to their important contributions to human health at different levels and with different mechanisms of action.

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