Mohammed Wasim Siddiqui
Jesus Fernando Ayala Zavala
Cheng-An (Andy) Hwang *Editors*

Postharvest Management Approaches for Maintaining Quality of Fresh Produce

Springer

Postharvest Management Approaches for Maintaining Quality of Fresh Produce

Mohammed Wasim Siddiqui
Jesus Fernando Ayala Zavala
Cheng-An (Andy) Hwang
Editors

Postharvest Management Approaches for Maintaining Quality of Fresh Produce

Springer

Editors
Mohammed Wasim Siddiqui
Food Science and Technology
Bihar Agricultural University
Sabour, Bhagalpur, Bihar, India

Jesus Fernando Ayala Zavala
Centro de Investigacion en Alimentacion
y Desarrollo, A.C.
Hermosillo, Sonora, Mexico

Cheng-An (Andy) Hwang
USDA-ARS
Wyndmoor, PA, USA

ISBN 978-3-319-23581-3 ISBN 978-3-319-23582-0 (eBook)
DOI 10.1007/978-3-319-23582-0

Library of Congress Control Number: 2015960447

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG Switzerland

To Our Beloved Parents
This Book
Is
Affectionately Dedicated

Preface

Maintaining the postharvest quality of fresh produce has long been a challenging task. In the past, several chemicals were used for postharvest treatment of fresh produce. These chemicals have been phased out, however, and replaced due to factors such as advances in technology a rise in health consciousness among consumers, and new environmental concerns. The safety and efficacy of postharvest treatments depends on the use of novel preservation technologies. The existing food laws have brought about several desirable changes in the logistics of postharvest handling and the value chain of fruits, vegetables, and other fresh produce. Synthetic pre- and post-storage treatment agents/molecules have been replaced by eco-friendly products. In the last couple of years, environmental and consumer friendly postharvest treatments have gained popularity across the globe.

The book "Postharvest Management Approaches for Maintaining Quality of Fresh Produce" is comprised of 12 chapters written by eminent experts of the developing and developed world. The book presents existing and novel management systems that are in use or have the potential to be used to maintain the postharvest quality of fresh produce in terms of microbiological safety, nutrition, and sensory quality.

Gaseous composition plays a vital role in the biochemical and physiological responses of fruits and vegetables, which in turn affect the postharvest quality. Chapter 1 discusses the importance of the changes in the respiration rate and its influence on the physiological and biochemical process, as well as its effect on the nutritional value and sensorial properties of fruits and vegetables. The endogenous signaling molecules play important roles in regulating/enhancing postharvest defense responses. These molecules delay the ripening process by inhibiting ethylene/CO_2 production and microbial infection, and maintain postharvest quality. Chapters 2–6 focus on the recent advances in exploring the roles of nitric oxide, hydrogen sulfide, salicylic acid, polyamines, and methyl jasmonate in regulating fruit senescence and microbial infection.

The antimicrobial power of plants and herb extracts has been recognized for centuries. In this volume, Chap. 7 includes a discussion of the use of essential oils as potential inhibitors of the quorum sensing mechanism, capable of controlling

bacterial spoilage and pathogenesis in food-related microorganisms. Endogenous plant growth regulators (PGR) are important regulators of many functions in plant development and physiology. Chapter 8 describes the major classes of PGR, including their nature, physiological functions, and horticultural practices. The chapter also covers their role in maintaining postharvest sensorial and nutritional quality and ripening or senescence processes. Chapter 9 discusses carbohydrates, focusing on their biological activity, in particular their antibacterial and antifungal activity, or their prebiotic effectiveness on postharvest quality preservation of fresh perishables. Chapter 10 explains the principle operation of each active packaging system and its effect on fresh produce quality and safety.

As an effective disinfectant, ozone can be employed in cold storage, washing systems, or process water sterilization. Chapter 11 discusses the generation, formation, properties, biocidal action, and phytotoxicity of ozone along with its mechanism of microbial inactivation. A chlorine-based solution has been one of the commonly used disinfectants for fresh produce, owing to its very potent oxidizing properties and cost-effectiveness. Chapter 12 discusses the characteristics and generation of ClO_2, the basis of its antimicrobial action, and its antimicrobial effects on the safety and quality of produce.

The editors are confident that this book will prove a standard reference work for the industries and researchers involved in postharvest management of fresh commodities. The editors would appreciate receiving new information and comments to assist in the future development of the next edition.

Bhagalpur, Bihar, India — Mohammed Wasim Siddiqui
Hermosillo, Sonora, Mexico — Jesus Fernando Ayala-Zavala
Wyndmoor, PA, USA — Cheng-An (Andy) Hwang

Acknowledgements

It is almost impossible to reveal the deepest sense of veneration to all without whose precious exhortation this book project could not have been completed. At the onset of the Acknowledgement, we ascribe all glory to the Gracious “Almighty God” from whom all blessings come. We would like to thank Him for His blessing to write this book.

With a profound and unfading sense of gratitude, we wish to express our sincere thanks to the Bihar Agricultural University, India, Centro de Investigacion en Alimentacion y Desarrollo, Mexico, and Agricultural Research Service, U.S. Department of Agriculture, USA for providing us with the opportunity and facilities to execute such an exciting project, and for supporting us in our research and other intellectual activities around the globe. We convey special thanks to our colleagues and other research team members for their support and encouragement, and for helping us to accomplish this venture. We would like to thank Ms. Susan Safren, Ms. Susan Westendorf, and Mr. Daniel Falatko of Springer Publishing for their continuous support throughout the duration of the project.

Our vocabulary remains insufficient to express our indebtedness to our beloved parents and family members for their infinitive love, cordial affection, incessant inspiration, and silent prayers to “God” for our well-being and confidence.

Contents

About the Editors

Mohammed Wasim Siddiqui, Ph.D. is an Assistant Professor and Scientist in the Department of Food Science and Post-Harvest Technology, Bihar Agricultural University, Sabour, India and author or co-author of 33 peer reviewed research articles, 26 book chapters, 2 manuals, and 18 conference papers. He has 10 edited and 1 authored books to his credit published by Elsevier, USA, CRC Press, USA, Springer, USA, & Apple Academic Press, USA. Dr. Siddiqui has established an international peer reviewed journal *Journal of Postharvest Technology*. He has been honoured to be the *Editor-in-Chief* of two book series entitled *Postharvest Biology and Technology* and *Innovations in Horticultural Science* being published from Apple Academic Press, New Jersey, USA. Dr. Siddiqui is a *Senior Acquisitions Editor* in Apple Academic Press, New Jersey, USA for Horticultural Science. He has been serving as an editorial board member and active reviewer of several international journals such as *LWT - Food Science and Technology* (Elsevier), *Food Science and Nutrition* (Wiley), *Acta Physiologiae Plantarum* (Springer), *Journal of Food Science and Technology* (Springer), *Indian Journal of Agricultural Science* (ICAR) etc.

Recently, Dr. Siddiqui is conferred with the Best Young Researcher Award – 2015 by GRABS Educational Trust, Chennai, India and the Young Scientist Award – 2015 by Venus International Foundation, Chennai, India. He was also a recipient of the Young Achiever Award – 2014 for the outstanding research work by the Society for Advancement of Human and Nature (SADHNA), Nauni, Himachal Pradesh, India, where he is an Honorary Board Member and Life Time Author. He has been an active member of organising committee of several national and international seminars/conferences/summits. He is one of key members in establishing the WORLD FOOD PRESERVATION CENTER (WFPC), LLC, USA. Presently, he is an active associate and supporter of WFPC, LLC, USA.

Dr. Siddiqui acquired B.Sc. (Agriculture) degree from Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, India. He received the M.Sc. (Horticulture) and Ph.D. (Horticulture) degrees from Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, India with specialization in the Postharvest Technology. He was awarded Maulana Azad National Fellowship Award from the University Grants Commission, New-Delhi, India. He is a member of 'Core Research Group' at the Bihar Agricultural University (BAU) where he is providing appropriate direction and assisting to sensitize priority of the research. He has received several grants from various funding agencies to carry out his research projects. Dr. Siddiqui has been associated to postharvest technology and processing aspects of horticultural crops. He is dynamically indulged in teaching (graduate and doctorate students) and research, and he has proved himself as an active scientist in the area of Postharvest Technology.

J. Fernando Ayala-Zavala, Ph.D. is a professor and research leader of the Laboratory of Emerging Technologies at CIAD in Hermosillo, Sonora, Mexico. He is the author of more than 59 peer-reviewed journal articles, 20 book chapters, and editor of several books and scientific journals; he has also participated in more than 35 international conferences. He is a Professor of Master and Doctoral studies, tutoring research thesis and imparting courses in topics related to postharvest of fruits and vegetables, minimal processing, bioactive compounds in plant tissues, functional foods, and science communication. Since the beginning of his career, Dr. Ayala-Zavala has been interested in the study and creation of basic and applied knowledge in the postharvest preservation of plant foods. Specifically, he has participated in projects related to the use of emerging technologies to ensure the safety and quality of horticultural products. His research interests include the use of natural compounds, irradiation, active packaging, and edible films as preservation techniques. In addition, Dr. Ayala-Zavala has been working with countries such as Italy, Spain, France, USA, Brazil, Argentina, Uruguay, Chile, and Panama to share experiences on the aforementioned topics.

Cheng-An (Andy) Hwang, Ph.D. obtained his Ph.D. in Food Science and Technology from the University of Tennessee. He is a research scientist with the Residue Chemistry and Predictive Microbiology Research Unit at the United State Department of Agriculture, conducting research in modeling food formulation, processing, and storage conditions on the behavior of food borne pathogens in food products. Prior to joining ARS, he was a microbiologist with the Nestlé Product Technology Center. He is an author or coauthor of 44 peer-reviewed journal articles and 7 book chapters. He is associate editor for the food microbiology and safety section of the *Journal of Food Science* and served on the editorial board of the *Journal of Food Protection*.

Chapter 1
Oxygen, Carbon Dioxide, and Nitrogen

M. Ovando-Martínez, C.A. Ruiz-Pardo, A.E. Quirós-Sauceda, G.R. Velderrain-Rodríguez, G.A. González-Aguilar, and J.F. Ayala-Zavala

Abbreviations

MAP	Modified atmosphere packaging
O_2	Oxygen
CO_2	Carbon dioxide
N_2	Nitrogen
PVC	Polyvinyl chloride
PET	Polyethylene terephthalate
PP	Polypropylene
PE	Polyethylene
PPO	Polyphenol oxidase
TCA	Glycolysis tricarboxylic acid
PAL	Phenylalanine ammonia lyase
ACC	1-Aminocyclopropane-1-carboxylic acid
ACS	ACC synthase
ACO	ACC oxidase
MIV	Minimum inhibitory volume

M. Ovando-Martínez • C.A. Ruiz-Pardo • A.E. Quirós-Sauceda • G.R. Velderrain-Rodríguez
G.A. González-Aguilar (✉) • J.F. Ayala-Zavala
Centro de Investigacion en Alimentacion y Desarrollo, A.C. (CIAD, AC),
Carretera a la Victoria Km 0.6, La Victoria, Hermosillo, Sonora 83000, Mexico
e-mail: maribel.ovandom@gmail.com; cinthya.ruizpa@live.com; quirosanaelena@hotmail.com; tavo_velderrain@hotmail.com; gustavo@ciad.mx; jayala@ciad.mx

M.W. Siddiqui et al. (eds.), *Postharvest Management Approaches for Maintaining Quality of Fresh Produce*, DOI 10.1007/978-3-319-23582-0_1

Introduction

The use of emergent technologies as modified atmosphere packaging (MAP) keeps the nutritional value and prolongs the shelf-life of the postharvest fruits and vegetables. The main gases produced in MAP under environmental conditions are oxygen (O_2), carbon dioxide (CO_2), and nitrogen (N_2). All of them have an important role during the MAP conditions applied in food products. Under MAP, the O_2 of the surrounding environment is consumed and CO_2 is released. Therefore, the biochemical and physiological responses caused in fruits and vegetables by MAP are reduction of the respiration rate, inhibition of the ethylene production, and increase of the transpiration process. Such responses are reflected in positive or negative changes in the quality of the food products as sensorial and microbiological properties up to certain percentages of O_2 and CO_2. However, because of differences in the botanical source, biochemical responses, physiological conditions of the edible plant food, processing and storage conditions, among others, the MAP conditions should vary among plant tissues. Basically, the use of MAP is to preserve the quality of the plant food product until this is ready to use. Because of that, this chapter discusses the importance of the changes in the respiration rate and its influence on the physiological and biochemical process under different MAP conditions and their effect on the nutritional value and sensorial properties of fruits and vegetables.

Overview: Modified Atmospheres

Mostly, all fruits and vegetables after harvest need to be treated in order to keep their microbiological, sensorial, nutritional, and organoleptic quality, which start to decline as a result of changes in the plant metabolism (Sen et al. 2012). In this matter, technologies as modified atmosphere packaging (MAP) are used as a preservation technique that (1) bear physical and physiological changes; (2) enhance nutritional quality, and (3) extend shelf-life of fruits and vegetables (Sen et al. 2012; Beckles 2012). It has been reported that under MAP, an appropriate gaseous atmosphere around the product packaged is generated, nitrogen (N_2), oxygen (O_2), and carbon dioxide (CO_2) being the main produced gases. Nitrogen displaces oxygen and prevents package collapse and oxidation. On the other hand, O_2 is responsible for several undesirable reactions in foods, including oxidation and rancidity of fats and oils, rapid ripening and senescence of fruits and vegetables, color changes, and spoilage due to microbial growth. Due to all these negative effects, O_2 is generally avoided in the MAP of many products. However, its presence in small quantities is necessary for many fruits and vegetables in order to sustain their basic process of aerobic respiration. Finally, CO_2 has a bacteriostatic effect and slows down the respiration of many products. However, the optimum level of each gas must be determined in order to maximize the positive and minimize the negative effects in the final quality of the product (Han 2005). Therefore, in MAP technique, the

natural respiration process in conjunction with the controlled gas exchange through a polymeric package results in an improvement of the nutritional quality and shelf-life of fruits and vegetables (Rai and Paul 2007).

Among the used packaging materials in the MAP industry for fruits and vegetables, most polymeric packs are still elaborated from four basic polymers: polyvinyl chloride (PVC), polyethylene terephthalate (PET), polypropylene (PP), and polyethylene (PE) (Lee and Rahman 2014; Ahvenainen 2003; Wani et al. 2014). In spite of the wide variety of packaging materials, the main characteristics taken into account for MAP are gas permeability, water vapor transmission rate, mechanical properties, transparency, type of package, and sealing reliability (Jouki and Khazaei 2014). Furthermore, these packs might apply in the two categories in which MAP is classified: passive and active modification. Passive modification happens when product is sealed in a package made with a selected film, and a desired atmosphere develops naturally as a consequence of product respiration and diffusion of gases through the film. On the other hand, active modification is created by replacing gases in the package with a desired mixture of gases (Jiang et al. 2010). It utilizes low O_2 concentration, and high CO_2, conditions used to control the microbiological growth, decrease the respiration rate of the product, preserve its quality, and extend its shelf-life (Ye et al. 2012).

Notwithstanding the MAP benefits mentioned above, unwanted physiological responses may also appear due to atmospheric modifications caused by differences among plant species, organ type, and development stage (Eason et al. 2007). For example, in asparagus, O_2 levels between 1.5 and 2.5 kPa and CO_2 concentrations around 10–15 kPa are atmosphere conditions recommended (Li and Zhang 2015), whilst for lettuce the presence of CO_2 is not recommended (Horev et al. 2012). Minimal changes in the MAP conditions among products can generate undesirable responses such as fermentation, disagreeable flavors, reduction in aroma biosynthesis, and induction of tissue injury. Instead of that, the target of MAP is to reduce the respiration rate, oxidative tissue damage, or discoloration; slow down the chlorophyll degradation; and reduce the ethylene production, which decrease the ripening and senescence rate, and other ethylene-mediated processes (Beaudry 1999). Moreover, application of O_2, CO_2, and N_2 has shown some technological improvement in the product quality, as flavor in greenhouse peaches (Xi et al. 2014), sensory attributes and in vitro antioxidant activity in olives (Dourtoglou et al. 2006), firmness and polyphenol oxidase (PPO) activity in shiitake mushrooms (Ye et al. 2012), and antimicrobial activity against *Listeria innocua* in leafy salads (Scifò et al. 2009), all of them subjected to MAP.

Additionally, the use of MAP has been coupled with alternative methods of disinfection as ultraviolet light treatments and coatings applications in minimally processed fruit and vegetables (López-Rubira et al. 2005). In this context, the intentional mixture of chemical and physical disinfection treatments, and gas packaging methods, can control the microbial growth in vegetables and fruit, resulting in longer shelf-life (Chun and Song 2013). For this reason, the responses caused by the MAP and its beneficial effects on the nutritional and sensorial properties of fruits and vegetables should be reviewed.

Physiological and Biochemical Responses

The use of modified atmosphere (MA) conditions is an emergent technology that helps to improve the shelf-life of fruits and vegetables after postharvest (Fonseca et al. 2002; Costa et al. 2011). Under MA, the plant foods consume the oxygen (O_2, respiration rate) of the surrounding environment and release carbon dioxide (CO_2, transpiration rate). The variation of the gases contained in the atmosphere slows down the respiration and increases the transpiration, affecting positively/negatively the quality properties of the food products up to certain percentages of O_2 and CO_2 (Oms-Oliu et al. 2008). The gases concentration into the atmosphere varies among different botanical sources, physiological status of the raw product, processing, storage, and temperature (Fonseca et al. 2002; Oms-Oliu et al. 2008). Because of this, MA conditions are used in order to minimize respiration rate of plant foods products and therefore extend its quality and sensorial properties. Hence, it is important to discuss how changes in the respiration rate influence the physiological and biochemical process related with the quality properties of the fruits and vegetables (Finnegan et al. 2013).

The respiration rate is related with maturation and ripening process of fruits and vegetables; this is because higher respiration rates tend to show minor storage life than those with lower respiration rate (Bapat et al. 2010). The respiration process can be divided into glycolysis, tricarboxylic acid (TCA) cycle, and mitochondrial electron transport (Fernie et al. 2004). The type and maturity stage are internal factors that affect respiration rate. Fruits and vegetables have shown differences in their respiration rate, even among varieties of the same product (Fonseca et al. 2002). The nonclimacteric ones present higher respiration rate in the early stage of development, which steadily declines during maturation (Mathooko 1996; Czarny et al. 2006; Paul et al. 2012). In the case of climacteric vegetable products, they show a peak of respiration and ethylene production (Fonseca et al. 2002). The major differences between climacteric and nonclimacteric vegetable tissues are the presence or absence of ethylene production, related with the regulation of ripening at various levels (Bapat et al. 2010), development of color and flavor, and softening in texture (Trinchero et al. 1999). Ethylene biosynthesis and its effect on fruits and vegetables are influenced by the gas composition of the atmosphere surroundings.

Under MA, CO_2 acts as an inducer and as a suppressor of the respiration rate depending on its concentration, temperature, and exposure time of the product to MA conditions (Mathooko 1996). MA with high CO_2 concentrations elicits different physiological responses in fruits and vegetables, where the type and magnitude of these responses depend on the tissue exposed and its age (Mathooko 1996). Among such responses are changes in the glycolysis and the TCA cycle (accumulation of succinate by inhibition of succinate dehydrogenase), decrease in pH and ATP level (Mathooko 1996; Gorny and Kader 1996; Angós et al. 2008; Fonseca et al. 2002), as well as the reduction of phenylalanine ammonia lyase (PAL) activity and phenolic compound synthesis (Angós et al. 2008). Absence of O_2 in the air leads to the anaerobic respiration producing ethylene, acetaldehyde, and by consequence the plant food

deterioration (Han 2005; Caleb et al. 2012; Fonseca et al. 2002; Oms-Oliu et al. 2008). Therefore, under MA with high CO_2 concentration, ethylene production is inhibited by the repressing of 1-aminocyclopropane-1-carboxylic acid (ACC) synthesis and activities of ACC synthase (ACS) and/or ACC oxidase (ACO), according to the Yang cycle, whilst moderate CO_2 concentrations enhance ethylene accumulation (Somboonkaew and Terry 2011; Ketsa et al. 2013). Due to that, the selection of optimum MA conditions is important to understand the action mode of the O_2/CO_2 ratio on fruits and vegetables cell metabolism, nutritional value, and shelf-life (Fig. 1.1).

Research about the study of MA in the physiology of fruits and vegetables was reported. For example Ding et al. (2002) reported that loquat fruit (*Eriobotrya japonica* L. cv Mogi) stored under MA conditions retained loquat organic acid levels, and total sugars were not affected; in addition, they realized that storage temperatures are important because when loquat fruit was stored at 20 °C it showed severe decay compared to that stored at 5 °C, which presented higher quality and minimal physiological disorders (Fig. 1.2). On the other hand, Argenta et al. (2002) reported that the exposure during short-term to high CO_2 concentrations leads to the accumulation of succinate, acetaldehyde, methanol, and ethanol causing brown heart in "Fuji" apples. The formation of these anaerobic products is due to inhibition of pyruvate dehydrogenase, induction of alcohol dehydrogenase and pyruvate decarboxylase under to stress of concentrations of O_2/CO_2 (Argenta et al. 2002). Plus to the accumulation of succinate, the presence of high CO_2 concentration conditions leads to a controlled decay and keeps strawberry firmness, but a negative response is observed in the internal and flesh color, attributed to the rise in the pH due to the reduction of malic and citric acids in strawberry (Holcroft and Kader 1999).

In addition, it has been reported that high O_2 concentrations help to reduce the adverse effects of high CO_2 concentrations, e.g., regulating decay in lettuce and grapefruit. The recommended ratio of these gases is around 80–85 % O_2 in combination with 15–20 % CO_2, to improve the sensorial quality and the antimicrobial properties in a wide range of fresh-cut produce (Angós et al. 2008). Angós et al. (2008) used low O_2/high CO_2 and high O_2/high-low CO_2 conditions to demonstrate the effects of these conditions on the respiration rate and browning in minimally processed potatoes. They reported that high O_2/CO_2 conditions decreased the respiration rate in the fresh-cut potatoes stored at 4 °C (14 days) compared to those stored at O_2/high-low CO_2 conditions. Moreover, acidity significantly decreases as compared to fresh potatoes after storage using low O_2/high CO_2 and high O_2/high-low CO_2 conditions. Regarding the antibrowning activity, only the same gas combinations showed the best results. On the other hand, Deng et al. (2005) studied the effect of high O_2 conditions on firmness, cell wall constituents, and hydrolases activities of "Kyoho" grapes (*Vitis vinifera* L. labrusca L.). These authors reported that grapes stored under high O_2 (40 % O_2 + 30 % CO_2 or 80 % O_2) presented greater firmness than those stored at normal O_2 conditions (storage in air). Also, there were less water-soluble pectins, more Na_2CO_3-soluble pectins, and higher hemicelulloses that air stored fruits. Further, high O_2 concentrations affected dramatically the polygalacturonase and β-galactosidase, moderately cellulose, and very low the pectinesterase activities (Fig. 1.3). They concluded that under this condition, fruit's firmness can be improved,

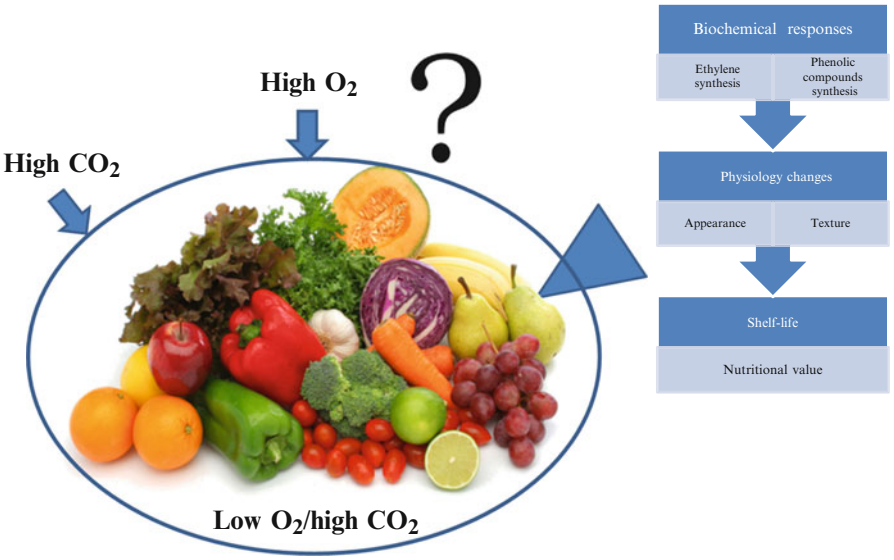


Fig. 1.1 Modified atmospheres and their effect on the shelf-life and nutritional value of fruits and vegetables

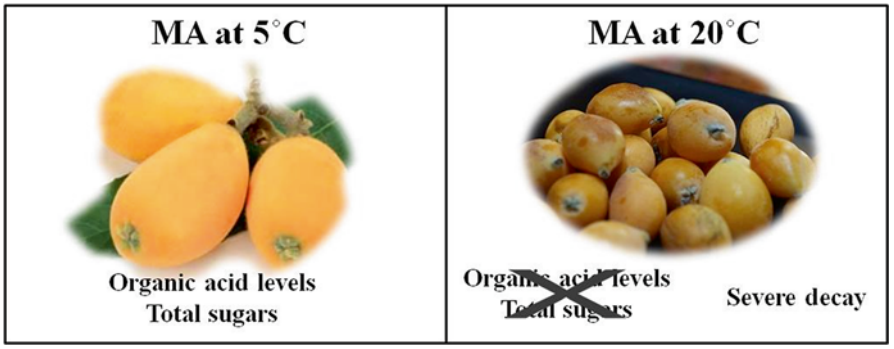


Fig. 1.2 Effect of temperature on fruit quality

and therefore its deterioration can be prolonged more. It is worth mentioning that both studies were done under controlled atmospheres.

Regarding the effect of the low O_2 and high CO_2 conditions on the bioactive compounds such as polyphenols, Holcroft and Kader (1999) reported that pH increment affects the color expression of anthocyanin pigments in strawberry under high CO_2. Also the authors suggest that elevated CO_2 atmospheres affect the pH differently among fruits with large and acidic vacuole than fruits with higher pH, or leafy tissues which do not accumulate high concentration of organic acids. Studies realized in sweet pomegranate reported a delayed in the total phenolic acids accumulation

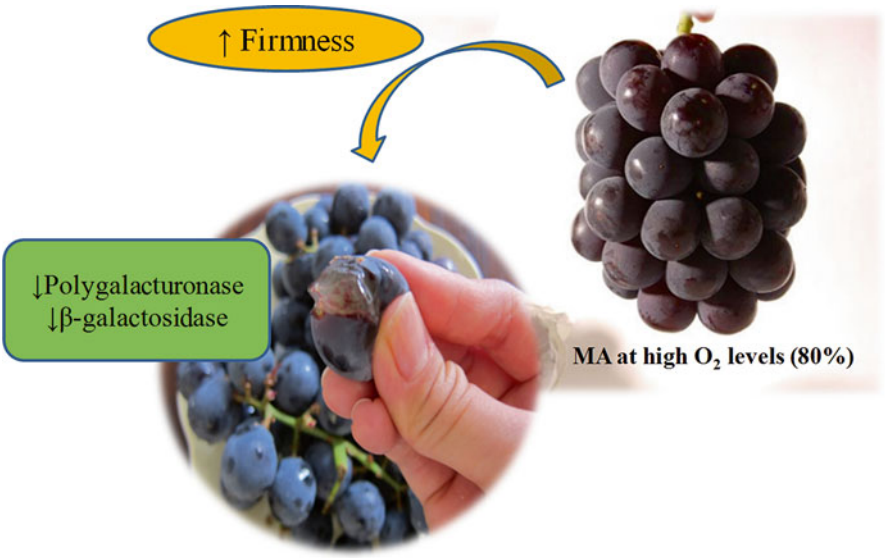


Fig. 1.3 Effect of high O_2 levels in MA on firmness quality

under high CO_2 concentrations and low temperature (Selcuk and Erkan 2014). According to Selcuk and Erkan (2014), this response could be due to the retarding ripening of the fruit with the MA conditions, which reduced the ethylene production, color change, among others, in addition to the PAL, chalcone synthase or anthocyanidin synthase, key enzymes in the phenolic compound biosynthesis, or probably to the reduced PPO or peroxidase activities, main enzymes responsible for the polyphenol degradation. On the other hand, Li et al. (2013) reported that high CO_2 atmospheres (2 % O_2 + 30 % CO_2) decreased the reactive oxygen species content and lipid peroxidation through the improvement in the antioxidant activity of *Pleurotus eryngii* (king oyster mushroom).

Fruit browning is the result of phenolic oxidation mediated by the PPO and PAL (promotes the catalytic conversion of L-phenylalanine into trans-cinnamic acid, which is transformed to phenols by different enzymatic reactions) in different botanical sources. However, their molecular mechanism and how it can be affected by MA storage are not well understood (Cheng et al. 2015). Recent studies made by Cheng et al. (2015) using MA with high CO_2 concentrations applied in "Yali" pear indicated that MA leads to changes in fruit browning, PPO activity, phenolic compound accumulation, and PPO and PAL gene expression, depending on the thickness of the package used. For example an MA packaging with 10 μm of thickness (MAP1) inhibited core browning of "Yali" pear through the retarded accumulation of phenolic acids and PPO activity, whilst MA packaging with 30 μm of thickness (MAP2) showed the opposed trend (Fig. 1.4). In addition, it was suggested that MAP1 downregulated and MAP2 upregulated the expression of PAL1, PAL2, and PbPPO1, genes correlated with changes in the fruit browning, phenolic compound content, and PPO activity.

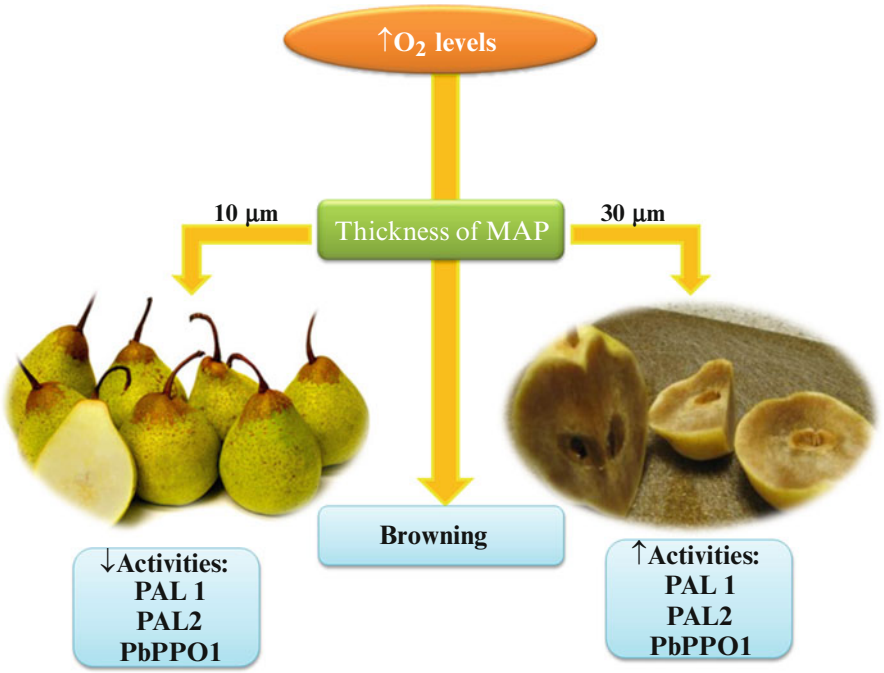


Fig. 1.4 Effect of MAP thickness on fruit browning

Shelf-Life

Even though the consumption of raw produces is nowadays popular, some food-borne pathogens have been isolated from commercially grown raw vegetables. Such products can become contaminated during harvesting, postharvest treatment, storage, or distribution steps (Mahmoud 2010). Thus, the preservation of fresh fruits and vegetables is a primary concern for both domestic demand and exportation in the food industry (Sawe et al. 2014). Although refrigeration is the most common preservation method for this type of food, this is not enough to destroy microorganisms, because this method simply retards the microbiological growth during storage. During the handling process of fresh fruits and vegetables before packaging, chemical treatment with chlorinated water is commonly adopted, this being the only step by which the number of pathogens and spoilage microorganisms can be reduced (Brilhante São José and Dantas Vanetti 2012). However, chlorate compounds can react with organic matter in these products and leading to the formation of trihalomethanes and haloacetic acids, both mutagenic and carcinogenic molecules. Therefore, among the postharvest technologies currently available for retention of overall fruit quality at low temperatures, MAP is a good choice, because mostly the gases used in this technology are not dangerous (Choi et al. 2015; Han 2005).

MAP in combination with a refrigeration method has been widely used to maintain the product safety and extend the shelf-life of fruits and vegetables conserved in polymeric packages (Abadias et al. 2012; Singh et al. 2014). CO_2 has been related with the inhibition of microbial growth, whereas a delay of quality in fresh fruits and vegetables is a consequence of low O_2 concentration (Horev et al. 2012). Both CO_2 and O_2 have long been used to reduce *Salmonella* outbreaks in raw whole and sliced tomatoes (Moreira et al. 2012; Niemira and Boyd 2013). In other study performed by Mukhopadhyay et al. (2014), it was observed that irradiated cherry tomatoes packaged under an active MAP (5.3 % CO_2 + 5.5 % O_2) showed a decrease on *Salmonella enterica serovar Typhimurium* population compared to the control without treatment. On the other hand, MAP has also been used against fungi contamination in fresh-cut products (Jouki and Khazaei 2014; Rivera et al. 2011; Taniwaki et al. 2001). In this regard, inhibitory activity of cinnamon and clove essential oil mixtures against important spoilage microorganism of foods with intermediate moisture content was tested. Essential oils were inoculated and sealed under a MA with low O_2 (<0.05–10 %) and high CO_2 (20 % or 40 %), both balanced with N_2. The minimum inhibitory volume (MIV) assay of the essential oil mixtures above 1000 μL resulted in the inhibition growth of *C. lipolytica* and *P. membranaefaciens*. In addition, at 2000 μL growth of *A. flavus, P. roqueforti*, *M. plumbeus*, *Eurotium* sp., *D. hansenii*, and *Z. rouxii* was inhibited. On the other hand, it was observed that the inhibition of *A. flavus* required the addition of 4000 μL. Then, the authors concluded that high ratios of cinnamon oil/clove oil inhibited the growth of *A. flavus* (Matan et al. 2006).

Furthermore, MAP application may extend storage life of fruits and vegetables by controlling respiration rate, senescence, and ripening (Finnegan et al. 2013), and by consequence increasing their organoleptic attributes (Díaz-Mula et al. 2011). Recent studies have shown the increase of chlorophyll retention (color) in broccoli (Büchert et al. 2011), better texture (softness and firmness) in carambola fruit (Ali et al. 2004), and good flavor (vitamin C) in green asparagus (Li and Zhang 2015), all of them treated under MAP storage conditions. Therefore, successful control of both respiration and perception by MAP can result in a fruit or vegetable product with high organoleptic quality. However, the control of these processes depends on the temperature. In this regard, the effect of the temperature on the quality of *Amaranthus cruentus* L. and *Solanum retroflexum* stored at 10 °C for 14 days under MAP was evaluated (Mampholo et al. 2015). The MAP was maintained at O_2 levels of 4.3 % and CO_2 levels of 7.3 % for *A. cruentus* leaves, whilst the MAP rate for *S. reflexum* was 5.6 % O_2 levels and 6.7 % CO_2. On the other hand, the color parameters L*, C increased and the h° decreased significantly with the increase in storage time. Therefore, changes in h° value with respect to the storage time for both samples tested were observed. Then, it was mentioned that changes in color properties of fresh products is a key factor on the consumers' acceptance. Due to this, food technologist might focus in the MA rates among the different plant tissues. In addition, the effect on the respiration rate by application of MA on shelf-life of green chilies packed at 8 ± 1 °C was studied. Among the results, it was found losses in weight, firmness, skin color, and ascorbic acid content in green chilies (Chitravathi et al. 2015). Hence, every fruit and vegetable will present differences in their storage life under MAP rate, as is shown in Table 1.1.

Table 1.1 Major changes in fresh products under MA application

Natural source	MA rate		Major changes	Temperature	References
	O_2	CO_2			
Pineapple 	50 %	50 %	Retarded the growth of aerobic microorganism and yeasts on pineapple Production of volatile metabolites	7 °C	Zhang et al. (2013)
Shiitake mushrooms 	100 %	0 %	Maintained nutritional compounds, color, and integrity	10 °C	Li et al. (2014)
Kohlrabi 	5 kPa	10–15 kPa	Improving of quality and retarding leaves' wilting	First 14 days at 0 °C, and then 3 days at 10 °C	Escalona et al. (2007)
Celery 	6 kPa	7 kPa	Improving of sensory quality, retaining of green color, and inhibition of microorganism growth	4 °C	Gómez and Artés (2005)
Fresh-cut papaya 	5 %	10 %	Sensory and quality characteristics retained. Extended the shelf-life	5 °C	Waghmare and Annapure (2013)
Fresh-cut tomato 	2 kPa	20 kPa	Juice accumulation, moisture condensation, water loss, and seed germination	5 °C	Gil et al. (2002)
Strawberries 	11–14 %	9–12 %	Extended shelf-life	5 °C	Nielsen and Leufvén (2008)
Mandarin 	19.8 %	1.2 %	Accumulation of acetaldehyde and ethanol	3 °C	Del-Valle et al. (2009)

Nutritional and Technological Value

Taking into account that MA is an emergent technology that does not imply the use of chemical reagents which affect the human health, the knowledge of the appropriate CO_2/O_2 ratio under MA can help to improve the quality properties and

nutritional value of different fruits and vegetables. However, it is important to know before apply such technology, the physiological characteristics of the fruit and vegetable in which the MA will be used. This is due to the existence of climacteric and nonclimacteric botanical sources, factor that affects the biochemical responses of the tissue under MA conditions, and by consequence plays a different role in the nutritional and technological value of fruits and vegetables treated with this technology.

According to the ripening process of fruits and vegetables, they can be divided in climacteric and nonclimacteric. The climacteric ones are associated with the increase of the respiration rate and ethylene production. By contrast, the nonclimacterics are characterized by the lack of ethylene-associated respiratory peak (Pech et al. 2008). It has been reported that ethylene plays an important role in the deterioration of fruits and vegetables, factor that affects their quality properties. Among those factors are the loss of the green color in immature fruits and vegetables, softening of them reducing their shelf-life, and changes in the flavor (Kader 1985). For example the ripening process of avocado as a climacteric fruit has been associated with the suppression of cellulase and polygalacturonase proteins, and the expression of the alcohol dehydrogenase, these related with the ripening rate and softening of this fruit under low O_2 concentrations (Kanellis et al. 1991). Mathooko (1996) described that MA with high CO_2 concentrations produces less effect on the respiration rate in nonclimacteric fruits and vegetables, because low levels of ethylene are produced compared to those climacteric ones, where the CO_2 delays or stops the ethylene synthesis.

A review in cantaloupe melons demonstrated that ethylene plays an important role in the regulation of ripening process and ripening rate, depending on the climacteric and nonclimacteric characteristics. However, the inhibition of ethylene production demonstrated that ethylene control many aspects of ripening, but not all of them. It has been reported that pulp color, sugar content increment, and loss of the acidity in nonclimacteric melons were observed, whereas yellowing of the rind, softening, aroma formation, and climacteric respirations were totally or partially attributed to climacteric melons (Pech et al. 2008). In essence, appearance and texture are two important parameters that determine the acceptability of fruit and vegetables. Furthermore, both of them are indicators of tissue deterioration because they can be used as a measure of freshness and quality in research and food industry (Toivonen and Brummell 2008). Therefore, the knowledge of the different biochemical responses of fruits and vegetables under different MA conditions depending on its ethylene dependence or ethylene nondependence will lead to elucidation of the cell wall hydrolysis mechanism and the metabolic pathways of different compounds (organic acids, carotenoids, anthocyanins, vitamin C, etc.) (Fig. 1.5). These kind of studies highlighted the interest in developing emergency technologies as MA which increase the nutritional value and sensorial properties and improve the technological applications through the regulation of fruit-specific processes such as ripening, knowing the gene expression under specific MA with a certain O_2/CO_2 concentrations.

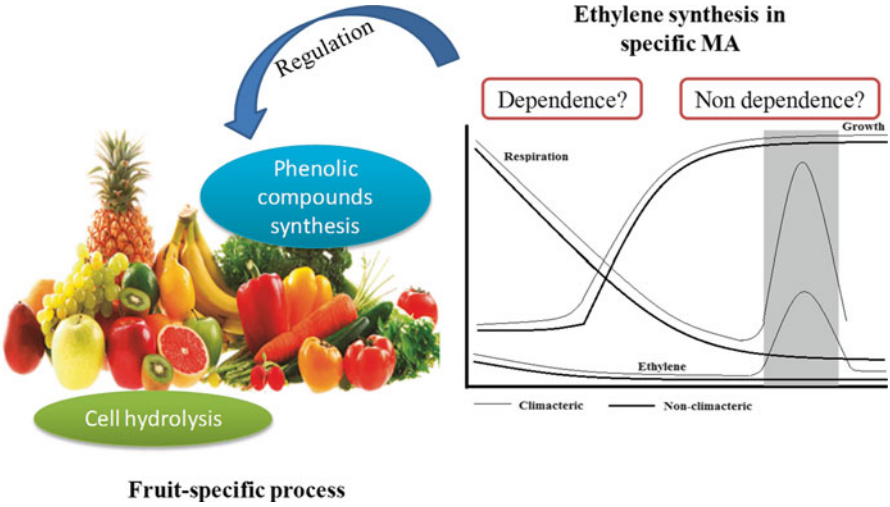


Figure 1.5 Regulation of fruit-specific processes when ethylene synthesis is controlled under specific MA conditions

Conclusions and Future Trends

- Biochemical and physiological responses of different plant species, organ type, and development stage caused by MAP need to be studied, in order to know how these affect the sensorial properties and nutritional quality of fresh produce.
- The knowledge of the different biochemical responses of fruits and vegetables under different MA conditions depending on its ethylene dependence or ethylene nondependence will lead to elucidation of the cell wall degrading mechanism and the metabolic pathways of different compounds involved in the quality of fresh produce.
- Gene expression studies of specific fresh produce submitted at certain MAP conditions are needed to regulate fresh produce-specific biochemical processes and improve specific physiological changes and increase the acceptability and/or nutritional value of the final product.

According with the information presented in this chapter, it is concluded that the use of MAP in plant foods preservation implies the knowledge of the physiological and biochemical functions, as well as the genetic expression of each vegetable tissue to know what levels of O_2, N_2, and CO_2 apply without producing negative responses in the end quality of the fresh product.

References

Abadias, M., Alegre, I., Oliveira, M., Altisent, R., & Viñas, I. (2012). Growth potential of Escherichia coli O157:H7 on fresh-cut fruits (melon and pineapple) and vegetables (carrot and escarole) stored under different conditions. *Food Control, 27*, 37–44.

Ahvenainen, R. (2003). 2-Active and intelligent packaging: An introduction. In R. Ahvenainen (Ed.), *Novel food packaging techniques*. Cambridge, UK: Woodhead Publishing.

Ali, Z. M., Chin, L.-H., Marimuthu, M., & Lazan, H. (2004). Low temperature storage and modified atmosphere packaging of carambola fruit and their effects on ripening related texture changes, wall modification and chilling injury symptoms. *Postharvest Biology and Technology, 33*, 181–192.

Angós, I., Vírseda, P., & Fernández, T. (2008). Control of respiration and color modification on minimally processed potatoes by means of low and high O_2/CO_2 atmospheres. *Postharvest Biology and Technology, 48*, 422–430.

Argenta, L. C., Fan, X., & Mattheis, J. P. (2002). Responses of 'Fuji' apples to short and long duration exposure to elevated CO_2 concentration. *Postharvest Biology and Technology, 24*, 13–24.

Bapat, V. A., Trivedi, P. K., Ghosh, A., Sane, V. A., Ganapathi, T. R., & Nath, P. (2010). Ripening of fleshy fruit: Molecular insight and the role of ethylene. *Biotechnology Advances, 28*, 94–107.

Beaudry, R. M. (1999). Effect of O_2 and CO_2 partial pressure on selected phenomena affecting fruit and vegetable quality. *Postharvest Biology and Technology, 15*, 293–303.

Beckles, D. M. (2012). Factors affecting the postharvest soluble solids and sugar content of tomato (Solanum lycopersicum L.) fruit. *Postharvest Biology and Technology, 63*, 129–140.

Brilhante São José, J. F., & Dantas Vanetti, M. C. (2012). Effect of ultrasound and commercial sanitizers in removing natural contaminants and Salmonella enterica Typhimurium on cherry tomatoes. *Food Control, 24*, 95–99.

Büchert, A. M., Civello, P. M., & Martínez, G. A. (2011). Effect of hot air, UV-C, white light and modified atmosphere treatments on expression of chlorophyll degrading genes in postharvest broccoli (Brassica oleracea L.) florets. *Scientia Horticulturae, 127*, 214–219.

Caleb, O. J., Mahajan, P. V., Opara, U. L., & Witthuhn, C. R. (2012). Modelling the respiration rates of pomegranate fruit and arils. *Postharvest Biology and Technology, 64*, 49–54.

Cheng, Y., Liu, L., Zhao, G., Shen, C., Yan, H., Guan, J., et al. (2015). The effects of modified atmosphere packaging on core browning and the expression patterns of PPO and PAL genes in 'Yali' pears during cold storage. *LWT - Food Science and Technology, 60*, 1243–1248.

Chitravathi, K., Chauhan, O., & Raju, P. (2015). Influence of modified atmosphere packaging on shelf-life of green chillies (Capsicum annuum L.). *Food Packaging and Shelf Life, 4*, 1–9.

Choi, D. S., Park, S. H., Choi, S. R., Kim, J. S., & Chun, H. H. (2015). The combined effects of ultraviolet-C irradiation and modified atmosphere packaging for inactivating Salmonella enterica serovar Typhimurium and extending the shelf life of cherry tomatoes during cold storage. *Food Packaging and Shelf Life, 3*, 19–30.

Chun, H. H., & Song, K. B. (2013). The combined effects of aqueous chlorine dioxide, fumaric acid, and ultraviolet-C with modified atmosphere packaging enriched in CO2 for inactivating preexisting microorganisms and Escherichia coli O157:H7 and Salmonella typhimurium inoculated on buckwheat sprouts. *Postharvest Biology and Technology, 86*, 118–124.

Costa, C., Lucera, A., Conte, A., Mastromatteo, M., Speranza, B., Antonacci, A., et al. (2011). Effects of passive and active modified atmosphere packaging conditions on ready-to-eat table grape. *Journal of Food Engineering, 102*, 115–121.

Czarny, J. C., Grichko, V. P., & Glick, B. R. (2006). Genetic modulation of ethylene biosynthesis and signaling in plants. *Biotechnology Advances, 24*, 410–419.

Del-Valle, V., Hernández-Muñoz, P., Catalá, R., & Gavara, R. (2009). Optimization of an equilibrium modified atmosphere packaging (EMAP) for minimally processed mandarin segments. *Journal of Food Engineering, 91*, 474–481.

Deng, Y., Wu, Y., & Li, Y. (2005). Changes in firmness, cell wall composition and cell wall hydrolases of grapes stored in high oxygen atmospheres. *Food Research International, 38*, 769–776.

Díaz-Mula, H. M., Martínez-Romero, D., Castillo, S., Serrano, M., & Valero, D. (2011). Modified atmosphere packaging of yellow and purple plum cultivars. 1. Effect on organoleptic quality. *Postharvest Biology and Technology, 61*, 103–109.

Ding, C.-K., Chachin, K., Ueda, Y., Imahori, Y., & Wang, C. Y. (2002). Modified atmosphere packaging maintains postharvest quality of loquat fruit. *Postharvest Biology and Technology, 24*, 341–348.

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.

Eason, J. R., Ryan, D., Page, B., Watson, L., & Coupe, S. A. (2007). Harvested broccoli (Brassica oleracea) responds to high carbon dioxide and low oxygen atmosphere by inducing stress-response genes. *Postharvest Biology and Technology, 43*, 358–365.

Escalona, V. H., Aguayo, E., & Artés, F. (2007). Modified atmosphere packaging improved quality of kohlrabi stems. *LWT - Food Science and Technology, 40*, 397–403.

Fernie, A. R., Carrari, F., & Sweetlove, L. J. (2004). Respiratory metabolism: Glycolysis, the TCA cycle and mitochondrial electron transport. *Current Opinion in Plant Biology, 7*, 254–261.

Finnegan, E., Mahajan, P. V., O'Connell, M., Francis, G. A., & O'Beirne, D. (2013). Modelling respiration in fresh-cut pineapple and prediction of gas permeability needs for optimal modified atmosphere packaging. *Postharvest Biology and Technology, 79*, 47–53.

Fonseca, S. C., Oliveira, F. A. R., & Brecht, J. K. (2002). Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: A review. *Journal of Food Engineering, 52*, 99–119.

Gil, M. I., Conesa, M. A., & Artés, F. (2002). Quality changes in fresh cut tomato as affected by modified atmosphere packaging. *Postharvest Biology and Technology, 25*, 199–207.

Gómez, P. A., & Artés, F. (2005). Improved keeping quality of minimally fresh processed celery sticks by modified atmosphere packaging. *LWT - Food Science and Technology, 38*, 323–329.

Gorny, J. R., & Kader, A. A. (1996). Regulation of ethylene biosynthesis in climacteric apple fruit by elevated CO_2 and reduced O_2 atmospheres. *Postharvest Biology and Technology, 9*, 311–323.

Han, J. H. (2005). *Innovations in food packaging*. Academic Press, California, USA.

Holcroft, D. M., & Kader, A. A. (1999). Controlled atmosphere-induced changes in pH and organic acid metabolism may affect color of stored strawberry fruit. *Postharvest Biology and Technology, 17*, 19–32.

Horev, B., Sela, S., Vinokur, Y., Gorbatsevich, E., Pinto, R., & Rodov, V. (2012). The effects of active and passive modified atmosphere packaging on the survival of Salmonella enterica serotype Typhimurium on washed romaine lettuce leaves. *Food Research International, 45*, 1129–1132.

Jiang, T., Luo, S., Chen, Q., Shen, L., & Ying, T. (2010). Effect of integrated application of gamma irradiation and modified atmosphere packaging on physicochemical and microbiological properties of shiitake mushroom (Lentinus edodes). *Food Chemistry, 122*, 761–767.

Jouki, M., & Khazaei, N. (2014). Effect of low-dose gamma radiation and active equilibrium modified atmosphere packaging on shelf life extension of fresh strawberry fruits. *Food Packaging and Shelf Life, 1*, 49–55.

Kader, A. A. (1985). Ethylene-induced senescence and physiological disorders in harvested horticultural crops. *HortScience, 20*, 54–57.

Kanellis, A. K., Solomos, T., & Roubelakis-Angelakis, K. A. (1991). Suppression of cellulase and polygalacturonase and induction of alcohol dehydrogenase isoenzymes in avocado fruit mesocarp subjected to low oxygen stress. *Plant Physiology, 96*, 269–274.

Ketsa, S., Wisutiamonkul, A., & Van Doorn, W. G. (2013). Apparent synergism between the positive effects of 1-MCP and modified atmosphere on storage life of banana fruit. *Postharvest Biology and Technology, 85*, 173–178.

Lee, S. J., & Rahman, A. T. M. M. (2014). Chapter 8—Intelligent packaging for food products. In J. H. Han (Ed.), *Innovations in food packaging* (2nd ed.). San Diego: Academic Press.

Li, Y., Ishikawa, Y., Satake, T., Kitazawa, H., Qiu, X., & Rungchang, S. (2014). Effect of active modified atmosphere packaging with different initial gas compositions on nutritional compounds of shiitake mushrooms (Lentinus edodes). *Postharvest Biology and Technology, 92*, 107–113.

Li, T., & Zhang, M. (2015). Effects of modified atmosphere package (MAP) with a silicon gum film window on the quality of stored green asparagus (Asparagus officinalis L) spears. *LWT - Food Science and Technology, 60*, 1046–1053.

Li, P., Zhang, X., Hu, H., Sun, Y., Wang, Y., & Zhao, Y. (2013). High carbon dioxide and low oxygen storage effects on reactive oxygen species metabolism in Pleurotus eryngii. *Postharvest Biology and Technology, 85*, 141–146.

López-Rubira, V., Conesa, A., Allende, A., & Artés, F. (2005). Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biology and Technology, 37*, 174–185.

Mahmoud, B. S. M. (2010). The effects of X-ray radiation on Escherichia coli O157:H7, Listeria monocytogenes, Salmonella enterica and Shigella flexneri inoculated on whole Roma tomatoes. *Food Microbiology, 27*, 1057–1063.

Mampholo, M. B., Sivakumar, D., & Van Rensburg, J. (2015). Variation in bioactive compounds and quality parameters in different modified atmosphere packaging during postharvest storage of traditional leafy vegetables (Amaranthus cruentus L and Solanum retroflexum). *Journal of Food Quality, 38*, 1–12.

Matan, N., Rimkeeree, H., Mawson, A., Chompreeda, P., Haruthaithanasan, V., & Parker, M. (2006). Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions. *International Journal of Food Microbiology, 107*, 180–185.

Mathooko, F. M. (1996). Regulation of respiratory metabolism in fruits and vegetables by carbon dioxide. *Postharvest Biology and Technology, 9*, 247–264.

Moreira, R. G., Puerta-Gomez, A. F., Kim, J., & Castell-Perez, M. E. (2012). Factors affecting radiation D-values (D10) of an Escherichia coli cocktail and Salmonella typhimurium LT2 inoculated in fresh produce. *Journal of Food Science, 77*, E104–E111.

Mukhopadhyay, S., Ukuku, D., Juneja, V., & Fan, X. (2014). Effects of UV-C treatment on inactivation of Salmonella enterica and Escherichia coli O157:H7 on grape tomato surface and stem scars, microbial loads, and quality. *Food Control, 44*, 110–117.

Nielsen, T., & Leufvén, A. (2008). The effect of modified atmosphere packaging on the quality of Honeoye and Korona strawberries. *Food Chemistry, 107*, 1053–1063.

Niemira, B. A., & Boyd, G. (2013). Influence of modified atmosphere and varying time in storage on the irradiation sensitivity of Salmonella on sliced roma tomatoes. *Radiation Physics and Chemistry, 90*, 120–124.

Oms-Oliu, G., Soliva-Fortuny, R., & Martín-Belloso, O. (2008). Modeling changes of headspace gas concentrations to describe the respiration of fresh-cut melon under low or superatmospheric oxygen atmospheres. *Journal of Food Engineering, 85*, 401–409.

Paul, V., Pandey, R., & Srivastava, G. C. (2012). The fading distinctions between classical patterns of ripening in climacteric and non-climacteric fruit and the ubiquity of ethylene—An overview. *Journal of Food Science and Technology, 49*, 1–21.

Pech, J.-C., Bouzayen, M., & Latché, A. (2008). Climacteric fruit ripening: Ethylene-dependent and independent regulation of ripening pathways in melon fruit. *Plant Science, 175*, 114–120.

Rai, D. R., & Paul, S. (2007). Transient state in-pack respiration rates of mushroom under modified atmosphere packaging based on enzyme kinetics. *Biosystems Engineering, 98*, 319–326.

Rivera, C. S., Venturini, M. E., Marco, P., Oria, R., & Blanco, D. (2011). Effects of electron-beam and gamma irradiation treatments on the microbial populations, respiratory activity and sensory characteristics of Tuber melanosporum truffles packaged under modified atmospheres. *Food Microbiology, 28*, 1252–1260.

Sawe, C. T., Onyango, C. M., & Njage, P. M. K. (2014). Current food safety management systems in fresh produce exporting industry are associated with lower performance due to context riskiness: Case study. *Food Control, 40*, 335–343.

Scifò, G. O., Randazzo, C. L., Restuccia, C., Fava, G., & Caggia, C. (2009). Listeria innocua growth in fresh cut mixed leafy salads packaged in modified atmosphere. *Food Control, 20*, 611–617.

Selcuk, N., & Erkan, M. (2014). Changes in antioxidant activity and postharvest quality of sweet pomegranates cv. Hicrannar under modified atmosphere packaging. *Postharvest Biology and Technology, 92*, 29–36.

Sen, C., Mishra, H., & Srivastav, P. (2012). Modified atmosphere packaging and active packaging of banana (Musa spp.): A review on control of ripening and extension of shelf life. *Journal of Stored Products and Postharvest Research, 3*, 122–132.

Singh, R., Giri, S. K., & Kotwaliwale, N. (2014). Shelf-life enhancement of green bell pepper (Capsicum annuum L.) under active modified atmosphere storage. *Food Packaging and Shelf Life, 1*, 101–112.

Somboonkaew, N., & Terry, L. A. (2011). Influence of temperature and packaging on physiological and chemical profiles of imported litchi fruit. *Food Research International, 44*, 1962–1969.

Taniwaki, M. H., Hocking, A. D., Pitt, J. I., & Fleet, G. H. (2001). Growth of fungi and mycotoxin production on cheese under modified atmospheres. *International Journal of Food Microbiology, 68*, 125–133.

Toivonen, P. M., & Brummell, D. A. (2008). Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. *Postharvest Biology and Technology, 48*, 1–14.

Trinchero, G. D., Sozzi, G. O., Cerri, A. M., Vilella, F., & Fraschina, A. A. (1999). Ripening-related changes in ethylene production, respiration rate and cell-wall enzyme activity in goldenberry (Physalis peruviana L.), a solanaceous species. *Postharvest Biology and Technology, 16*, 139–145.

Waghmare, R. B., & Annapure, U. S. (2013). Combined effect of chemical treatment and/or modified atmosphere packaging (MAP) on quality of fresh-cut papaya. *Postharvest Biology and Technology, 85*, 147–153.

Wani, A. A., Singh, P., & Langowski, H. C. (2014). Food technologies: Packaging. In Y. Motarjemi (Ed.), *Encyclopedia of food safety*. Waltham: Academic Press.

Xi, W., Zhang, Q., Lu, X., Wei, C., Yu, S., & Zhou, Z. (2014). Improvement of flavour quality and consumer acceptance during postharvest ripening in greenhouse peaches by carbon dioxide enrichment. *Food Chemistry, 164*, 219–227.

Ye, J.-J., Li, J.-R., Han, X.-X., Zhang, L., Jiang, T.-J., & Miao, X. (2012). Effects of active modified atmosphere packaging on postharvest quality of shiitake mushrooms (Lentinula edodes) stored at cold storage. *Journal of Integrative Agriculture, 11*, 474–482.

Zhang, B.-Y., Samapundo, S., Pothakos, V., De Baenst, I., Sürengil, G., Noseda, B., et al. (2013). Effect of atmospheres combining high oxygen and carbon dioxide levels on microbial spoilage and sensory quality of fresh-cut pineapple. *Postharvest Biology and Technology, 86*, 73–84.

Chapter 2
Nitric Oxide

Cintia Mazzucotelli, María G. Goñi, Sara I. Roura, Gustavo González-Aguilar, and J. Fernando Ayala-Zavala

Introduction

Nitric oxide (NO) is a free radical gas, which acts as a multifunctional signaling molecule in plants and animals (Wendehenne et al. 2001). Ya'acov and Haramaty (1996) reported emission of NO from plants; since then NO has been the subject of several studies on agriculture and food technology as an alternative to chemical treatments. At the beginning, NO studies in plants were focused on the phytotoxic properties of the oxides of nitrogen (NO_2, N_2O_3, NO_2^-, NO_3^-) and their effect since considerable amounts of them are produced naturally (Lamattina et al. 2003). Since then, subsequent investigations have linked endogenous NO to several physiological processes including modulation of endogenous ethylene and vegetative stress, water loss, root growth and fruit and flower formation, plant immunity, anthocyanin

C. Mazzucotelli • S.I. Roura
Consejo Nacional de Ciencia y Tecnología (CONICET), Avenida Rivadavia 1917, C1033AAJ CABA, Argentina

Grupo de Investigación en Ingeniería de Alimentos, Facultad de Ingeniería, UNMdP, Juan B. Justo 4302, Mar del Plata 7600, Argentina

M.G. Goñi (✉)
Consejo Nacional de Ciencia y Tecnología (CONICET), Avenida Rivadavia 1917, C1033AAJ CABA, Argentina

Grupo de Investigación en Ingeniería de Alimentos, Facultad de Ingeniería, UNMdP, Juan B. Justo 4302, Mar del Plata 7600, Argentina

Centro de Investigación en Alimentación y Desarrollo, AC (CIAD, AC), Carretera a la Victoria Km 0.6, Hermosillo, Sonora 83000, México
e-mail: ggoni@fi.mdp.edu.ar

G. González-Aguilar • J.F. Ayala-Zavala
Centro de Investigación en Alimentación y Desarrollo, AC (CIAD, AC), Carretera a la Victoria Km 0.6, Hermosillo, Sonora 83000, México

M.W. Siddiqui et al. (eds.), *Postharvest Management Approaches for Maintaining Quality of Fresh Produce*, DOI 10.1007/978-3-319-23582-0_2

biosynthesis, and chlorophyll production (Ku et al. 2000; Ya'acov and Pinchasov 2000; Del Río et al. 2004; García-Mata and Lamattina 2001).

NO plays an important role in many physiological processes in plants, and it can produce either beneficial or harmful effects, depending on the concentration and location in plant cells. NO present in the plant tissue could alleviate the harmfulness of reactive oxygen species (ROS), and reacts with other target molecules, and regulates the expression of stress-responsive genes under various stress conditions (Qiao and Fan 2008). It also plays important role in control various pathophysiological and developmental processes in plants (Lamattina et al. 2003; Neill et al. 2003). Additionally, several studies demonstrated NO effects on senescence and ripening in several fruits by suppressed respiration rate, ethylene biosynthesis, disease incidence, delayed peel color changes, and reduced enzyme activity (Ku et al. 2000; Duan et al. 2007; Manjunatha et al. 2010).

Postharvest application of NO has been shown to be effective in extending the postharvest life of a range of flowers, fruits, and vegetables when applied as a short-term fumigation treatment at low concentrations. This chapter is focused on the effect that NO application in fresh fruits and vegetables has in its metabolism and how these metabolic responses affect postharvest quality and product safety. However, the gaseous nature of NO is a barrier to its commercial usage. The construction of the infrastructure necessary to undertake large-scale fumigation is substantial and requires a measure of technical operational expertise. Efficient usage is particularly problematical when the site of production is geographically isolated and in developing countries. Therefore, a new alternative has emerged and NO donor technology was developed. In that case, solid compounds that store NO chemically but allow it to be regenerated under appropriate physical conditions are applied (Hou et al. 1999). Several examples of NO application are presented, along with a detail of the application methodology and the compounds used as NO donors.

Biological Factors Involved in the Senescence of Harvested Fruits and Vegetables

The causes of postharvest losses in quality fresh fruits and vegetables are many and depend on the type of product, its morphological structure, composition, developmental stages, and general physiology (Kader 2002). However, the main causes of deterioration are a result of their continuous metabolic activity after harvest, among which respiration, ethylene production, and enzymatic browning are the most important, and it is well established that postharvest quality of fresh fruits and vegetables cannot be improved further but it can be retained till their consumption if the rate of metabolic activities is reduced by applying the appropriate postharvest handling technologies (Kader 2002).

One of the main parameters while determining the metabolic activity of fruits and vegetables is their respiration rate, which is usually associated with the commodity deterioration (Wu 2010). Respiratory rate of produce after harvest is

reversely proportional to its storage life, meaning the higher the rate of respiration, the shorter the storability (Kader and Saltveit 2003). Respiration rate of a produce is dependent on a wide range of variables, including commodity, maturity state, and several environmental factors. Among the external factors affecting respiratory rate of fresh fruits and vegetables after harvest, temperature and gas composition surrounding the horticultural produce are two very important variables to be taken into consideration.

Ethylene (C_2H_2) is a gaseous phytohormone produced for all tissues of higher plants and by some microorganisms (Cao et al. 2008) and it regulates many aspects of growth, development, and senescence and is physiologically active in trace amounts (<0.1 ppm). Although ethylene action may have beneficial effects on certain product attributes, as stimulating ripening of climacteric fruit or promoting de-greening of citrus (Saltveit 1999), in most harvested horticultural products, ethylene is associated with degradation, fruit ripening, senescence, and abscission of plant organ (Saltveit 1999; Kader and Saltveit 2003). Ethylene accelerates chlorophyll degradation causing yellowing of green tissues, and induces abscission of leaves and flowers, softening of fruit, and several physiological disorders (Kader and Saltveit 2003). Since exposure to ethylene can be detrimental to most fresh horticultural commodities, ethylene is of major concern to all produce handlers during postharvest storage and handling.

Browning in fresh vegetables, also a detrimental quality, is a result of the oxidation of phenolic compounds (Tomás-Barberán and Espin 2001). Plant cells contain phenolic compounds which, in the presence of oxygen, easily oxidize to quinones by the action of enzymes, mainly polyphenol oxidase (PPO) and peroxidase (POD). Quinones in turn oxidize and polymerize producing brown compounds, which are responsible for superficial and/or deep tissue browning. Therefore, browning in plants is a result of cell injury in their tissues due to lost compartmentalization. When cellular damage occurs, the disruption of cellular compartments and the loss of membrane integrity allow the enzymes (such as PPO) and substrates (such as polyphenols) to mix and hence initiate several browning reactions (Duan et al. 2007; Pristijono et al. 2006).

Chemical Properties of NO

Nitric oxide (NO) is a small, uncharged, scarcely polar molecule; thus it can freely diffuse across membranes from one compartment to the other. Under normal atmosphere conditions, NO is a free radical lipophilic diatomic gas. Its small Stokes' radius and neutral charge allow rapid membrane diffusion (Lamattina et al. 2003). From a chemical point of view it is a free radical; however, it can adopt an energetically more favorable electron structure by gaining or losing an electron, so that NO can exist as three interchangeable species: the radical ($NO^{\bullet}$), the nitrosonium cation (NO^{+}), and the nitroxyl radical (NO^{-}) (Hasanuzzaman et al. 2010; Neill et al. 2003; Simontacchi et al. 2013) (Fig. 2.1).

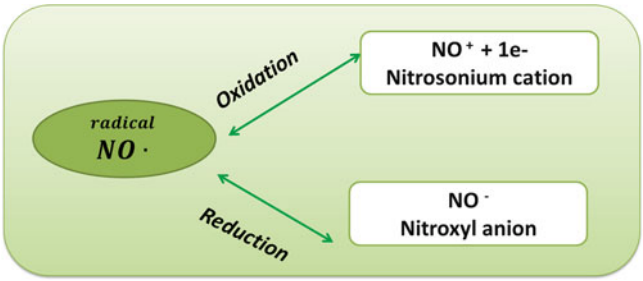


Fig. 2.1 NO forms with biological activity

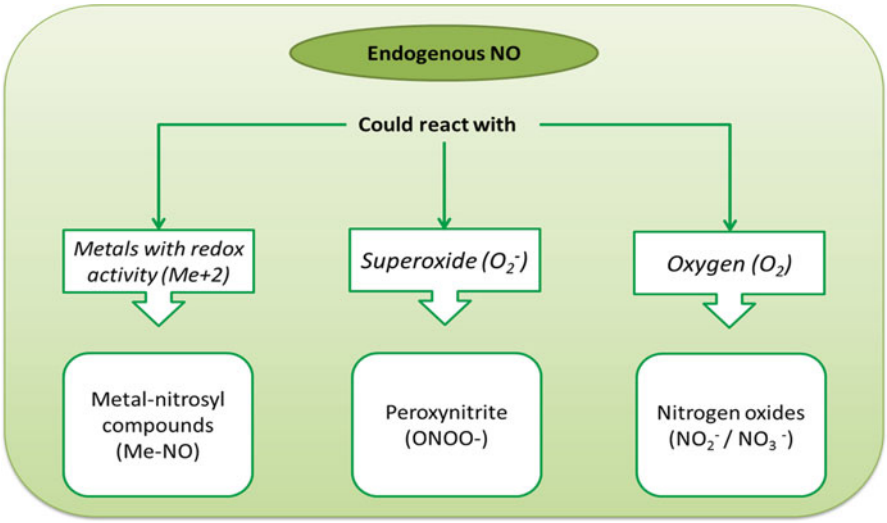


Fig. 2.2 Chemical reactions of endogenous NO and its products

NO reacts with oxygen to produce other nitrogen oxides with different stabilities and decay rates, its reactivity related to its single electron in its 2p-¼ antibonding orbital (Stamler 1994; Lamattina et al. 2003). The un-paired electron is responsible for its elevated reactivity with oxygen (O_2), superoxide ($O_2^{\bullet-}$), other N base compounds, and transition metals (Fig. 2.2). The complex redox chemistry of NO contributes to provide a general mechanism for cell redox homeostasis regulation, which can exert a protective action against oxidative stress (Stamler 1994; Wink et al. 1993).

The Fenton-type reaction between H_2O_2 and redox-active metals produces the hydroxyl radical ($OH^{\bullet}$), which is a powerful oxidant (Stamler 1994). NO can attenuate the Fenton oxidative damage preventing the formation of oxidants by scavenging either iron or superoxide and thus limiting hydroxyl radical formation (Wink et al. 1993). Several studies have also demonstrated that NO can act as a chain-breaking antioxidant arresting lipid peroxidative reactions (Rubbo et al. 1994). On the other

hand, NO can also react with superoxide anion ($O_2^{\bullet -}$), thus leading to the formation of the strong oxidant peroxinitrite ($ONOO^-$), which can oxidize thiol residues to sulfenic and sulfonic acids producing a cytotoxic effect (Radi et al. 1991).

Nitric Oxide in Plants

Some years ago, NO was considered as a highly toxic compound due to its free radical nature (Beckman et al. 1990). However, the discovery of NO signaling role in regulation of cardiovascular system has changed the paradigm concerning the cytotoxicity (Loscalzo and Welch 1995; Balligand et al. 1993). Later, the discovery of its biological functions has been elucidated (Ya'acov 1996). Since then, researching the function and metabolism of NO in plants has gained considerable attention in recent years. Some examples of its application are presented in Table 2.1.

NO has shown to play a crucial role in the regulation of several plant physiological processes, including growth and development. NO can act as a key signaling molecule in different intracellular processes in plants (Crawford and Guo 2005; Ya'acov 1996; Wendehenne et al. 2004; Lamattina et al. 2003). Moreover, NO is emitted from plants under stress situations, as well as under normal growth conditions (Arasimowicz and Floryszak-Wieczorek 2007).

The physiological function of NO in plants implicates the induction of different processes, including the expression of defense-related genes against abiotic and biotic stress (Durner et al. 1998; Wendehenne et al. 2004; Klessig et al. 2000) and apoptosis/programmed cell death (Clarke et al. 2000; Beligni et al. 2002), maturation and senescence (Badiyan et al. 2004; Singh et al. 2009), stomatal closure (García-Mata and Lamattina 2001; Neill et al. 2002), seed germination (Beligni and Lamattina 2000; Kopyra and Gwóźdź 2003), root development (Correa-Aragunde et al. 2004; Pagnussat et al. 2002), and so many other examples. However, the effects of NO on different types of cells have been proved to be either protective or toxic, also depending on the concentration and situation (Hasanuzzaman et al. 2010). As a consequence, more research is needed to assess the specific effects of NO in different fruits and vegetables in order to be successfully applied.

As shown in previous studies, endogenously produced NO gas appears to be a natural plant growth regulator in a wide variety of both climacteric and non-climacteric fruits, flowers, vegetables, and legume sprouts (Leshem et al. 2000; Ya'acov et al. 1998; Bredt 1999). In biological systems, NO can be generated enzymatically or nonenzymatically (Wendehenne et al. 2001). The most extensively described NO-producing enzymes have been nitric oxide synthase (NOS) and nitrate reductase (NR) (Hasanuzzaman et al. 2010).

Published data suggest that drought and salinity induce NO generation which activates cellular processes that afford some protection against the oxidative stress associated with these conditions (Neill et al. 2008). Their study suggested an emerging model of stress responses in which ABA has several ameliorative functions. These include the rapid induction of stomatal closure to reduce water

Table 2.1 Effects of NO application in fruits and vegetables

NO donor	Treated produce	Postharvest effect	References
Endogenous NO, induced by chilling stress	Loquat (*E. japonica* Lindl. cv. Luoyangqing)	Alleviated chilling injuries	Xu et al. (2012)
NO gas	Strawberry (*Fragaria ananassa* Duch cv. Pajaro)	50 % extension of shelf life (less mold growth, delayed softening, and color changes)	Wills et al. (2000)
	Cucumber (*Cucumis sativus* L.)	Alleviate chilling injuries system (maintained membrane integrity). Delayed lipid peroxidation, $O_2^{\bullet-}$ and H_2O_2 production. Increased SOD, CAT, APX, and POD activities. Increased DPPH-radical scavenging activity	Yang et al. (2011)
	Chinese Winter jujube (*Zizyphus jujuba* Mill. Cv. Dongzao)	Reduced PPO and PAL activities. Delayed maturation (maintaining low levels of anthocyanins, TF, TSS). Reduced AA oxidation	Zhu et al. (2009)
	Tomato (*Lycopersicum lycopersicum* L. cv. Abunda)	Reduced net photosynthesis	Bruggink et al. (1988)
	Japanese plums (*Prunus salicina* Lindell)	Reduced respiration rate, ethylene production. Delayed ripening by 2–3 days (no color changes or tissue softening). Alleviated chilling injuries symptoms	Singh et al. (2009)
	Peach (*Prunus persica* L. Batsch., cv. Feicheng)	Reduced LOX and ACC oxidase activities and ethylene production	Zhu et al. (2006)
	Mango (*Mangifera indica* L. cv. Kensington Pride)	Alleviated chilling injuries. Suppressed ethylene production. Delayed fruit softening and color development	Zaharah and Singh (2011)
	Mushrooms (*Russula griseocarnosa*)	Increased AOX. Increased TF and flavonoids contents. Increased PAL and chalcome synthase	Dong et al. (2012)
SNP	Litchi (*Litchi chinensis* Sonn.)	Reduced pericarp browning. Increased anthocyanin and TP content. Enhanced AOX. 8 days extension of shelf life	Barman et al. (2014)
	Bamboo shoots (*Phyllostachys violascens*)	Inhibited PPO, POD and PAL activities. Maintained TP content. Delayed external browning and tissue lignification	Yang et al. (2010)
	Logan fruit (*Dimocarpus longan* Lour. cv. Shixia)	In vitro inhibition of PPO and POD activities. Lower pulp breakdown and pericarp browning. Maintained TSS and AA content	Duan et al. (2007)

(continued)

Table 2.1 (continued)

NO donor	Treated produce	Postharvest effect	References
	Strawberry (*Fragaria ananassa* L.)	Inhibited ethylene production. Reduced respiration rate, ACC accumulation, ACC synthase activity but it did not affect ACC oxidase activity	Zhu and Zhou (2007)
	Banana (*Musa spp.* cv. Brazil)	Reduced ethylene production, inhibited degreening of the peel, and delayed softening of the pulp	Cheng et al. (2009)
DETA/NO	Mushrooms (*Agaricus bisporus*)	Maintained firmness. Delayed cap opening and browning. Increased TP content and AA retention. Reduced H_2O_2 accumulation and oxidative stress (less free radical accumulation). Inhibited PPO activity and increased CAT, SOD, and APX activities. Extended shelf life up to 12 days	Jiang et al. (2011)
	Fresh-cut apple slices	Delayed surface browning. Reduced TP total phenols, ion leakage, and respiration rate. Inhibited PPO activity. It did not affect ethylene production or lipid peroxide levels	Huque et al. (2013)

SNP sodium nitroprusside, DETA/NO 2,2′-(hydroxynitrosohydrazino)-bisethanamine (diethylene-triamine nitric oxide), *ACC* 1-aminocyclopropane-1-carboxylic acid, *AOX* antioxidant activity/capacity, *PPO* polyphenol oxidase, *POD* peroxidase, *LOX* lipoxygenase, *APX* ascorbate peroxidase, *CAT* catalase, *TP* total phenolics, *TSS* total soluble solids, *AA* ascorbic acid

loss due to transpiration and the activation of antioxidant defenses to combat oxidative stress. These are two processes that both involve NO as a key signaling intermediate.

NO and Ethylene in Plant Senescence

The hypothesis that endogenously produced NO gas is a natural plant growth regulator was experimentally tested by Ya'acov et al. (1998). Experimentation encompassed a wide spectrum of both climacteric and non-climacteric varieties of fruits, flowers, vegetables, and legume sprout species. Specific NO sensor revealed that maturation and senescence go hand in hand with a significant decrease of NO emission.

Fruit and vegetable senescence is the last stage in their development, consisting in the degradation and translocation of molecules to other growing tissues (Noodén et al. 1997). Degradation of chlorophyll, proteins, antioxidants, and water imbalance are

processes involved in the senescence of plants, processes that are highly regulated by hormones. As mentioned above, ethylene is largely known as a key hormone that accelerates leaf, flower, and fruit senescence. In contrast, NO has been observed participating in active growth and delaying the development of the senescence syndrome in plants (Lamattina et al. 2003; Leshem and Wills 1998). Young plant tissues presented high NO rate emission, but it decreased during maturation showing ethylene formation, an opposite trend (Leshem and Wills 1998). This result implies that NO and ethylene productions are inversely affected during plant development. A specific association between these two signaling molecules has been established in several previous studies. NO appears to be able to decrease ethylene emission through the downregulation of its synthesis (Manjunatha et al. 2010; Zhu and Zhou 2007). Not all the elements of the ethylene synthesis pathway affected by NO are clearly established. However, it is consistently observed in several reports that the final step in ethylene synthesis catalyzed by 1-aminocyclopropane carboxylic acid oxidase is downregulated by NO. Furthermore, the S-nitrosylation by NO inhibits the activity of enzymes involved in ethylene synthesis (Zhu et al. 2008; Zaharah and Singh 2011).

NO and Plant Water Loss

Stomata are pores formed by two differentiated cells (guard cells) located in the epidermis of terrestrial plants. The opening of the stomatal pore is regulated to facilitate CO_2 uptake, for CO_2 fixation during photosynthesis and to avoid water loss due to transpiration (Simontacchi et al. 2013).

Environmental parameters such as light, CO_2 level, humidity, soil water status, and biotic stresses regulate stomatal aperture (Hetherington and Woodward 2003). In addition, stomatal movements are effected by osmotic fluxes of water across the tonoplast and plasma membrane, such fluxes being driven by movements of K^+ and Cl^- ions through specific channels that are activated and deactivated in response to various stimuli such as abscisic acid (ABA) content (Neill et al. 2003). Interestingly, it was demonstrated that NO is required in ABA signaling both for stomatal closure induction and for inhibition of light-induced stomatal opening (Garcia-Mata and Lamattina 2007; Zhang et al. 2007). The first report on NO regulation of stomatal movement showed that the NO donor SNP-induced stomatal closure in three different plant species *Vicia faba*, *Salpichroa origanifolia*, *and Tradescantia* spp. (García-Mata and Lamattina 2001). In following studies, it was also demonstrated that NO is required for the ABA-regulated signaling pathway leading to stomatal closure (Neill et al. 2002; García-Mata and Lamattina 2002).

Stomatal closure, initiated by ABA, is effected through a complex symphony of intracellular signaling in which NO appears to be a key component. Exogenous NO application induces stomatal closure, ABA triggers NO generation, removal of NO by scavengers inhibits stomatal closure in response to ABA, and ABA-induced stomatal closure is reduced in mutants that are impaired in NO generation (Neill et al. 2008).

NO and Antioxidant Properties in Plants

Higher plants growing in natural environments experience various abiotic stresses. H_2O_2 and NO free radicals are produced and cause oxidative damage to plants under various abiotic stress conditions (Uchida et al. 2002). Since its discovery as an endogenous free radical, NO has been proposed to be either cytotoxic or cytoprotective. The presence of an unpaired electron within the NO molecule makes it a reactive species and is also the origin of its duality (Lamattina et al. 2003). NO interacts with reactive oxygen species (ROS) in various ways and it might exert an antioxidant effect against various stresses (Beligni and Lamattina 1999; Soheila et al. 2001). NO modulation of superoxide radical formation and inhibition of lipid peroxidation also illustrate its potential role as a potent antioxidant (Wink et al. 1993; Neill et al. 2003; Duan et al. 2007). On the other hand, it has been known for years that NO forms various reactive nitrogen oxide species (RNOS) in the presence of oxygen which can be deleterious to macromolecules, such as lipids, proteins, and DNA (Wink et al. 1993). Thus, an excess of NO can result in nitrosative stress; therefore a favorable balance of ROS/NO is important to maintain a healthy environment for the plant tissue (Neill et al. 2003).

NO and Defense-Related Genes in Plants

ROS are believed to perform multiple roles during plant defense responses to microbial attack, acting in the initial defense and possibly as cellular signaling molecules. In animals, NO is an important redox-active signaling molecule. It is known that infection of resistant, but not susceptible, tobacco with tobacco mosaic virus resulted in enhanced NO synthase activity (Durner et al. 1998). Furthermore, administration of NO donors or recombinant mammalian NOS to tobacco plants or tobacco suspension cells triggered expression of the defense-related genes encoding pathogenesis-related 1 protein and phenylalanine ammonia lyase (PAL). These genes were also induced by cyclic GMP (cGMP) and cyclic ADP-ribose, two molecules that can serve as second messengers for NO signaling in mammals. Consistent with cGMP acting as a second messenger in tobacco (Durner et al. 1998), NO treatment induced dramatic and transient increases in endogenous cGMP levels. Furthermore, NO-induced activation of PAL was blocked by 6-anilino-5,8-quinoline dione and 1H-(1,2,4)-oxadiazole[4,3-a]quinoxalin-1-one, two inhibitors of guanylate cyclase. Although 6-anilino-5, 8-quinolinedione fully blocked PAL activation, inhibition by 1H-(1,2,4)-oxadiazole[4,3-a]quinoxalin-1-one was not entirely complete, suggesting the existence of cGMP-independent, as well as cGMP-dependent, NO signaling.

Exogenous NO Application in Plants

Application Methods for NO

Postharvest application of NO or its donor compounds has been shown to be effective in extending the postharvest life of fruits and vegetables when applied as a short-term fumigation treatment at low concentrations (Table 2.2). It has been shown that exogenous NO delayed ripening (Leshem et al. 2000), inhibited ethylene biosynthesis (Eum et al. 2009; Zhu et al. 2008), inhibited cut-surface browning (Pristijono et al. 2006; Wills et al. 2008), and enhanced resistance to post-harvest diseases (Zhu et al. 2008; Zhu and Zhou 2007).

One of the most widely used methods of application is the fumigation of the postharvest horticultural product with NO in a low concentration. A common problem during application of NO is that, in air in contact with O_2, NO rapidly converts to NO_2 with half-life of 5–12 s (Wills et al. 2000). Thus, the application of exogenous NO requires fumigation in an oxygen-depleted atmosphere. This can be achieved by initially displacing oxygen with nitrogen or argon (which could be more expensive) in the atmosphere around produce, followed by the inclusion of low concentrations of NO into the O_2-depleted atmosphere for 2–24 h. It appears that optimal effective NO concentrations and fumigation duration vary between different commodities. Therefore, the need for NO to be applied in an oxygen-free atmosphere will require some innovation in the design and operation of the fumigation process (Leshem et al. 2000).

Wills et al. (2000) studied the extension in postharvest life by applying NO on strawberries. NO was then added by injection (4–500 μL NO/L of N_2, 2 h). NO at 5–10 μL/L to strawberries for 2 h consistently extended postharvest life by 50 % compared to strawberries held in air. In a similar way, Zhu et al. (2006) studied the inhibition of ethylene biosynthesis by NO application in China peach fruit during storage. Peaches were placed in 30 L sealed containers and flushed with nitrogen gas to displace all oxygen. NO was then injected (5, 10, and 15 μL/L, 3 h). Zhu et al. (2009) and Yang et al. (2011) applied the same treatment to study the effects of NO fumigation on phenolic metabolism of postharvest Chinese winter jujube and to reduced chilling injury in cucumber, respectively.

Zaharah and Singh (2011) studied NO postharvest fumigation to alleviate chilling injury and delaying fruit ripening in mango. Mangos were fumigated with different concentrations of NO (0, 5, 10, 20, and 40 μL/L N_2) by injection of the mix gases into a sealed container for 2 h. Opposed to the previous cases, the O_2 gas in the container was not depleted because the authors consider that NO was sufficiently stable at the low concentrations and short treatment times required for the fruit to be treated in normal air.

In another study, Wills et al. (2008) found that browning of fresh-cut lettuce was inhibited by short-term exposure to NO when applied by two different methods. First, NO was applied by fumigation of the cut lettuce with the desired

Table 2.2 Application methods used for exogenous NO in fruits and vegetables

Application method	NO donor	Treated produce	Concentration	References
Fumigation	NO gas	Strawberry (*Fragaria ananassa* Duch cv. Pajaro)	1–4000 μL/L	Wills et al. (2000)
		Cut lettuce slices (*Lactuca sativa* L.)	5–1000 μL/L	Wills et al. (2008)
		Mango (*Mangifera indica* L. cv. Kensington Pride)	10–40 μL/L	Zaharah and Singh (2011)
		Mushrooms (*Russula griseocarnosa*)	10–30 μL/L	Dong et al. (2012)
Immersion	SNP	Litchi (*Litchi chinensis* Sonn.)	0.5–2.0 mM	Barman et al. (2014)
		Strawberry (*Fragaria ananassa* L.)	1–10 μmol/L	Zhu and Zhou (2007)
		Logan fruit (*Dimocarpus longan* Lour. cv. Shixia)	1 mM	Duan et al. (2007)
	DETA/NO	Mushrooms (*Agaricus bisporus*)	0.5–2.0 mM	Jiang et al. (2011)
		Fresh-cut apple slices (*Malus domestica* cv. Borkh)	10 mg/L	Huque et al. (2013)
		Cut lettuce slices (*Lactuca sativa* L.)	10–1000 mg/L	Wills et al. (2008)

concentration, for 1–4 h at 20 °C. Second, NO was applied through a NO donor: 2,20-(hydroxylnitrosohydrazino)-bisethanamine (DETA/NO). This compound, a diazeniumdiolate, has gained considerable interest among researchers due to its high water solubility, chemical stability, and ease of synthesis (Hrabie and Keefer 2002). Its interest for use on horticultural crops resides in its 50 % NO content and its ability to released two equivalents of NO gas per molecule. DETA/NO was applied by dipping lettuce plants in solution with the desired concentration, from 15 s to 60 min at 20 °C (Wills et al. 2008). DETA/NO solution and NO gas application resulted in longer postharvest life than control plants. Moreover, cut lettuce plants dipped in 500 mg/L DETA/NO for 60 s or 5 min had a longer postharvest life than lettuce plants fumigated with 500 μL/L of NO gas for 2 h. However, the application of 1000 μL/L of NO gas strongly inhibited browning on the cut surface, but it caused rapid severe tissue damage and loss of color to the leaf surface (Wills et al. 2008).

Another donor of NO utilized in the treatment postharvest of horticultural products is sodium nitroprusside (SNP). Zhu and Zhou (2007) studied the effect of NO on ethylene production in strawberry fruit during storage and assayed the treatment

of strawberry samples by dipping in 1.0, 5.0, or 10.0 μmol/L SNP aqueous solutions for 2 h at 25 °C. In the same way, Duan et al. (2007) investigated the effect of NO on pericarp browning of harvested longan fruit, and treated the samples with nitroprusside (SNP), as a NO donor. Fruits were dipped for 5 min in 1 mM SNP solution at 28 °C. After dipping, the fruits were air-dried for 30 min, packed in polyethylene bags, and stored. Duan et al. (2007) also studied the application of NO to reduce pericarp browning and to maintain bioactive antioxidants level in litchi fruits. Treatments were performed by dipping the fruits in aqueous solutions of SNP (0.5 mM, 1.0 mM, or 2.0 mM) containing Tween-20 (2 g/L) as surfactant, at 25 °C for 5 min. Afterwards, fruits were air-dried, packed in corrugated fiber board boxes, and stored (30 ± 2 °C temperature and 85 ± 5 % RH) for 8 days.

Effect of Exogenous NO on Ripening and Senescence Fruits and Vegetables

Evidence of the interplay between NO and ethylene in the maturation and senescence of plant tissues suggests an antagonistic effect of both gases during these stages of plant development. This antagonistic effect has been used for the manipulation of the development of undesirable biochemical modifications of edible plants. Exogenous NO treatment was successfully used for the extension of postharvest life of several horticultural products (Lamattina et al. 2003; Simontacchi et al. 2013).

NO treatment delays the climacteric peak of ethylene production and the progress of fruit ripening on several species. Anti-senescent action of NO on plant tissues has been proposed to take place via the inhibition of ethylene biosynthesis (Leshem and Wills 1998; Zhu et al. 2006). The mechanism of action of NO via ethylene inhibition is not completely understood, but many researchers have proposed different mechanisms according to the results of their investigations. Aminocyclopropanecarboxylate (ACC) oxidase is a key enzyme that catalyzes the conversion of ACC to ethylene, which is a rate-determining step in the ethylene biosynthesis pathway. Eum et al. (2009) confirmed that inhibition of ethylene biosynthesis in NO-fumigated tomatoes was due to decreased and delayed expression of ACC oxidase genes. However, another study on strawberries revealed that NO decreased the activity of ACC synthase, but not ACC oxidase (Zhu and Zhou 2007).

Tierney et al. (2005) explained that the binding of ACC to the Fe^{2+} in ACC oxidase serves to activate the enzyme. ACC oxidase in climacteric peaches can also form a stable ternary ACC-ACC oxidase-NO complex with NO and ACC. This complex cannot be changed into an iron-oxo species that affects ACC oxidation yielding ethylene. Therefore, ethylene cannot be produced in this way. These results also elucidate the accumulation of ACC and the decrease in ACC oxidase activity in the peach fruit that were treated with 5 and 10 μL/L NO (Zhu et al. 2006). Thus, the exogenous application of NO significantly delayed maturation and senescence in fruits where NO effect was stoichiometrically related to ethylene

suppression (Ya'acov et al. 1998). For example, treatment with NO extended postharvest life of strawberry (Wills et al. 2000; Zhu and Zhou 2007), pear (Sozzi et al. 2003), broccoli, green bean, bok choy (Soegiarto and Wills 2004), peach (Zhu et al. 2006), mango (Zaharah and Singh 2011), tomato (Eum et al. 2009), and carnations (Bowyer et al. 2003).

Associated with ethylene production, there is a rise in the respiratory activity and in ROS generation that are involved in the acceleration of deteriorative processes during ripening. Respiration activity is proportional to the product deterioration, since many organic compounds are metabolized (Kader 2002). It was also observed that NO decreases respiration through the inhibition of cytochrome c oxidase activity in plant mitochondria (Millar and Day 1996).

Fruit softening appears to play a significant role in fruit quality, which are significantly influenced by ripeness. Ethylene is directly involved in increasing the activities of fruit-softening enzymes (pectin esterase, endoglucanase, exo- and endo polygalacturonase) (Khan and Singh 2007; Manjunatha et al. 2010). It is, therefore, possible that inhibition of ethylene production through NO treatments helps to retard the fruit-softening process (Singh et al. 2009). NO treatment reduced the degree of disintegration of the cell membranes and electrolyte leakage, which resulted in better retention of the cellular components such as pigments, titratable acidity, soluble solids, and free antioxidant compounds, particularly the ascorbic acid, which fresh fruits are most valued for (Zhang et al. 2007; Flores et al. 2008). Application of exogenous NO on postharvest fruits has been reported to retard softening during storage and ripening of peaches with 5 or 10 μL/L NO (Flores et al. 2008; Zhu et al. 2006) and kiwifruit with 1 μmol/L NO (Zhu et al. 2008). As a gas that has dual characteristics, a higher concentration of NO was detrimental; for example 15 μL/L NO applied in peaches and 2 μmol/L NO applied in kiwifruit enhanced softening (Singh et al. 2009). In peaches, higher NO concentrations exhibited toxicity and harmed the fruit, possibly by forming RNOS with superoxide ions (Zhu et al. 2006). The RNOS could injure biological systems by oxidation, nitrosation, and nitration (Wink and Mitchell 1998). In jujube fruits, treatment with 30 μL/L NO promoted ripening of the harvested fruits, which also exhibited signs of NO toxicity (Zhu et al. 2009).

NO and Browning Development in Postharvest Fruits and Vegetables

An important factor causing loss of quality in many fruits and vegetables is the development of browning on cut or damage surfaces (Duan et al. 2007). Browning reactions are generally assumed to be a direct consequence of PPO and POD activities on polyphenols, to form quinones which ultimately polymerize to produce the brown polymers that are responsible for the changes in color and appearance of fresh-cut fruits and vegetables (Li-Qin et al. 2009; Pristijono et al. 2006).

The initial event in the oxidative browning process is the breakdown of membranes within the cells of plant tissues, and browning is associated with the loss of membrane integrity, which occurs during tissue deterioration and senescence. Once the compartmentalization of the cells begins to fail, PPO and/or POD can act on phenol substrates (Degl'innocenti et al. 2005). Besides, phenylalanine ammonia lyase (PAL) is the first key enzyme involved in the biosynthesis of phenols in fruits and it can be induced by various stress conditions, but mainly by wounding. Increase in PAL activity was related to tissue browning of fresh fruits and vegetables during storage (Tomás-Barberán and Espin 2001; Zhu et al. 2009). Therefore, delaying or reducing enzymatic browning should be a relevant way to extend storage life and maintain postharvest quality of fruits and vegetables.

Treatment of these products with NO for the inhibition of browning surface has been widely reported. Duan et al. (2007) studied the inhibition of the pericarp browning in longan fruit, and found that treatment with SNP inhibited activities of PPO, POD, and PAL during storage. The same behavior was reported for the NO treatment in other products, where a significantly browning reduction was observed, as in cut surface of lettuces (Wills et al. 2008), litchi (Barman et al. 2014), jujube fruit (Zhu et al. 2009), mango (Zaharah and Singh 2011), peach slices (Li-Qin et al. 2009), apple slices (Pristijono et al. 2006), among many others.

One of the proposed mechanisms for inhibiting PPO activity takes into account the structure of the enzyme and possible interactions with the NO molecule. The active site of PPO includes two copper atoms and exists in three states: "met," "deoxy," and "oxy." Of the two copper atoms of the active site in PPO, $Cu_{(A)}$ is liganded by three histidines, which are highly stable. In contrast, the structure accounting for $Cu_{(B)}$ binding is less stable, although in general, three histidines are involved as $Cu_{(A)}$ ligands (Radi 1996). It was speculated that low concentration of NO could react with $Cu_{(B)}$ of PPO to form copper–nitrosyl complexes (NO-$Cu_{(B)}$-PPO), which could change the normal structure of the active site in PPO and thus, reduce PPO activity (Zhu et al. 2009). Low PPO activity inhibited the oxidation of phenols and inhibited the degradation of anthocyanins to brown products. However, indirect effects of NO involving the formation of RNOS may become significant at high concentrations (Wink and Mitchell 1998) and should be considered. A similar mechanism is proposed for PAL enzyme inhibition by NO. PAL is an oligomeric enzyme, consisting of four identical subunits with an electrophilic center of dehydroalanyl radical in its active site (Schuster and Retey 1995). The active site might reacts with NO, leading to inhibition of PAL activity (Wink and Mitchell 1998).

NO and Other Beneficial Aspects in Postharvest of Fruits and Vegetables

Another effect observed for the NO treatment of postharvest fresh produce, is improvement in chilling tolerance and reduced incidence of chilling injury in several fruits. Yang et al. (2011) studied the application of NO in cucumber fruit and found that NO significantly reduced its chilling injury symptoms and increased the

activities of antioxidant enzymes as catalase, peroxidase, ascorbate peroxidase, and superoxide dismutase, under chilling stress. It has been reported that the improvement of chilling tolerance in harvested horticultural crops is related to enhancement in activities of antioxidant enzymes (Yang et al. 2011). In similar studies, it was reported that NO application was able to alleviate chilling injury symptoms in plums (Singh et al. 2009), peach (Li-Qin et al. 2009), and mango (Zaharah and Singh 2011).

Barman et al. (2014) reported that SNP treatment significantly reduced loss of moisture from the litchi fruit when compared to control, and propose that its effect might be due to NO released from SNP, which maintained cell integrity and permeability of tissues, therefore reducing moisture transference rate from the fruits and vegetables (Ku et al. 2000; Barman et al. 2014). In addition, exogenous application of NO donors can reduce stomatal apertures and thereby reduce transpiration in several species (García-Mata and Lamattina 2001; Neill et al. 2003).

NO, as a bioactive molecule, was found to function as pro-oxidant as well as antioxidant. Kopyra and Gwóźdź (2003) found that SNP, a NO donor, stimulates seed germination and root growth of lupin (*Lupinus luteus L.* cv. Ventus). Seed germination was promoted when SNP was applied (0.1 and 800 μM SNP). The promoting effect of NO on seed germination persisted even in the presence of heavy metals (Pb, Cd) and sodium chloride, which can also be perceived as an added advantage. Pretreatment of lupin seedlings for 24 h with 10 μM SNP also presented a significant reduction of the detrimental effect of abiotic stress on root growth and morphology. The inhibitory effect of heavy metals on root growth was accompanied by increased activity of SOD, which in roots pre-treated with SNP was significantly higher. Some changes in the activity of other antioxidant enzymes, such as POX, and catalase were also detected as a consequence of NO. Similar results were found for Uchida et al. (2002) for rice seedling under saline and heat stress.

As it is well known, the production of ROS in plants increases under stress conditions and causes oxidative damage. Shi et al. (2007) studied the effects of exogenous SNP on both, the ROS metabolism in mitochondria and functions of plasma membrane (PM) and tonoplast in cucumber seedlings under saline stress (100 mM NaCl). Saline stress induced significant accumulation of H_2O_2 and led to serious lipid peroxidation in cucumber mitochondria. Exogenous application of SNP (50 μM) stimulated ROS-scavenging enzymes and reduced accumulation of H_2O_2 in mitochondria of cucumber roots.

Conclusions and Future Trends

Several studies have shown the effect NO on plant metabolism and its application has proved to be useful to maintain postharvest quality in fruits and vegetables, extend storage life, and reduce the incidence of stress-related injuries. However, more research is needed to completely elucidate the metabolic pathways associated to its biological activity in plants (Neill et al. 2008). Stronger knowledge of the NO

metabolism may be a key advantage to broaden its agronomical application, to new produce or in different environmental conditions that have yet to be tested, like salinity or drought stress. Even though NO is a short-lived molecule that reacts with oxygen, it is an extremely active one and the mechanisms by which either NO or its donors are participating in NO signaling are also important, and needs further study. Another unanswered question is the mechanism by which NO is perceived in cells (Neill et al. 2008).

There is also a remaining need to develop improved and more efficient methods to apply NO when in O_2-rich atmospheres, since oxidation of NO could post a complication. The need to deplete O_2 from the surrounding atmosphere of the produce during fumigation makes difficult to implement NO fumigation in some cases, like preharvest application in situ, or for longer periods of time when the absence of O_2 may result in undesired changes in the metabolism of the fruits and vegetables that are treated.

As in all biological systems (like live fresh fruits and vegetables are), generalization of the effects of NO found in a specific set of conditions for a produce to a different one, or to a different set of conditions, is few and not very accurate. Therefore new studies are needed to apply NO to different commodities or in changing growing or storage conditions than those presented in the existing literature. However, as previously mentioned, knowledge obtained so far is promising in the advantage of NO application in agriculture and food technology to preserve postharvest quality of fresh fruits and vegetables.

In the present chapter, NO has been presented as a promising pre and postharvest agriculture agent to reduce losses and maintain quality during postharvest storage and handling. All this evidence shows that NO might affect different metabolic traits leading to the improvement of extension and quality of plant products for human consumption. The results reported in the presented studies suggest that exogenous application of NO could delay the fruit ripening process and senescence, reduced losses due to stress-related injuries, water loss, browning, among others.

The utilization of NO in fruits and vegetables production has potential commercial value. However, considerable work is still required to demonstrate a cost-effective benefit to specific produce and more research is needed to widening its application in the fresh produce industry.

References

Arasimowicz, M., & Floryszak-Wieczorek, J. (2007). Nitric oxide as a bioactive signalling molecule in plant stress responses. *Plant Science, 172*, 876–887.

Badiyan, D., Wills, R., & Bowyer, M. (2004). Use of a nitric oxide donor compound to extend the vase life of cut flowers. *HortScience, 39*, 1371–1372.

Balligand, J.-L., Kelly, R. A., Marsden, P. A., Smith, T. W., & Michel, T. (1993). Control of cardiac muscle cell function by an endogenous nitric oxide signaling system. *Proceedings of the National Academy of Sciences of the United States of America, 90*, 347–351.

Barman, K., Siddiqui, M. W., Patel, V., & Prasad, M. (2014). Nitric oxide reduces pericarp browning and preserves bioactive antioxidants in litchi. *Scientia Horticulturae, 171*, 71–77.

Beckman, J. S., Beckman, T. W., Chen, J., Marshall, P. A., & Freeman, B. A. (1990). Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide. *Proceedings of the National Academy of Sciences of the United States of America, 87*, 1620–1624.

Beligni, M. V., Fath, A., Bethke, P. C., Lamattina, L., & Jones, R. L. (2002). Nitric oxide acts as an antioxidant and delays programmed cell death in barley aleurone layers. *Plant Physiology, 129*, 1642–1650.

Beligni, M. V., & Lamattina, L. (1999). Nitric oxide counteracts cytotoxic processes mediated by reactive oxygen species in plant tissues. *Planta, 208*, 337–344.

Beligni, M. V., & Lamattina, L. (2000). Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants. *Planta, 210*, 215–221.

Bowyer, M., Wills, R., Badiyan, D., & Ku, V. (2003). Extending the postharvest life of carnations with nitric oxide—comparison of fumigation and in vivo delivery. *Postharvest Biology and Technology, 30*, 281–286.

Bredt, D. S. (1999). Endogenous nitric oxide synthesis: Biological functions and pathophysiology. *Free Radical Research, 31*, 577–596.

Bruggink, G. T., Wolting, H. G., Dassen, J. H. A., & Bus, V. G. M. (1988). The effect of nitric oxide fumigation at two CO_2 concentrations on net photosynthesis and stomatal resistance of tomato (*Lycopersicon lycopersicum* L. cv. Abunda). *New phytologist, 110*(2), 185–191.

Cao, Y.-R., Chen, S.-Y., & Zhang, J.-S. (2008). Ethylene signaling regulates salt stress response: An overview. *Plant Signaling & Behavior, 3*, 761–763.

Cheng, G., Yang, E., Lu, W., Jia, Y., Jiang, Y., & Duan, X. (2009). Effect of nitric oxide on ethylene synthesis and softening of banana fruit slice during ripening. *Journal of Agricultural and Food Chemistry, 57*(13), 5799–5804.

Clarke, A., Desikan, R., Hurst, R. D., Hancock, J. T., & Neill, S. J. (2000). NO way back: Nitric oxide and programmed cell death in Arabidopsis thaliana suspension cultures. *The Plant Journal, 24*, 667–677.

Correa-Aragunde, N., Graziano, M., & Lamattina, L. (2004). Nitric oxide plays a central role in determining lateral root development in tomato. *Planta, 218*, 900–905.

Crawford, N. M., & Guo, F.-Q. (2005). New insights into nitric oxide metabolism and regulatory functions. *Trends in Plant Science, 10*, 195–200.

Degl'innocenti, E., Guidi, L., Pardossi, A., & Tognoni, F. (2005). Biochemical study of leaf browning in minimally processed leaves of lettuce (Lactuca sativa L. var. Acephala). *Journal of Agricultural and Food Chemistry, 53*, 9980–9984.

Del Río, L. A., Corpas, F. J., & Barroso, J. B. (2004). Nitric oxide and nitric oxide synthase activity in plants. *Phytochemistry, 65*, 783–792.

Dong, J., Zhang, M., Lu, L., Sun, L., & Xu, M. (2012). Nitric oxide fumigation stimulates flavonoid and phenolic accumulation and enhances antioxidant activity of mushroom. *Food chemistry, 135*(3), 1220–1225.

Duan, X., Su, X., You, Y., Qu, H., Li, Y., & Jiang, Y. (2007). Effect of nitric oxide on pericarp browning of harvested longan fruit in relation to phenolic metabolism. *Food Chemistry, 104*, 571–576.

Durner, J., Wendehenne, D., & Klessig, D. F. (1998). Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 10328–10333.

Eum, H. L., Kim, H. B., Choi, S. B., & Lee, S. K. (2009). Regulation of ethylene biosynthesis by nitric oxide in tomato (Solanum lycopersicum L.) fruit harvested at different ripening stages. *European Food Research and Technology, 228*, 331–338.

Flores, F. B., Sánchez-Bel, P., Valdenegro, M., Romojaro, F., Martínez-Madrid, M. C., & Egea, M. I. (2008). Effects of a pretreatment with nitric oxide on peach (Prunus persica L.) storage at room temperature. *European Food Research and Technology, 227*, 1599–1611.

García-Mata, C., & Lamattina, L. (2001). Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress. *Plant Physiology, 126*, 1196–1204.

García-Mata, C., & Lamattina, L. (2002). Nitric oxide and abscisic acid cross talk in guard cells. *Plant Physiology, 128*, 790–792.

Garcia-Mata, C., & Lamattina, L. (2007). Abscisic acid (ABA) inhibits light-induced stomatal opening through calcium-and nitric oxide-mediated signaling pathways. *Nitric Oxide, 17*, 143–151.

Hasanuzzaman, M., Hossain, M. A., & Fujita, M. (2010). Physiological and biochemical mechanisms of nitric oxide induced abiotic stress tolerance in plants. *American Journal of Plant Physiology, 5*, 295–324.

Hetherington, A. M., & Woodward, F. I. (2003). The role of stomata in sensing and driving environmental change. *Nature, 424*, 901–908.

Hou, Y., Janczuk, A., & Wang, P. (1999). Current trends in the development of nitric oxide donors. *Current Pharmaceutical Design, 5*, 417–442.

Hrabie, J. A., & Keefer, L. K. (2002). Chemistry of the nitric oxide-releasing diazeniumdiolate ("nitrosohydroxylamine") functional group and its oxygen-substituted derivatives. *Chemical Reviews, 102*, 1135–1154.

Huque, R., Wills, R. B. H., Pristijono, P., & Golding, J. B. (2013). Effect of nitric oxide (NO) and associated control treatments on the metabolism of fresh-cut apple slices in relation to development of surface browning. *Postharvest Biology and Technology, 78*, 16–23.

Jiang, T., Zheng, X., Li, J., Jing, G., Cai, L., & Ying, T. (2011). Integrated application of nitric oxide and modified atmosphere packaging to improve quality retention of button mushroom *(Agaricus bisporus). Food Chemistry, 126*(4), 1693–1699.

Kader, A. A. (2002). Biology and technology: An overview. *Postharvest Technology of Horticultural Crops, 3311*, 39–48.

Kader, A. A., & Saltveit, M. E. (2003). Respiration and gas exchange. *Postharvest Physiology and Pathology of Vegetables, 2*, 7–29.

Khan, A. S., & Singh, Z. (2007). 1-MCP regulates ethylene biosynthesis and fruit softening during ripening of 'Tegan Blue' plum. *Postharvest Biology and Technology, 43*, 298–306.

Klessig, D. F., Durner, J., Noad, R., Navarre, D. A., Wendehenne, D., Kumar, D., Zhou, J. M., Shah, J., Zhang, S., & Kachroo, P. (2000). Nitric oxide and salicylic acid signaling in plant defense. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 8849–8855.

Kopyra, M., & Gwóźdź, E. A. (2003). Nitric oxide stimulates seed germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of Lupinus luteus. *Plant Physiology and Biochemistry, 41*, 1011–1017.

Ku, V. V., Wills, R. B., & Leshem, Y. A. Y. (2000). Use of nitric oxide to reduce postharvest water loss from horticultural produce. *Journal of Horticultural Science and Biotechnology, 75*, 268–270.

Lamattina, L., García-Mata, C., Graziano, M., & Pagnussat, G. (2003). Nitric oxide: The versatility of an extensive signal molecule. *Annual Review of Plant Biology, 54*, 109–136.

Leshem, Y., & Wills, R. (1998). Harnessing senescence delaying gases nitric oxide and nitrous oxide: A novel approach to postharvest control of fresh horticultural produce. *Biologia Plantarum, 41*, 1–10.

Leshem, Y., Wills, R., & Ku, V. (2000). *Applications of nitric oxide (NO) for postharvest control*. 4th International Conference on Postharvest Science (Vol. 553, pp. 571–576).

Li-Qin, Z., Jie, Z., Shu-Hua, Z., & Lai-Hui, G. (2009). Inhibition of browning on the surface of peach slices by short-term exposure to nitric oxide and ascorbic acid. *Food Chemistry, 114*, 174–179.

Loscalzo, J., & Welch, G. (1995). Nitric oxide and its role in the cardiovascular system. *Progress in Cardiovascular Diseases, 38*, 87–104.

Manjunatha, G., Lokesh, V., & Neelwarne, B. (2010). Nitric oxide in fruit ripening: Trends and opportunities. *Biotechnology Advances, 28*, 489–499.

Millar, A. H., & Day, D. A. (1996). Nitric oxide inhibits the cytochrome oxidase but not the alternative oxidase of plant mitochondria. *FEBS Letters, 398*, 155–158.

Neill, S., Barros, R., Bright, J., Desikan, R., Hancock, J., Harrison, J., Morris, P., Ribeiro, D., & Wilson, I. (2008). Nitric oxide, stomatal closure, and abiotic stress. *Journal of Experimental Botany, 59*, 165–176.

Neill, S. J., Desikan, R., Clarke, A., & Hancock, J. T. (2002). Nitric oxide is a novel component of abscisic acid signaling in stomatal guard cells. *Plant Physiology, 128*, 13–16.

Neill, S. J., Desikan, R., & Hancock, J. T. (2003). Nitric oxide signalling in plants. *New Phytologist, 159*, 11–35.

Noodén, L. D., Guiamét, J. J., & John, I. (1997). Senescence mechanisms. *Physiologia Plantarum, 101*, 746–753.

Pagnussat, G. C., Simontacchi, M., Puntarulo, S., & Lamattina, L. (2002). Nitric oxide is required for root organogenesis. *Plant Physiology, 129*, 954–956.

Pristijono, P., Wills, R., & Golding, J. (2006). Inhibition of browning on the surface of apple slices by short term exposure to nitric oxide (NO) gas. *Postharvest Biology and Technology, 42*, 256–259.

Qiao, W., & Fan, L. M. (2008). Nitric oxide signaling in plant responses to abiotic stresses. *Journal of Integrative Plant Biology, 50*, 1238–1246.

Radi, R. (1996). Reactions of nitric oxide with metalloproteins. *Chemical Research in Toxicology, 9*, 828–835.

Radi, R., Beckman, J. S., Bush, K. M., & Freeman, B. A. (1991). Peroxynitrite-induced membrane lipid peroxidation: The cytotoxic potential of superoxide and nitric oxide. *Archives of Biochemistry and Biophysics, 288*, 481–487.

Rubbo, H., Radi, R., Trujillo, M., Telleri, R., Kalyanaraman, B., Barnes, S., Kirk, M., & Freeman, B. A. (1994). Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. Formation of novel nitrogen-containing oxidized lipid derivatives. *Journal of Biological Chemistry, 269*, 26066–26075.

Saltveit, M. E. (1999). Effect of ethylene on quality of fresh fruits and vegetables. *Postharvest Biology and Technology, 15*, 279–292.

Schuster, B., & Retey, J. (1995). The mechanism of action of phenylalanine ammonia-lyase: The role of prosthetic dehydroalanine. *Proceedings of the National Academy of Sciences of the United States of America, 92*, 8433–8437.

Shi, Q., Ding, F., Wang, X., & Wei, M. (2007). Exogenous nitric oxide protect cucumber roots against oxidative stress induced by salt stress. *Plant Physiology and Biochemistry, 45*, 542–550.

Simontacchi, M., García-Mata, C., Bartoli, C. G., Santa-María, G. E., & Lamattina, L. (2013). Nitric oxide as a key component in hormone-regulated processes. *Plant Cell Reports, 32*, 853–866.

Singh, S., Singh, Z., & Swinny, E. (2009). Postharvest nitric oxide fumigation delays fruit ripening and alleviates chilling injury during cold storage of Japanese plums (Prunus salicina Lindell). *Postharvest Biology and Technology, 53*, 101–108.

Soegiarto, L., & Wills, R. (2004). Short term fumigation with nitric oxide gas in air to extend the postharvest life of broccoli, green bean, and bok choy. *HortTechnology, 14*, 538–540.

Soheila, A.-H., John, C. F., Jordan, B., & Thomas, B. (2001). Early signaling components in ultraviolet-B responses: Distinct roles for different reactive oxygen species and nitric oxide. *FEBS Letters, 489*, 237–242.

Sozzi, G. O., Trinchero, G. D., & Fraschina, A. A. (2003). Delayed ripening of 'Bartlett' pears treated with nitric oxide. *Journal of Horticultural Science and Biotechnology, 78*, 899–903.

Stamler, J. S. (1994). Redox signaling: Nitrosylation and related target interactions of nitric oxide. *Cell, 78*, 931–936.

Tierney, D. L., Rocklin, A. M., Lipscomb, J. D., Que, L., & Hoffman, B. M. (2005). ENDOR studies of the ligation and structure of the non-heme iron site in ACC oxidase. *Journal of the American Chemical Society, 127*, 7005–7013.

Tomás-Barberán, F. A., & Espin, J. C. (2001). Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *Journal of the Science of Food and Agriculture, 81*, 853–876.

Uchida, A., Jagendorf, A. T., Hibino, T., Takabe, T., & Takabe, T. (2002). Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. *Plant Science, 163*, 515–523.

Wendehenne, D., Durner, J., & Klessig, D. F. (2004). Nitric oxide: A new player in plant signalling and defence responses. *Current Opinion in Plant Biology, 7*, 449–455.

Wendehenne, D., Pugin, A., Klessig, D. F., & Durner, J. (2001). Nitric oxide: Comparative synthesis and signaling in animal and plant cells. *Trends in Plant Science, 6*, 177–183.

Wills, R., Ku, V., & Leshem, Y. (2000). Fumigation with nitric oxide to extend the postharvest life of strawberries. *Postharvest Biology and Technology, 18*, 75–79.

Wills, R., Pristijono, P., & Golding, J. (2008). Browning on the surface of cut lettuce slices inhibited by short term exposure to nitric oxide (NO). *Food Chemistry, 107*, 1387–1392.

Wink, D. A., Hanbauer, I., Krishna, M. C., Degraff, W., Gamson, J., & Mitchell, J. B. (1993). Nitric oxide protects against cellular damage and cytotoxicity from reactive oxygen species. *Proceedings of the National Academy of Sciences of the United States of America, 90*, 9813–9817.

Wink, D. A., & Mitchell, J. B. (1998). Chemical biology of nitric oxide: Insights into regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide. *Free Radical Biology and Medicine, 25*, 434–456.

Wu, C.-T. (2010). An overview of postharvest biology and technology of fruits and vegetables. *Technology on reducing post harvest losses and maintaining quality of fruits and vegetables*, 2–11.

Xu, M., Dong, J., Zhang, M., Xu, X., & Sun, L. (2012). Cold-induced endogenous nitric oxide generation plays a role in chilling tolerance of loquat fruit during postharvest storage. *Postharvest biology and technology, 65*, 5–12.

Ya'acov, Y. L. (1996). Nitric oxide in biological systems. *Plant Growth Regulation, 18*, 155–159.

Ya'acov, Y. L., & Haramaty, E. (1996). The characterization and contrasting effects of the nitric oxide free radical in vegetative stress and senescence of Pisum sativum Linn. foliage. *Journal of Plant Physiology, 148*, 258–263.

Ya'acov, Y. L., & Pinchasov, Y. (2000). Non-invasive photoacoustic spectroscopic determination of relative endogenous nitric oxide and ethylene content stoichiometry during the ripening of strawberries Fragaria anannasa (Duch.) and avocados Persea americana (Mill.). *Journal of Experimental Botany, 51*, 1471–1473.

Ya'acov, Y. L., Wills, R. B., & Ku, V. V.-V. (1998). Evidence for the function of the free radical gas—nitric oxide (NO•)—as an endogenous maturation and senescence regulating factor in higher plants. *Plant Physiology and Biochemistry, 36*, 825–833.

Yang, H., Wu, F., & Cheng, J. (2011). Reduced chilling injury in cucumber by nitric oxide and the antioxidant response. *Food Chemistry, 127*, 1237–1242.

Yang, H., Zhou, C., Wu, F., & Cheng, J. (2010). Effect of nitric oxide on browning and lignification of peeled bamboo shoots. *Postharvest Biology and Technology, 57*(1), 72–76.

Zaharah, S., & Singh, Z. (2011). Postharvest nitric oxide fumigation alleviates chilling injury, delays fruit ripening and maintains quality in cold-stored 'Kensington Pride' mango. *Postharvest Biology and Technology, 60*, 202–210.

Zhang, M., Duan, L., Tian, X., He, Z., Li, J., Wang, B., & Li, Z. (2007). Uniconazole-induced tolerance of soybean to water deficit stress in relation to changes in photosynthesis, hormones and antioxidant system. *Journal of Plant Physiology, 164*, 709–717.

Zhu, S., Liu, M., & Zhou, J. (2006). Inhibition by nitric oxide of ethylene biosynthesis and lipoxygenase activity in peach fruit during storage. *Postharvest Biology and Technology, 42*, 41–48.

Zhu, S., Sun, L., Liu, M., & Zhou, J. (2008). Effect of nitric oxide on reactive oxygen species and antioxidant enzymes in kiwifruit during storage. *Journal of the Science of Food and Agriculture, 88*, 2324–2331.

Zhu, S., Sun, L., & Zhou, J. (2009). Effects of nitric oxide fumigation on phenolic metabolism of postharvest Chinese winter jujube (Zizyphus jujuba Mill. cv. Dongzao) in relation to fruit quality. *LWT-Food Science and Technology, 42*, 1009–1014.

Zhu, S.-H., & Zhou, J. (2007). Effect of nitric oxide on ethylene production in strawberry fruit during storage. *Food Chemistry, 100*, 1517–1522.

Chapter 3
Hydrogen Sulfide

A.E. Quirós-Sauceda, G.R. Velderrain-Rodríguez, M. Ovando-Martínez, M.G. Goñi, G.A. González-Aguilar, and J.F. Ayala-Zavala

Hydrogen Sulfide General Description

Hydrogen sulfide (H_2S) is a colorless, flammable, extremely hazardous gas with a strong odor of "rotten egg" that can be smelt at levels of 0.02 μl L^{-1} and higher (Fig. 3.1) (Lloyd 2006; Beauchamp et al. 1984). H_2S toxicity has been substantiated for almost 300 years due to its implication in several mass extinctions, including one at the end of the Permian period that wiped out more than three-quarters of all species on Earth (Kump et al. 2005; Erwin 1993). However, more recently, H_2S has been added to nitric oxide (NO) and carbon monoxide (CO) as a newly categorized group of biologically active gases termed gasotransmitters and gasomediators, due to its capacity to control a range of physiological responses in animals (Wang 2003; Mancardi et al. 2009). Many studies have revealed that H_2S in low concentrations can act as a signaling molecule in animals, and participate in various biological processes, such as smooth muscle, relaxation, brain development, blood pressure, and inflammation (Chen et al. 2011). Therefore, it is reasonable to suspect that this gas molecule has a similar role in plants and can affect a range of their physiological responses and could be used to control postharvest plant function (Zhang et al. 2010).

A.E. Quirós-Sauceda • G.R. Velderrain-Rodríguez • M. Ovando-Martínez
G.A. González-Aguilar • J.F. Ayala-Zavala (✉)
Centro de Investigacion en Alimentacion y Desarrollo, A.C. (CIAD, AC),
Carretera a la Victoria Km 0.6, La Victoria, Hermosillo, Sonora 83000, Mexico
e-mail: quirosanaelena@hotmail.com; tavo_velderrain@hotmail.com;
maribel.ovandom@gmail.com; gustavo@ciad.mx; jayala@ciad.mx

M.G. Goñi
Consejo Nacional de Ciencia y Tecnología (CONICET), Avenida Rivadavia, Argentina

Grupo de Investigación en Ingeniería de Alimentos, Facultad de Ingeniería, UNMdP,
Juan B. Justo 4302, Mar del Plata 7600, Argentina
e-mail: ggoni@fi.mdp.edu.ar

M.W. Siddiqui et al. (eds.), *Postharvest Management Approaches for Maintaining Quality of Fresh Produce*, DOI 10.1007/978-3-319-23582-0_3

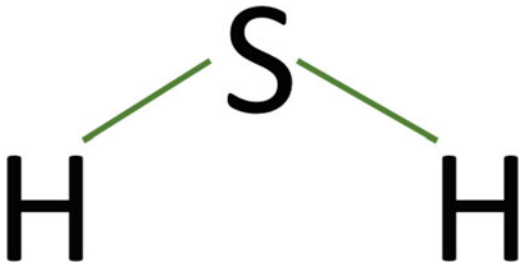


Fig. 3.1 Hydrogen sulfide chemical structure

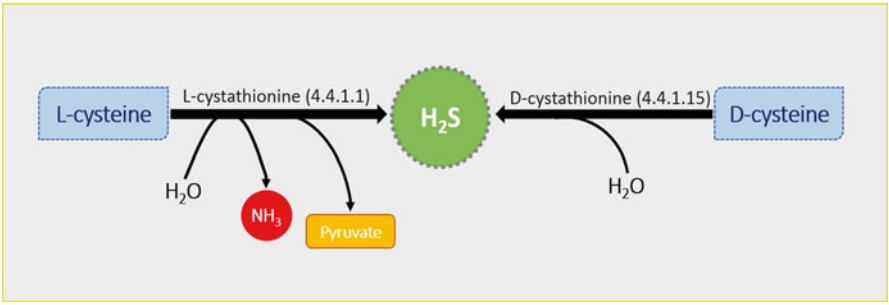


Fig. 3.2 Hydrogen sulfide metabolism in plants

H_2S is synthesized in mammalian tissues via endogenous enzymes and by non-enzymatic pathways (e.g., reduction of thiols and thiol-containing molecules) (Li et al. 2011). Moreover, there is evidence that H_2S in plants is released from cysteine via reversible *O*-acetylserine (thiol)lyase (OAS-TL) reaction, catalyzed by several L- and D-cysteine-specific desulfhydrase candidates. L-Cystathionine specifically metabolized L-cysteine to produce H_2S, pyruvate, and ammonium, while D-CYSTATHIONINE only decomposes D-cysteine and produces H_2S (Fig. 3.2) (Wirtz et al. 2004; Chen et al. 2011). Although the biochemical properties of the H_2S make it difficult to study, this gas can be measured in biological systems using a method based on the formation of methylene blue from sulfide and *N*,*N*-dimethyl-p-phenylenediamine in the presence of Fe^{3+} and its spectrophotometric detection at 675 nm (Hancock et al. 2012). Also, other methods include the use of gas chromatography which assess the release of H_2S and the use of fluorescent probes for the gas (Liu et al. 2011a; Sasakura et al. 2011). Such and other similar assays can be applied to detect H_2S in plants and their environments.

Hydrogen Sulfide in Plants

H_2S is often thought to be a phytotoxin, being harmful to the growth and development of plants. However, there is accumulating evidence that H_2S also could act as a gaseous regulator in plants. For example, 35 years ago it was found to inhibit oxygen release from young seedling of six rice cultivars. Likewise, in some cultivars of rice, nutrient uptake was also reduced, while in others it was increased. Phosphorous uptake was also inhibited in this plant species (Joshi et al. 1975). Also, a constant fumigation of H_2S (3000 parts per billion) caused lesions on leaves, defoliation, and reduced growth of *Medicago*, grapes, lettuce, sugars beets, and pine. Interestingly, lower levels of fumigation (100 part per billion) caused a significant increase in the growth of *Medicago*, lettuce, and sugar beet (Thompson and Kats 1978). More recently, a study showed that the H_2S donor NaSH would alleviate the osmotic-induced decrease in chlorophyll concentration in sweet potato. Furthermore, spraying NaSH increased the activity of the antioxidant enzymes superoxide dismutase, catalase, and ascorbate peroxidase while decreasing the concentration of reactive oxygen species (ROS) such as hydrogen peroxide and lipoxygenase (Zhang et al. 2009). These results clearly suggested that H_2S can have intracellular effects which impinge on cell signaling events in the plant cells.

For H_2S to have an effect on plants cells, it has to be present and in a high enough concentration. There are two main sources of H_2S to which a plant cell may wish to respond: from the environment or from within (Fig. 3.3). The aerial parts and roots of plants may be often exposed to atmospheric H_2S because it is commonly emitted

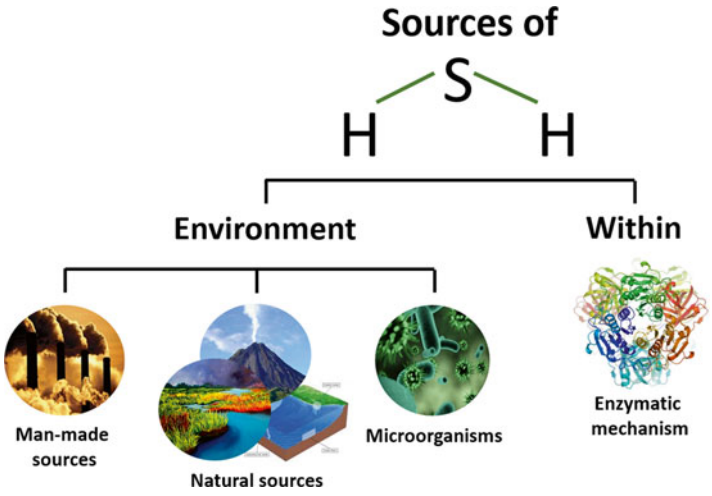


Fig. 3.3 Hydrogen sulfide sources

from many natural and man-made sources. Natural sources of H_2S include the discharge from volcanoes, coastal marine sediments, or anoxic soils such as found in marshland (Aiuppa et al. 2005; Hansen et al. 1978; Morse et al. 1987). Man-made sources of this gas are also common, and include waste treatment installations, agricultural industries, and geothermal power plants. It has also been found to be at surprisingly high concentrations in some urban environments with car catalytic converters, being suggested as a potential source (Zhang et al. 2008; Aneja et al. 2008; Bacci et al. 2000). However, the fact that plants respond to such exogenous sources of H_2S does not necessarily indicate that it has a signaling role. Often, the responses of the plants are associated to toxic levels of these compounds. After all, it is because of its phytotoxic effects at high levels that H_2S has become well known.

Some authors reported that to truly be a cell signaling molecule, H_2S must be generated by plant cells. Many species of plant have been found to generate H_2S using a light-dependent activity and includes cucumber, squash, pumpkin, soybean, and cotton, among other plants (Wilson et al. 1978). Intracellular sources of H_2S would include the production by enzymatic mechanisms. As mentioned before, it appears that the enzymes responsible for H_2S in plants are desulfhydrases. A plastid located cysteine desulfhydrase has been reported in *Arabidopsis*, while others report the presence of a similar enzyme in the mitochondria (Léon et al. 2002; Riemenschneider et al. 2005). However, activities of such enzymes are not static and have been shown to change after some circumstances, for example, pathogen challenge (Bloem et al. 2004). This would be expected if the enzymes are to perform a role in the creation of a molecule which is to act as a signal. Other enzymes have also been proposed as being able to generate H_2S too, and includes D-cysteine desulfhydrase that produces pyruvate, ammonia, and H_2S, and an enzyme involved in cyanide metabolism, β-cyanoalanine synthase, which converts cysteine and cyanide to *β*-cyanoalanine and H_2S (Papenbrock et al. 2007; García et al. 2010).

Bacteria have been shown to generate H_2S too. Microorganisms that are invading plants, such as pathogenic bacteria, may be able to release H_2S, which will then affect the activities of the plant. Recently, Bloem et al. (2012) reported that H_2S emissions were assessed in oilseed rape (*Brassica napus* L.) and after fungal infection with *Sclerotinia sclerotiorum*. It was found that infection caused a significant rise in H_2S release, but they also reported that under different conditions, depending on the air concentration and the sulfur demand of the plant that H_2S was sometimes taken up and not released. This may suggest that H_2S is important for sulfur metabolism rather than acting solely as a signal.

Hydrogen Sulfide as Regulator of Plant Physiological Processes

Plant physiology is concerned with the fundamental processes of plants, its survival, metabolic activities, water relations, mineral nutrition, development, movement, irritability, organization, growth, and transport processes (Nilsen and Orcutt 1996).

Table 3.1 Effects of hydrogen sulfide in plant physiological processes

Positive effects	Negative effects
• Increase antioxidant activity of enzymes • Abiotic stress tolerance • Root organogenesis • Resistance to drought and heavy metal toxicity • Stomatal aperture • Promote chloroplast biogenesis • Alleviates oxidative damage against osmotic stress • Promote seed germination	• Leaves' lesions, defoliation, and reduced growth • Decrease chlorophyll concentration • Inhibition of photosynthesis • Decrease germination time

Likewise, a lot of physiological processes of plants are regulated by interactions among different plant growth molecules due to plant cells are able to sense and respond to a wide range of external and internal signals. Plant regulators are considered organic compounds, other than nutrients, which in small amounts promote, inhibit, or otherwise affect the physiological processes of plants (Moore 1979).

Although at present there is no direct evidence that H_2S acts as an endogenous regulator or signal molecule in plants, the induction of L-cysteine desulfhydrase upon pathogen attack, emission of H_2S from plants exposed to SO_2 injury, abiotic stress tolerance in plants supplied with endogenous H_2S donor, and its involvement in guard cell signaling and root organogenesis, all suggest that this is indeed the case (Hällgren and Fredriksson 1982; Bloem et al. 2004; García-Mata and Lamattina 2010). Moreover, it is now known that H_2S causes inhibition of photosynthesis at high concentrations and that it can decrease the time to germination, but also increases the resilience to drought and heavy metal toxicity (Lisjak et al. 2011; Chen et al. 2011; Oren et al. 1979; Dooley et al. 2013). Recent emerging evidence has also suggested a possible signaling role for stomatal apertures, and in promoting chloroplast biogenesis (Hancock et al. 2012; García-Mata and Lamattina 2010; Chen et al. 2011). In this sense, several research groups are now focusing on H_2S and its role as a signal in plants (Table 3.1).

Mediator of Stomatal Movements

Stomata are by far the most influential components in gas exchange and their movements control transpiration. Consequently, stomata are important regulators of plant growth and development (Liu et al. 2011b). Although previous studies have shown stomata respond to a variety of environmental stresses, such as drought, cold, high CO_2 concentration, and phytohormone, little is known about the signal transduction mechanisms that function in guard cells. The effects of H_2S as a newly identified signal molecule on regulation of stomatal aperture have been recently emerging.

Liu et al. (2011b) reported that H_2S and NO are involved in the signal transduction pathway of ethylene-induced stomatal closure, and that in *Arabidopsis*, H_2S may represent a novel downstream indicator of NO during ethylene-induced stomatal movement. Other studies have taken advantage of the use of H_2S donors. The most used donor is the compound sodium hydrosulfide (NaSH) which will dissociate rapidly to generate a very short burst of H_2S. However, H_2S can be relatively high if high concentrations of NaSH are used. Lisjak et al. (2011) showed a H2S-mediated stomatal opening in *Arabidopsis*. This was seen in plants treated with both NaSH, giving a relatively short burst of H_2S, or with GYY4137 (a forerunner) giving a longer more prolonged exposure to H_2S. With leaves which had not been pre-opened in the light, the effects of both NaSH and GYY4137 were larger. This work was repeated in *Capsicum annuum* and similar opening was induced by the treatment with both H_2S donors. Other work has shown that stomatal conductance was increased by carbonyl sulfide (COS) and it was suggested that H_2S mediates this effect which produced from COS hydrolysis. However, clearly further work is required (Stimler et al. 2010).

On the other hand, García-Mata and Lamattina (2010) reported a different effect of H_2S on stomatal movements. They found that exogenous H_2S induces stomatal closure and this effect is impaired by (1) the ATP-binding cassette (ABC) transporter inhibitor glibenclamide; (2) scavenging H_2S or inhibition of the enzyme responsible for endogenous H_2S synthesis partially blocks ABA-dependent stomatal closure; and (3) H_2S treatment increases relative water content and protects plants against drought stress. In conclusion, their results indicate that H_2S induces stomatal closure and participates in ABA-dependent signaling, possibly through the regulation of ABC transporters in guard cells. Jin et al. (2011) showed similar results, where exogenous H_2S released by its donors induced stomatal closure in *Arabidopsis*. Also, Hou et al. (2013) suggest that the enzyme D-/L-cysteine desulfhydrase that generated H_2S is involved in the regulation of ethylene-induced stomatal closure in *Arabidopsis*.

Regulation of Senescence

Plant senescence is considered as a highly regulated physiological process that leads to plant death (Thomas et al. 2003). Accumulating evidence shows that H_2S plays various physiological roles in plants, such as senescence of cut flowers. Zhang et al. (2011) reported that H_2S was found to delay flower opening and senescence in various cut flowers and branches. Cut explants of these plants were cultured in solution containing different concentrations of the H_2S donor, NaHS. H_2S donor treatment prolonged the vase time of cut flowers and prevented senescence in a dose-dependent manner. Also, they measured the levels of malondialdehyde (MDA) as an indicator of oxidative damage to cells and showed that it was inversely related to endogenous H_2S concentration in explants. Flowers that had senesced showed higher levels of MDA and lower amounts of H_2S. Furthermore, NaHS treatment

increased the activities of catalase, superoxide dismutase, ascorbate peroxidase, and guaiacol peroxidase, and sustained much lower levels of H_2O_2 and O_2^- in cut flowers. In conclusion, the study implies that H_2S is involved in improving longevity of cut flowers and functions in activity of antioxidant enzymes in plants.

Moreover, H_2S participation in the regulation of ripening and senescence in postharvest fruits remains unknown. A study investigated the effect of H_2S on postharvest shelf life and antioxidant metabolism in strawberry fruits. Fumigation with H_2S gas released from the H_2S donor NaHS prolonged postharvest shelf life of strawberry fruits in a dose-dependent manner. Strawberry fruits fumigated with various concentrations of H_2S sustained significantly lower rot index and higher fruit firmness, and kept lower respiration intensity and polygalacturonase activities than controls. Further investigation showed that H_2S treatment maintained higher activities of catalase, guaiacol peroxidase, ascorbate peroxidase, and glutathione reductase and lower activities of lipoxygenase relative to untreated controls. H_2S also reduced malondialdehyde, hydrogen peroxidase, and superoxide anion to levels below control fruits during storage. Moreover, H_2S treatment maintained higher contents of reducing sugars, soluble proteins, free amino acid, and endogenous H_2S in fruits. These data indicated that H_2S plays an antioxidative role in prolonging postharvest shelf life and senescence of strawberry fruits (Hu et al. 2012).

Photosynthetic Response

It is well known that the increase in photosynthesis can be achieved by enhancing the activity of ribulose-1, 5-bisphosphate carboxylase (RuBISCO) (Krantev et al. 2008). Changes in RuBISCO synthesis have been primarily explained by changes in transcript abundance of its genes in response to various external and/or internal signals (Nishimura et al. 2008; Suzuki et al. 2010). In addition, the oxidation of glycolate to glyoxylate in higher plants is catalyzed by glycolate oxidase, which is located in the peroxisomes and performs an essential role in the oxidative photorespiration cycle accompanying photosynthetic CO_2 assimilation (Zelitch et al. 2009). Meanwhile, photorespiration also involves a cooperative interaction among enzymes localized in chloroplasts, mitochondria, and peroxisomes, and is performed by the glycolate pathway (Yamaguchi and Nishimura 2000). A previous study showed the effect of H_2S as a biologically active gas on photosynthesis, it was reported that excess sulfide (1 mM) resulted in inhibition of photosystem II (PSII) in cyanobacteria and tobacco chloroplasts (Oren et al. 1979) and that a high sulfide concentration (2 mM) depressed the growth and photosynthesis in a mangrove plant (Lin and Sternberg 1992). However, it is not clear whether a low concentration of H_2S is involved in regulation of photosynthesis in plants.

Chen et al. (2011) used a NaHS as a donor of H_2S to understand further the roles of this gas in physiological processes of photosynthesis and grana lamella formation in *S. oleracea*. The results indicated that photosynthesis, RuBISCO, OAS-TL, and L-cysteine desulfhydrase activities and other photosynthetic characteristics were

altered by exogenous application of a low concentration of NaHS. The number of grana lamellae stacking into functional chloroplasts was also increased markedly. Furthermore, it was demonstrated that seedlings treated with 100 μM NaHS increased the expression of RuBISCO genes but significantly decreased the gene expression of glycolate oxidase and cytochrome oxidase. They concluded that H_2S acts as a signaling molecule that participates in enhancing photosynthesis and chloroplast development during *S. oleracea* growth.

On the other hand, highly reducing sediments are prevalent in sea grass environments. Under anoxic conditions, H_2S can accumulate as an end product of anaerobic respiration at levels which may be toxic to halophytes. The photosynthetic response of *Zostera marina* L. (eelgrass) to manipulations in sediment sulfide concentration and light regimes was examined by Goodman et al. (1995). Sediment sulfide levels were enriched using Na_2S and lowered using $FeSO_4$. Photosynthesis vs. irradiance relationships were determined experimentally at ten light levels throughout the 21 day experiment. Photoadaptation was detected in response to the previous 4-day light history of the plants, as maximum photosynthesis decreased in response to lower daily light levels. Negative impacts of sulfide on eelgrass in this study were observed, increases in the light intensity at which gross photosynthesis equals respiration, and decreases in the initial slope of the photosynthesis-irradiance curve. The effects of eutrophication through reduced light and increased sediment sulfide were additive. Elevated sediment sulfide levels may contribute to sea grass loss in stressed areas as the potential for utilization of available light is reduced.

Pre- and Postharvest Applications of Hydrogen Sulfide

Due to the abovementioned roles that H_2S plays in various physiological roles in plants, new studies are trying to apply this chemical as a novel pre- and postharvest technology applied in fresh produce to control their physiological processes. Its application in fresh produce could be through H_2S donors, such as NaHS, or saturated solutions obtained by passing H_2S gas into carbon dioxide-free water (Zhu et al. 2014; Li et al. 2012). However, both H_2S application methods obtain similar results as it is reported by several studies. For example, Hu et al. (2014a) stated that when H_2S is applied to mulberry fruits using an aqueous solution of NaHS, it could result in a decrease in the ripening rate as well as a lowering in the respiration intensity and anthocyanin content. Likewise, Zhu et al. (2014) applied H_2S in kiwifruits by dipping them in gas-saturated water solutions. They found that some antioxidant-related enzymes, such as CAT, SOD, APX, and POD significantly increased after H_2S treatment, delaying its senescence.

This small gas, along with others such as carbon monoxide (CO) and nitric oxide (NO), has been target of novel research projects with the purpose of developing new postharvest technologies (Abdollahi et al. 2013; Li et al. 2014; Fu et al. 2014). Some of the oldest pesticides, such as calcium polysulfide, can release H_2S, especially when the pesticide solution is acidified (Smilanick and Sorenson 2001). The

knowledge provided by the elucidation of role of H_2S in the above mentioned physiological responses "stomatal movements" and "photosynthetic responses," is essential to the novel technologies applied to alleviate abiotic types of stress and provide tolerance in plants (Shi et al. 2013). For example, Zhang et al. (2009) found that the application of NaHS as H_2S donor can alleviate osmotic stress and prevent chlorophyll losses in seedling leaves of sweet potato (*Ipomoea batatas*). However, and excessive air exposure to H_2S (3000 parts per billion) could result in leaves lesions, defoliation, and reduced growth of plants (Li et al. 2013). Moreover, at proper concentrations, H_2S could act as a powerful preharvest technology alleviating even root tip death in pea seedlings induced by flooding of soils (Cheng et al. 2013).

On the other hand, H_2S can also be successfully applied as postharvest technology to preserve fresh produce. In this context, a recent study suggested that the application of aqueous solutions of NaHS (0.5–2.5 mM) releases about 0.05–0.5 ppm of H_2S gas in a sealed container, which was enough to prolong the storage of pears at 20 °C (Hu et al. 2014b). Moreover, Zhu et al. (2014) found that treatments with 45 and 90 H_2S, applied as a saturated aqueous solution, could delay maturation and senescence of kiwifruits and maintain higher quality attributes. These results suggest that H_2S could be applied as pre- and postharvest technology to plants and their fruits, either if they are climacteric or non-climacteric fruits.

Shelf Life Prolongation of Food Products

The shelf life of fresh produce is one of the most important objectives of food industry and many research groups around the world. In that sense, the application of natural compounds as technology to preserve foods has been suggested by several authors (González-Aguilar et al. 2010; Mastromatteo et al. 2010; Juneja et al. 2012). Applications of H_2S to fresh produce reduce the damage of ROS by upregulating antioxidant enzymes (Zhu et al. 2014). According to Hu et al. (2012), strawberry fruits fumigated with H_2S maintained higher contents of reducing sugars, soluble proteins, free amino acids, and endogenous H_2S due to the antioxidative role in the postharvest life of this fruits. The same protective effect was observed by Hu et al. (2014a) in a study where they applied NaSH as H_2S donor to mulberry fruits resulting in an enhancement of antioxidant enzyme activity. Likewise, Fu et al. (2014) suggested that besides the enhancement in the antioxidant enzyme activity in fruits after treatment, H_2S acts also as an antifungal agent inhibiting the growth of *Saccharomyces cerevisiae*, *Rhizopus oryzae*, *Candida albicans*, and several foodborne bacteria.

Hu et al. (2014b) reported similar inhibition of pathogens *Aspergillus niger* and *Penicillium expansum* in pear fruits after fumigation with H_2S. These antimicrobial and antifungal properties are similar to those reported to NO, which has shown growth inhibition of *Aspergillus niger*, *Monilinia fructicola*, *Penicillium italicum*, and *Rhizopus nigricans* (Manjunatha et al. 2010). As both gases have similar chemical properties, both enhance antioxidant enzyme activity, inhibit pathogens, and

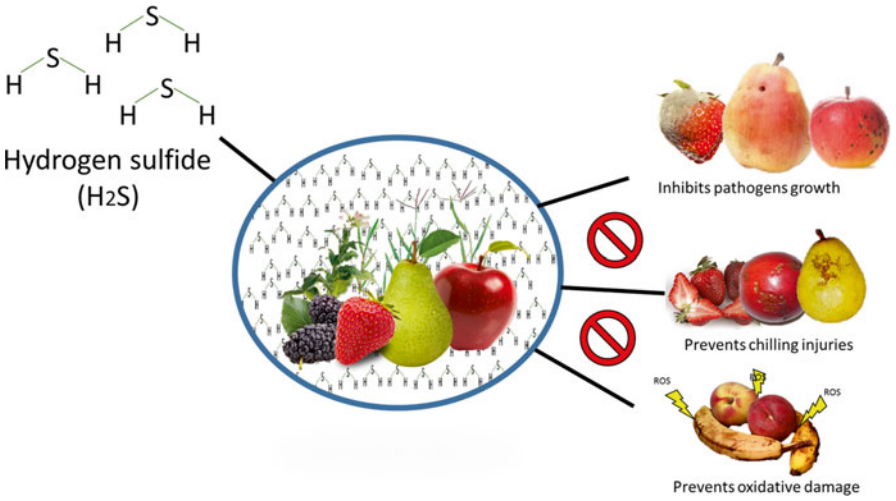


Fig. 3.4 Benefits of hydrogen sulfide application to postharvest shelf life of fruits

also protect fresh produce against chilling injury (Luo et al. 2015; Zaharah and Singh 2011) as well as remarkable benefits to the enhancement of fresh produce when applied, both could be applied in combination to achieve a cooperative effect in the shelf life extension of food products. For example, Chang et al. (2014) observed that there was a greater effect prolonging the postharvest shelf life of strawberry fruit combining H_2S and NO than that effect observed when used separately (Fig. 3.4).

Conclusions and Future Trends

So far, the role of H_2S molecules has been underestimated because this was considered an undesirable phytotoxin which causes deleterious effects on plant growth. However, it has been demonstrated through diverse research projects that, in proper amounts, H_2S could perform signaling functions that drive or enhance physiological responses in plant tissues. These recent breakthroughs over the truly effects of H_2S are leading to the development of novel technologies to preserve or enhance diverse quality attributes in plant tissues, or as well directly over fresh produce prolonging its postharvest shelf life storage. Likewise, besides the prevention of spoilage microorganism growth, the effect of H_2S as a signaling molecule triggers a series of events that induce and enhance the activity of antioxidant enzymes which may result in an added value to the plant tissue of food product treated.

- The studies about the synergistic effects of H_2S with other gases or signaling molecules are scarce and should be addressed in order to properly understand how H_2S could interact with other natural molecules and its biological effect.

- There's a lack of studies that evaluate the effect over firmness and color and bioactive compound content changes after H_2S treatments.
- Further studies should be addressing the use of H_2S molecules in emergent technologies such as MAP or active packaging to prolong shelf life of either climacteric or non-climacteric fruits and vegetables.

References

Abdollahi, R., Asghari, M., Esmaiili, M., & Abdollahi, A. (2013). Postharvest nitric oxide treatment effectively reduced decays of selva strawberry fruit. *Nitric Oxide, 3*, 5.459.

Aiuppa, A., Inguaggiato, S., Mcgonigle, A., O'Dwyer, M., Oppenheimer, C., Padgett, M., et al. (2005). H 2 S fluxes from Mt. Etna, Stromboli, and Vulcano (Italy) and implications for the sulfur budget at volcanoes. *Geochimica et Cosmochimica Acta, 69*, 1861–1871.

Aneja, V. P., Schlesinger, W. H., & Erisman, J. W. (2008). Farming pollution. *Nature Geoscience, 1*, 409–411.

Bacci, E., Gaggi, C., Lanzillotti, E., Ferrozzi, S., & Valli, L. (2000). Geothermal power plants at Mt. Amiata (Tuscany–Italy): Mercury and hydrogen sulphide deposition revealed by vegetation. *Chemosphere, 40*, 907–911.

Beauchamp, R., Bus, J. S., Popp, J. A., Boreiko, C. J., Andjelkovich, D. A., & Leber, P. (1984). A critical review of the literature on hydrogen sulfide toxicity. *CRC Critical Reviews in Toxicology, 13*, 25–97.

Bloem, E., Haneklaus, S., Kesselmeier, J. R., & Schnug, E. (2012). Sulfur fertilization and fungal infections affect the exchange of H2S and COS from agricultural crops. *Journal of Agricultural and Food Chemistry, 60*, 7588–7596.

Bloem, E., Riemenschneider, A., Volker, J., Papenbrock, J., Schmidt, A., Salac, I., et al. (2004). Sulphur supply and infection with Pyrenopeziza brassicae influence L-cysteine desulphydrase activity in Brassica napus L. *Journal of Experimental Botany, 55*, 2305–2312.

Chang, Z., Jingying, S., Liqin, Z., Changle, L., & Qingguo, W. (2014). Cooperative effects of hydrogen sulfide and nitric oxide on delaying softening and decay of strawberry. *International Journal of Agricultural and Biological Engineering, 7*, 114–122.

Chen, J., Wu, F.-H., Wang, W.-H., Zheng, C.-J., Lin, G.-H., Dong, X.-J., et al. (2011). Hydrogen sulphide enhances photosynthesis through promoting chloroplast biogenesis, photosynthetic enzyme expression, and thiol redox modification in Spinacia oleracea seedlings. *Journal of Experimental Botany, 62*, 4481–4493.

Cheng, W., Zhang, L., Jiao, C., Su, M., Yang, T., Zhou, L., et al. (2013). Hydrogen sulfide alleviates hypoxia-induced root tip death in Pisum sativum. *Plant Physiology and Biochemistry, 70*, 278–286.

Dooley, F. D., Wyllie-Echeverria, S., Roth, M. B., & Ward, P. D. (2013). Tolerance and response of Zostera marina seedlings to hydrogen sulfide. *Aquatic Botany, 105*, 7–10.

Erwin, D. H. (1993). *The great Paleozoic crisis; life and death in the Permian* (Critical moments in paleobiology and earth history series). New York: Columbia University Press.

Fu, L.-H., Hu, K.-D., Hu, L.-Y., Li, Y.-H., Hu, L.-B., Yan, H., et al. (2014). An antifungal role of hydrogen sulfide on the postharvest pathogens *Aspergillus niger* and *Penicillium italicum. PloS One, 9*, e104206.

García, I., Castellano, J. M., Vioque, B., Solano, R., Gotor, C., & Romero, L. C. (2010). Mitochondrial β-cyanoalanine synthase is essential for root hair formation in Arabidopsis thaliana. *The Plant Cell Online, 22*, 3268–3279.

García-Mata, C., & Lamattina, L. (2010). Hydrogen sulphide, a novel gasotransmitter involved in guard cell signalling. *New Phytologist, 188*, 977–984.

González-Aguilar, G. A., Ayala-Zavala, J., Olivas, G., DE LA Rosa, L., & Álvarez-Parrilla, E. (2010). Preserving quality of fresh-cut products using safe technologies. *Journal für Verbraucherschutz und Lebensmittelsicherheit, 5*, 65–72.

Goodman, J. L., Moore, K. A., & Dennison, W. C. (1995). Photosynthetic responses of eelgrass (Zostera marina L.) to light and sediment sulfide in a shallow barrier island lagoon. *Aquatic Botany, 50*, 37–47.

Hällgren, J.-E., & Fredriksson, S.-Å. (1982). Emission of hydrogen sulfide from sulfur dioxide-fumigated pine trees. *Plant Physiology, 70*, 456–459.

Hancock, J. T., Lisjak, M., Teklic, T., Wilson, I. D., & Whiteman, M. (2012). Hydrogen sulphide and signalling in plants. *Plant Sciences Reviews, 2011*, 33.

Hansen, M. H., Ingvorsen, K., & Jøgensen, B. B. (1978). Mechanisms of hydrogen sulfide release from coastal marine sediments to the atmosphere. *Limnology and Oceanography, 23*, 68–76.

Hou, Z., Wang, L., Liu, J., Hou, L., & Liu, X. (2013). Hydrogen sulfide regulates ethylene-induced stomatal closure in Arabidopsis thaliana. *Journal of Integrative Plant Biology, 55*, 277–289.

Hu, L.-Y., Hu, S.-L., Wu, J., Li, Y.-H., Zheng, J.-L., Wei, Z.-J., et al. (2012). Hydrogen sulfide prolongs postharvest shelf life of strawberry and plays an antioxidative role in fruits. *Journal of Agricultural and Food Chemistry, 60*, 8684–8693.

Hu, H., Shen, W., & Li, P. (2014a). Effects of hydrogen sulphide on quality and antioxidant capacity of mulberry fruit. *International Journal of Food Science & Technology, 49*, 399–409.

Hu, K.-D., Wang, Q., Hu, L.-Y., Gao, S.-P., Wu, J., Li, Y.-H., et al. (2014b). Hydrogen sulfide prolongs postharvest storage of fresh-cut pears (Pyrus pyrifolia) by alleviation of oxidative damage and inhibition of fungal growth. *PloS One, 9*, e85524.

Jin, Z., Shen, J., Qiao, Z., Yang, G., Wang, R., & Pei, Y. (2011). Hydrogen sulfide improves drought resistance in Arabidopsis thaliana. *Biochemical and Biophysical Research Communications, 414*, 481–486.

Joshi, M., Ibrahim, I., & Hollis, J. (1975). Hydrogen sulfide: Effects on the physiology of rice plants and relation to straighthead disease. *Phytopathology, 65*, 1165–1170.

Juneja, V. K., Dwivedi, H. P., & Yan, X. (2012). Novel natural food antimicrobials*. *Annual Review of Food Science and Technology, 3*, 381–403.

Krantev, A., Yordanova, R., Janda, T., Szalai, G., & Popova, L. (2008). Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants. *Journal of Plant Physiology, 165*, 920–931.

Kump, L. R., Pavlov, A., & Arthur, M. A. (2005). Massive release of hydrogen sulfide to the surface ocean and atmosphere during intervals of oceanic anoxia. *Geology, 33*, 397–400.

Léon, S., Touraine, B., Briat, J., & Lobréaux, S. (2002). The AtNFS2 gene from Arabidopsis thaliana encodes a NifS-like plastidial cysteine desulphurase. *Biochemical Journal, 366*, 557–564.

Li, Z.-G., Gong, M., Xie, H., Yang, L., & Li, J. (2012). Hydrogen sulfide donor sodium hydrosulfide-induced heat tolerance in tobacco (Nicotiana tabacum L) suspension cultured cells and involvement of Ca 2+ and calmodulin. *Plant Science, 185*, 185–189.

Li, L., Rose, P., & Moore, P. K. (2011). Hydrogen sulfide and cell signaling. *Annual Review of Pharmacology and Toxicology, 51*, 169–187.

Li, Z. G., Yang, S. Z., Long, W. B., Yang, G. X., & Shen, Z. Z. (2013). Hydrogen sulphide may be a novel downstream signal molecule in nitric oxide-induced heat tolerance of maize (Zea mays L.) seedlings. *Plant, Cell & Environment, 36*, 1564–1572.

Li, Q., Zhang, S., & Mao, Y. (2014). Effect of carbon monoxide on active oxygen metabolism of postharvest jujube. *Journal of Food Technology Research, 1*, 146–155.

Lin, G., & Sternberg, L. D. S. (1992). Effect of growth form, salinity, nutrient and sulfide on photosynthesis, carbon isotope discrimination and growth of red mangrove (Rhizophora mangle L.). *Functional Plant Biology, 19*, 509–517.

Lisjak, M., Teklić, T., Wilson, I. D., Wood, M., Whiteman, M., & Hancock, J. T. (2011). Hydrogen sulfide effects on stomatal apertures. *Plant Signaling & Behavior, 6*, 1444–1446.

Liu, J., Hou, L., Liu, G., Liu, X., & Wang, X. (2011a). Hydrogen sulfide induced by nitric oxide mediates ethylene-induced stomatal closure of Arabidopsis thaliana. *Chinese Science Bulletin, 56*, 3547–3553.

Liu, C., Pan, J., Li, S., Zhao, Y., Wu, L. Y., Berkman, C. E., et al. (2011b). Capture and visualization of hydrogen sulfide by a fluorescent probe. *Angewandte Chemie, 123*, 10511–10513.

Lloyd, D. (2006). Hydrogen sulfide: Clandestine microbial messenger? *Trends in Microbiology, 14*, 456–462.

Luo, Z., Li, D., Du, R., & Mou, W. (2015). Hydrogen sulfide alleviates chilling injury of banana fruit by enhanced antioxidant system and proline content. *Scientia Horticulturae, 183*, 144–151.

Mancardi, D., Penna, C., Merlino, A., Del Soldato, P., Wink, D. A., & Pagliaro, P. (2009). Physiological and pharmacological features of the novel gasotransmitter: Hydrogen sulfide. *Biochimica et Biophysica Acta-Bioenergetics, 1787*, 864–872.

Manjunatha, G., Lokesh, V., & Neelwarne, B. (2010). Nitric oxide in fruit ripening: Trends and opportunities. *Biotechnology Advances, 28*, 489–499.

Mastromatteo, M., Conte, A., & DEL Nobile, M. (2010). Combined use of modified atmosphere packaging and natural compounds for food preservation. *Food Engineering Reviews, 2*, 28–38.

Moore, T. C. (1979). *Biochemistry and physiology of plant hormones*. New York: Springer.

Morse, J. W., Millero, F. J., Cornwell, J. C., & Rickard, D. (1987). The chemistry of the hydrogen sulfide and iron sulfide systems in natural waters. *Earth-Science Reviews, 24*, 1–42.

Nilsen, E. T., & Orcutt, D. M. (1996). *Physiology of plants under stress Abiotic factors*. New York: John Wiley and Sons Inc.

Nishimura, K., Ogawa, T., Ashida, H., & Yokota, A. (2008). Molecular mechanisms of RuBisCO biosynthesis in higher plants. *Plant Biotechnology, 25*, 285–290.

Oren, A., Padan, E., & Malkin, S. (1979). Sulfide inhibition of photosystem II in cyanobacteria (blue-green algae) and tobacco chloroplasts. *Biochimica et Biophysica Acta-Bioenergetics, 546*, 270–279.

Papenbrock, J., Riemenschneider, A., Kamp, A., Schulz-Vogt, H. N., & Schmidt, A. (2007). Characterization of cysteine-degrading and H2S-releasing enzymes of higher plants-from the field to the test tube and back. *Plant Biology, 9*, 582–588.

Riemenschneider, A., Wegele, R., Schmidt, A., & Papenbrock, J. (2005). Isolation and characterization of a D-cysteine desulfhydrase protein from Arabidopsis thaliana. *FEBS Journal, 272*, 1291–1304.

Sasakura, K., Hanaoka, K., Shibuya, N., Mikami, Y., Kimura, Y., Komatsu, T., et al. (2011). Development of a highly selective fluorescence probe for hydrogen sulfide. *Journal of the American Chemical Society, 133*, 18003–18005.

Shi, H., Ye, T., & Chan, Z. (2013). Exogenous application of hydrogen sulfide donor sodium hydrosulfide enhanced multiple abiotic stress tolerance in bermudagrass (Cynodon dactylon (L). Pers.). *Plant Physiology and Biochemistry, 71*, 226–234.

Smilanick, J., & Sorenson, D. (2001). Control of postharvest decay of citrus fruit with calcium polysulfide. *Postharvest Biology and Technology, 21*, 157–168.

Stimler, K., Montzka, S. A., Berry, J. A., Rudich, Y., & Yakir, D. (2010). Relationships between carbonyl sulfide (COS) and CO2 during leaf gas exchange. *New Phytologist, 186*, 869–878.

Suzuki, Y., Kihara-Doi, T., Kawazu, T., Miyake, C., & Makino, A. (2010). Differences in Rubisco content and its synthesis in leaves at different positions in Eucalyptus globulus seedlings. *Plant, Cell & Environment, 33*, 1314–1323.

Thomas, H., Ougham, H. J., Wagstaff, C., & Stead, A. D. (2003). Defining senescence and death. *Journal of Experimental Botany, 54*, 1127–1132.

Thompson, C. R., & Kats, G. (1978). Effects of continuous hydrogen sulfide fumigation on crop and forest plants. *Environmental Science & Technology, 12*, 550–553.

Wang, R. (2003). The gasotransmitter role of hydrogen sulfide. *Antioxidants and Redox Signaling, 5*, 493–501.

Wilson, L. G., Bressan, R. A., & Filner, P. (1978). Light-dependent emission of hydrogen sulfide from plants. *Plant Physiology, 61*, 184–189.

Wirtz, M., Droux, M., & Hell, R. (2004). O-acetylserine (thiol) lyase: An enigmatic enzyme of plant cysteine biosynthesis revisited in Arabidopsis thaliana. *Journal of Experimental Botany, 55*, 1785–1798.

Yamaguchi, K., & Nishimura, M. (2000). Reduction to below threshold levels of glycolate oxidase activities in transgenic tobacco enhances photoinhibition during irradiation. *Plant and Cell Physiology, 41*, 1397–1406.

Zaharah, S., & Singh, Z. (2011). Postharvest nitric oxide fumigation alleviates chilling injury, delays fruit ripening and maintains quality in cold-stored 'Kensington Pride' mango. *Postharvest Biology and Technology, 60*, 202–210.

Zelitch, I., Schultes, N. P., Peterson, R. B., Brown, P., & Brutnell, T. P. (2009). High glycolate oxidase activity is required for survival of maize in normal air. *Plant Physiology, 149*, 195–204.

Zhang, L., DE Schryver, P., DE Gusseme, B., DE Muynck, W., Boon, N., & Verstraete, W. (2008). Chemical and biological technologies for hydrogen sulfide emission control in sewer systems: A review. *Water Research, 42*, 1–12.

Zhang, H., Hu, S.-L., Zhang, Z.-J., Hu, L.-Y., Jiang, C.-X., Wei, Z.-J., et al. (2011). Hydrogen sulfide acts as a regulator of flower senescence in plants. *Postharvest Biology and Technology, 60*, 251–257.

Zhang, H., Tan, Z. Q., Hu, L. Y., Wang, S. H., Luo, J. P., & Jones, R. L. (2010). Hydrogen sulfide alleviates aluminum toxicity in germinating wheat seedlings. *Journal of Integrative Plant Biology, 52*, 556–567.

Zhang, H., Ye, Y.-K., Wang, S.-H., Luo, J.-P., Tang, J., & Ma, D. F. (2009). Hydrogen sulfide counteracts chlorophyll loss in sweetpotato seedling leaves and alleviates oxidative damage against osmotic stress. *Plant Growth Regulation, 58*, 243–250.

Zhu, L., Wang, W., Shi, J., Zhang, W., Shen, Y., Du, H., et al. (2014). Hydrogen sulfide extends the postharvest life and enhances antioxidant activity of kiwifruit during storage. *Journal of the Science of Food and Agriculture, 94*, 2699–2704.

Chapter 4
Salicylic Acid

Kalyan Barman, Swati Sharma, Pushpa Kumari, and Mohammed Wasim Siddiqui

Introduction

Fresh fruits and vegetables are highly perishable after harvest which causes a significant loss of the harvested produce. Synthetic chemicals and fungicides are repeatedly used for several years to minimize the decay and postharvest loss of fruits and vegetables. However, indiscriminate and continuous application of chemicals over the produce and its harmful effects on human health and environment lead the scientists to search safe compounds that might be helpful in preserving the quality of harvested produce. Moreover, repeated application of synthetic fungicides may develop pathogen resistance against the synthetic fungicides.

Salicylic acid (SA) or monohydroxy benzoic acid belongs to a group of phenolic compounds which is present ubiquitously throughout the plant kingdom. It is an endogenous signal molecule, involved in regulating a variety of physiological processes in plants. SA plays an important role in regulating several plant developmental processes like thermogenesis, photosynthesis, respiration, transpiration, stomatal closure, seed germination, cell growth, sex polarization, ion uptake and transport, disease resistance, senescence-associated gene expression, and crop yield (Klessig and Malamy 1994; Clarke et al. 2004; Morris et al. 2000; Rajou et al. 2006; Harper and Balke 1981; Khan et al. 2003). SA is a major component of signal transduction

K. Barman (✉) • P. Kumari
Department of Horticulture (Fruit & Fruit Technology), Bihar Agricultural University, Sabour, Bhagalpur, Bihar 813210, India
e-mail: barman.kalyan@gmail.com

S. Sharma
ICAR - National Research Centre on Litchi, Mushahari Farm, Mushahari, Muzaffarpur, Bihar 842002, India

M.W. Siddiqui
Department of Food Science and Postharvest Technology, Bihar Agricultural University, Sabour, Bhagalpur, Bihar 813210, India

M.W. Siddiqui et al. (eds.), *Postharvest Management Approaches for Maintaining Quality of Fresh Produce*, DOI 10.1007/978-3-319-23582-0_4

pathway which plays a pivotal role in inducing multiple mode of resistance to plants (Asghari and Aghdam 2010). SA has also been reported to involve in activating local and systemic disease resistance in response to pathogen attack (Enyedl et al. 1992; Alverez 2000). Moreover, it also modulates the response of plant to various abiotic stresses like drought, salinity, heat shock, chilling stress, and UV light (Ding and Wang 2003; Ding et al. 2001). During recent years, SA has received particular attention in extending shelf life and preserving postharvest quality of fresh horticultural produce due to its anti-ripening, anti-senescence properties and enhancing resistance to different biotic and abiotic stresses (Table 4.1).

Brief History of Salicylic Acid

During fourth century B.C., women were encouraged to chew leaves of willow plant to relieve pain during childbirth (Raskin 1992). Despite wide popularity of willow leaves and bark as a folk remedy of pain, its medicinal properties were not studied until mid-1700 by Reverend Edward Stone in Oxfordshire, England. In the year 1828, Johann A. Buchner, a German scientist, extracted and purified a small quantity of a yellowish substance, and he called it salicin. Later in 1838 an Italian chemist, Raffaele Piria, split salicin into a sugar and an aromatic compound which could be converted to acid and named acide salicylice or salicylic acid (Vlot et al. 2009). In the year 1859, first chemical synthesis of SA was carried out by Hermann Kolbe and his coworkers which led large-scale production of SA for medicinal use. However, its unpleasant taste and long-term side effects restricted its wide use. Later, an employee of Bayer pharmaceutical company, Felix Hoffmann, identified that acetylation of SA yielded a compound which is better tolerated without affecting its desirable qualities. In the year 1899, this acetyl salicylic acid was given the trade name of aspirin for its marketing as a pain-killer medicine.

Salicylic Acid Biosynthesis and Metabolism

In plants, biosynthesis of SA takes place via two distinct pathways that involve the primary metabolite chorismate (Garcion and Métraux 2006). In one pathway, L-phenylalanine derived from chorismate is converted into SA either via benzoate intermediates or from coumaric acid intermediates through a series of enzymatic reactions. In another pathway, chorismate is converted to isochorismate by the enzyme isochorismate synthase (ICS) which later converted to SA by the enzyme isochrismate pyruvate lyase (IPL) (Strawn et al. 2007; Verberne et al. 2000; Wildermuth et al. 2001) (Fig. 4.1). It has been reported that SA produced from chorismate in chloroplasts is responsible for inducing local and systemic acquired resistance in plants (Wildermuth et al. 2001). In the first pathway, L-phenylalanine is first converted to cinnamic acid by the enzyme phynylalanine ammonia lyase (PAL). Two routes for the conversion of cinnamic acid to SA exist which differ in

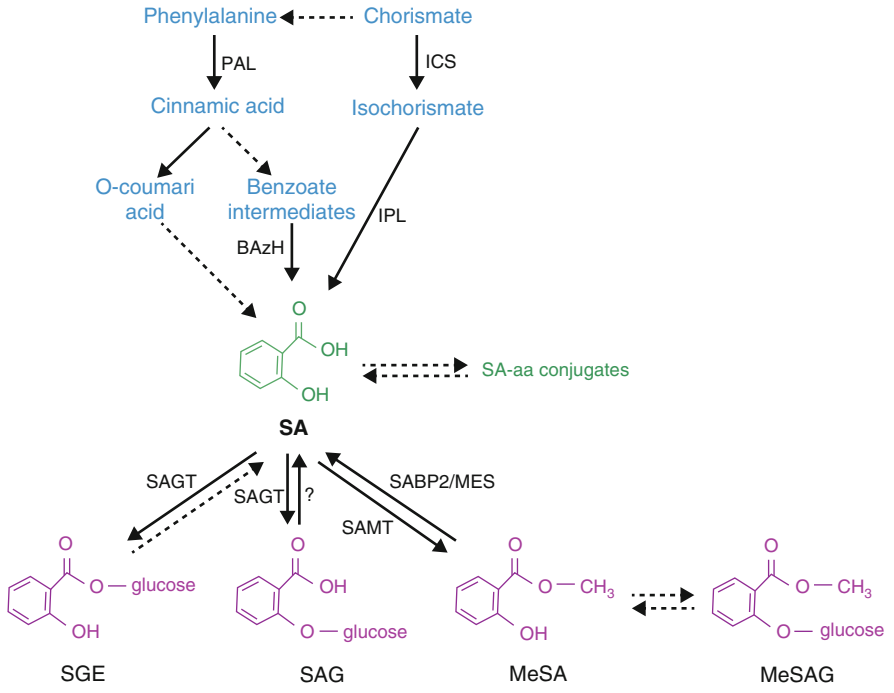


Fig. 4.1 Biosynthesis pathway and metabolism of salicylic acid. Abbreviations: *PAL* phenylalanine ammonia lyase, *ICS* isochorismate synthase, *IPL* isochorismate pyruvate lyase, *BA2H* benzoic acid-2-hydroxylase, *SA* salicylic acid, *SAGT* SA glucosyltransferase, *aa* amino acid, *SAMT* SA methyltransferase, *SABP*$_2$ SA-binding protein 2, *MES* methyl esterase, *SGE* salicyloyl glucose ester, *SAG* SA *O*-β-glucoside, *MeSA* methyl salicylate, *MeSAG* methyl salicylate *O*-β-glucoside (source: Vlot et al. 2009)

hydroxylation of aromatic ring. In one route, side chain of cinnamic acid is oxidized to form benzoic acid, which later undergoes hydroxylation in *ortho* position to form SA. Such biosynthetic pathway of SA has been reported in tobacco (Yalpani et al. 1993) and rice (Silverman et al. 1995). In another pathway, cinnamic acid first hydroxylated into *ortho*-coumaric acid followed by oxidation of the side chain takes place to form SA (Sticher et al. 1997). In this, enzyme trans-cinnamate-4-hydroxylate catalyzes conversion into SA which was identified in pea seedling (Russell and Conn 1967), *Quercus pedunculata* (Alibert and Ranjeva 1971, 1972), and *Melilotus alba* (Gestetner and Conn 1974). According to earlier studies on tobacco, synthesis of SA was reported to occur from free benzoic acid however; later results revealed that benzoyl glucose, a conjugated form of benzoic acid, is the precursor of SA (Yalpani et al. 1993; Chong et al. 2001).

Salicylic acid forms conjugates with different molecules either by glycosylation or by esterification (Popova et al. 1997). A major portion of SA produced in plants is converted into SA *O*-β-glucoside (SAG) by the enzyme SA-glucosyltransferase (SAGT) which has been found in suspension cultures of *Mallotus japonicus* (Tanaka et al. 1990) and roots of *Avena sativa* seedlings (Balke and Schulz 1987; Yalpani

Table 4.1 Various effects of salicylic acid on fruits and vegetables during postharvest storage

Crop	Effects of salicylic acid (SA)	References
Chinese water chestnut	Reduced browning, maintained eating quality, reduced disease incidence and activities of PPO, POD, and PAL enzymes	Peng and Jiang (2006)
Tomato	Reduced weight loss, decay percentage, delayed changes in total soluble solids, titratable acidity, sugar accumulation, chlorophyll degradation, carotenoids accumulation, enhanced storage life, alleviated chilling injury	Pila et al. (2010), Aghdam et al. (2012)
Sweet cherry	Delayed ripening and associated changes in firmness, acidity, color, enhanced total phenolics, anthocyanins and antioxidant capacity, induced disease resistance, increased activities of CAT, glutathione peroxidase, chitinase and β-1,3-glucanase, PAL and POD	Valero et al. (2011), Chan et al. (2008), Xu and Tian (2008), Yao and Tian (2005)
Sugar apple	Reduced respiration, ethylene production rates, softening and decay rates, increased activities of SOD, POD, CAT, APX, and decreased activity of LOX enzymes	Mo et al. (2008)
Strawberry	Superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), Reduced weight loss, ethylene production, fungal decay, maintain higher firmness and vitamin C	Babalar et al. (2007), Shafiee et al. (2010)
Pomegranate	Alleviated chilling injury, reduced PAL activity, respiration rate, and preserved phenolics, anthocyanins, antioxidant capacity, sugars, and organic acids	Sayyari et al. (2009, 2011)
Plum	Reduced chilling injury, respiration and ethylene production rates, disease incidence, reduced PPO and POD activity	Luo et al. (2011)
Pineapple	Reduced internal browning, inhibited PPO and PAL activities, delayed decline in ascorbic acid content	Lu et al. (2011)
Pepper	Increased cuticle thickness of fruit pericarp, vitamin C, carotenoids content, invertase activity, and reduced POD activity	Elwan and El-Hamahmy (2009)
Asparagus	Improved color and maintained chlorophyll, phenolics, flavonoid, ascorbic acid content, and antioxidant activity	Wei et al. (2011)
Banana	Delayed ripening, decreased fruit softening, pulp:peel ratio, reducing sugar content, respiration rate, and enzyme activity of invertase, cellulase, xylanase, and polygalacturonase	Srivastava and Dwivedi (2000)
Mango	Alleviate chilling injury, reduce weight loss, fruit softening, disease incidence, PME and PG activities, and preserved carotenoids, phenolics, and antioxidant capacity of fruit	Barman and Asrey (2014)
Litchi	Reduced pericarp browning, decay percentage, electrolyte leakage, weight loss, PPO activity and maintained higher anthocyanin content	Kumar et al. (2013)

PPO polyphenol oxidase, *POD* peroxidase, *PAL* phenylalanine ammonia lyase, *SOD* superoxide dismutase, *CAT* catalase, *APX* ascorbate peroxidase, *LOX* lipoxygenase

et al. 1992). In Arabidopsis, two SAGT enzymes have been found: one converts SA into SAG while the other forms less abundant SA derivative, salicyloyl glucose ester (SGE) (Dean and Delaney 2008). Synthesis of SA takes place in the chloroplast whereas in tobacco SAGT is present in cytosol (Dean et al. 2005; Garcion et al. 2008; Strawn et al. 2007). SAG is transported into vacuole from cytosol, where it remains as inactive storage that later converted to SA (Dean and Mills 2004; Dean et al. 2005).

Role of Salicylic Acid in Postharvest Management of Horticultural Crops

Effect on Ethylene Biosynthesis

Ethylene plays an important role in the postharvest physiology of horticultural crops by inducing fruit ripening and senescence. During fruit ripening, a series of biochemical and physiological changes (Table 4.2) take place that initiate the processes of senescence. Ethylene is produced from L-methionine that includes the intermediates SAM (S-adenosyl methionine) and ACC (1-aminocyclopropane-1-carboxylic acid) (Fig. 4.2). The two enzymes responsible for the conversion of SAM to ACC and ACC to ethylene are ACC synthase and ACC oxidase, respectively. SA decreases the ethylene biosynthesis by reducing the activity of ACC synthase and/or ACC oxidase enzymes. Exogenous application of SA and its derivative acetyl salicylic acid (ASA) significantly decreases ethylene production in cultured pear cell, apple and pear fruit tissue, mung bean hypocotyl, cell suspension culture of carrot, and several fruit crops (Babalar et al. 2007; Romani et al. 1989; Leslie and Romani 1988). In banana, exogenous application of SA delayed ripening of fruit probably through reducing ethylene production (Srivastava and Dwivedi 2000). Similarly in kiwifruit, postharvest application of acetyl salicylic acid (1.0 mmol/L) decreased the endogenous production of ethylene during early stages of fruit ripening (Zhang et al. 2003). In strawberry fruit, preharvest application of SA (2 mmol/L) at three stages of vegetative growth, fruit development

Table 4.2 Changes associated with fruit ripening (source: Wills et al. 2007)

Changes in color
Changes in respiration rate
Changes in ethylene production rate
Softening of fruit due to changes in composition of pectic substances
Changes in carbohydrate composition
Changes in organic acid
Changes in protein
Production of flavor volatiles
Development of wax on peel

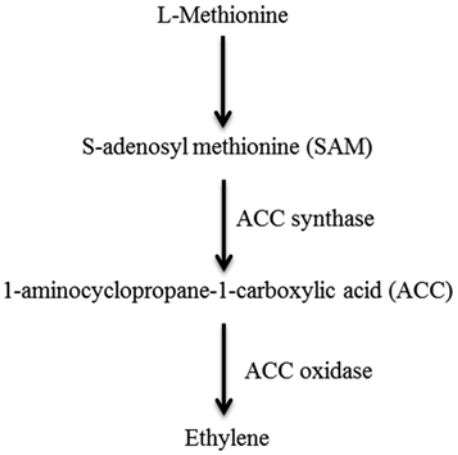


Fig. 4.2 Biosynthesis pathway of ethylene

stage, and after harvest was found effective in reducing ethylene production. However, single application of SA at postharvest stage was found more effective than its application at vegetative growth and fruit development stage (Babalar et al. 2007). Application of SA in other fruits like mango, plum, and sugar apple was also reported highly effective in reducing biosynthesis of ethylene (Barman and Asrey 2014; Luo et al. 2011; Mo et al. 2008).

Effect on Fruit Firmness

With the onset of ripening, firmness of fruit decreases progressively. During this period, activities of cell wall and membrane-degrading enzymes like pectin methyl esterase (PME), polygalacturonase (PG), and lipoxygenase (LOX) increase which causes faster decrease in fruit firmness. Application of SA has been reported to reduce ethylene production, thereby reducing the activities of above cell wall and membrane-degrading enzymes (Srivastava and Dwivedi 2000; Zhang et al. 2003). A positive correlation between LOX activity and ethylene biosynthesis in fruit tissue has been reported by Marcelle (1991). Postharvest treatment of sweet cherry ('Cristalina' and 'Prime Giant') with salicylic acid (SA) or acetyl salicylic acid (ASA) at 1 mM concentration delayed loss of fruit firmness during storage under cold temperature for 20 days (Valero et al. 2011). In banana, 1000 μM SA application delayed fruit softening by reducing activities of fruit-softening enzymes, namely cellulase, polygalacturonase (PG), and xylanase (Srivastava and Dwivedi 2000). In another study, immersion treatment of banana fruit in 0.8 mmol L^{-1} SA solution for 4 h was found to be effective in maintaining higher firmness (Huigang et al. 2009). Barman and Asrey (2014) reported that mango fruits, when treated with SA and stored at 8 °C, maintained significantly higher firmness than control. The authors mentioned that higher fruit firmness in SA-treated fruit was

attributed to lower activities of PME and PG enzymes than control. Combined application of SA with calcium chloride was also found to be effective in increasing firmness of kiwifruit during cold storage at 1 °C up to 60 days (Kazemi et al. 2011). Zhang et al. (2003) also reported a positive correlation between fruit firmness and endogenous free SA content in kiwifruit. In strawberry, application of SA (2 mM) in nutrient solution maintained higher firmness of fruit than control (Shafiee et al. 2010).

Effect on Alleviating Chilling Injury

Low-temperature storage is an effective and commercial means for extending shelf life of horticultural produce. However, fruits and vegetables of tropical and subtropical origin are prone to chilling injury (CI) while stored at low but nonfreezing temperatures below the critical point. CI causes skin pitting, discoloration of peel and flesh, shriveling and sunken lesions on the peel, uneven ripening, poor color, reduced aroma, the development of off-flavors, and increased susceptibility to fungal or bacterial decay (Nair et al. 2003; Nair and Singh 2009). As a result, market value of the fruit is affected negatively and reduces its shelf life. This CI consists of primary (reversible) and secondary (irreversible) events. In primary event, when susceptible produce are exposed to chilling temperatures, it causes phase change of membrane lipids from liquid to gel state, production of reactive oxygen species (ROS), and dissociation of enzymes and other proteins into their structural subunits (Lyons 1973; Wills et al. 2007). These ROS cause oxidative burst and damage subcellular components including membranes. Later, primary events dispose the susceptible tissues to develop secondary events, a cascade of deteriorative reactions, causing electrolyte leakage, impaired metabolic process, cell autolysis, and cell death (Parkin et al. 1989; Sevillano et al. 2009). Treatment of fruit and vegetables with SA has been reported highly effective in alleviating chilling injury during postharvest storage at low temperature. Application of SA induced expression of ROS avoidance genes and ROS scavenging genes like SOD, CAT, and APX. As a result, it increased antioxidant capacity of cells which subsequently reduced chilling injury (Asghari and Aghdam 2010) (Fig. 4.3). Moreover, it also induces synthesis and accumulation of heat-shock proteins (HSPs) which confers protection of horticultural produce against CI (Tian et al. 2007). Wang et al. (2006) reported that SA (1.0 mM) pretreatment of peach fruit alleviated CI during storage at 0 °C for 28 days. They mentioned that stress tolerance of SA-treated fruit was attributed to enhanced antioxidant systems and accumulation of HSPs (HSP101 and HSP73) in the cells. SA treatment enhanced activities of reduced-to-oxidized ascorbate ratio (AsA/DHAsA), reduced-to-oxidized glutathione ratio (GSH/GSSG), ascorbate peroxidase, and glutathione reductase in the fruit compared to control. In pomegranate, immersion treatment of fruit with 2.0 mM SA reduced CI and electrolyte leakage of fruit during low-temperature storage (Sayyari et al. 2009). Similar dose of SA was also found effective in mango (Barman and Asrey 2014). In tomato and

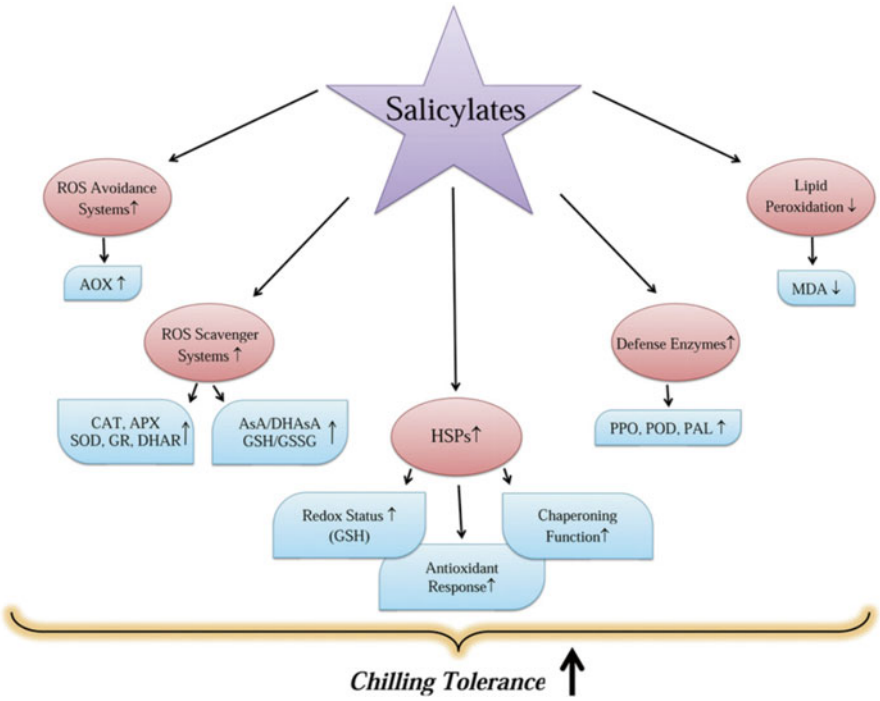


Fig. 4.3 Induction of chilling tolerance by SA (source: Asghari and Aghdam 2010)

cucumber, SA treatment reduced chilling-induced membrane lipid peroxidation and accumulation of malondialdehyde (MDA) during storage at 1 °C (Aghdam et al. 2012; Cao et al. 2009). In case of plum, SA treatment reduced CI by increasing accumulation of polyamines and reducing electrolyte leakage and MDA accumulation (Luo et al. 2011).

Effect on Reducing Disease Incidence

Plants continuously remain exposed to a variety of pathogenic attack in the environment. However, they have several inherent mechanisms to defend themselves from these pathogenic microorganisms. One such mechanism involves accumulation of large quantities of SA in response to pathogenic attack (Hayat et al. 2010). Malamy et al. (1990) reported that large amount of SA was accumulated in the leaves of tobacco mosaic virus (TMV)-resistant tobacco cultivar Xanthi when it was inoculated with TMV. Similar increase in endogenous SA was noted in cucumber plant upon infection with *Colletotrichum lagenarium*, *Pseudomonas syringae*, or tobacco necrosis virus (Metraux et al. 1990; Rasmussen et al. 1991; Smith et al. 1991). Similar to these findings, exogenous application of SA or its synthetic analogue

acetyl salicylic acid (ASA) provides tolerance or reduces disease incidence in fruits and vegetables against various pathogens during postharvest storage (Malamy and Klessig 1992). When plants are attacked by the pathogen, it activates some defence mechanisms viz. local acquired resistance (LAR) and systemic acquired resistance (SAR) against the pathogens (Vlot et al. 2009). Initially, defence responses are activated at the infection site (LAR), which subsequently triggered systemic defence response to protect undamaged plant parts from invasion by the pathogen. This systemic acquired resistance (SAR) activates specific set of *PR* genes which encode for proteins with antimicrobial activity (Durrant and Dong 2004; Van Loon et al. 2006). Meena et al. (2001) reported that SA induced expression of a range of defence genes like chitinase, β-1,3-glucanase, and peroxidase. It also increases level of SA in plants which decreases expression of ascorbate peroxidase (APX) and catalase (CAT) genes that lead to increase in H_2O_2, which acts as secondary messenger for activation of LAR and SAR (Fig. 4.4) (Klessig and Malamy 1994; Tian et al. 2007). Transgenic plants and mutants in which SA signaling is impaired are not capable of developing SAR and do not show activation of *PR* genes upon infection by pathogens (Durrant and Dong 2004; Pieterse et al. 2009).

In mango, Zeng et al. (2006) reported that treatment of fruit with SA increased the activity of β-1,3-glucanase and production of H_2O_2 and superoxide radicals than control after 8 days of storage. In another study, postharvest application of SA

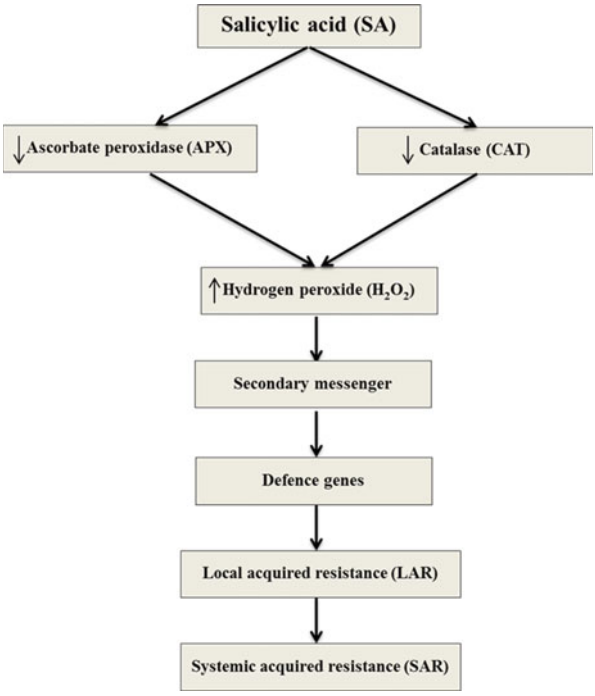


Fig. 4.4 Effect of SA on inducing disease resistance (source: Asghari and Aghdam 2010)

(2.0 mM) in mango was found highly effective in reducing disease incidence during low-temperature storage at 8 °C for up to 30 days (Barman and Asrey 2014). Immersion treatment of banana fruit in 0.8 mmol L^{-1} SA solution for 4 h reduced disease incidence (Hui-gang et al. 2009). Chan et al. (2008) reported that SA-induced disease resistance against *Penicillium expansum* in sweet cherry fruit depends upon maturity stage, the less mature fruit being more resistant to diseases. SA was found to induce disease resistance by activating antioxidant proteins like POD, CAT, and SOD. Similar response was found when sugar apple fruits were treated with SA (Mo et al. 2008). Cao et al. (2006) reported that young pear fruits, when sprayed with SA, increased activities of defensive enzymes like chitinase, POD, and PAL. In another study, treatment of SA (2.0 mM) induced disease resistance by stimulating activities of PPO, PAL, and β-1,3-glucanase in sweet cherry fruit (Qin et al. 2003; Chan and Tian 2006). In fresh-cut Chinese water chestnut, treatment with 2.0 and 4.0 mM SA completely checked disease development up to 12 days of storage (Peng and Jiang 2006). In tomato, treatment of fruit with 0.4 mM SA significantly reduced decay percentage than control (Pila et al. 2010). Preharvest treatment of strawberry plants at vegetative stage and fruit development stage followed by postharvest treatment of fruits with 1 and 2 mmol L^{-1} was found highly effective in reducing fungal decay of fruit, in a concentration-dependant manner (Babalar et al. 2007).

SA has also been reported to enhance biocontrol efficacy of antagonistic yeast. Combined application of 10 μg ml^{-1} SA with *Cryptococcus laurentii* improved the biocontrol efficacy of yeast against blue mould rot caused by *Penicillium expansum* on apple in a concentration-dependant manner (Yu and Zheng 2006). However, SA was less effective at higher or lower concentrations. Similarly in sweet cherry, 0.5 mM SA reduced the disease incidence of blue mould (*Penicillium expansum*) and alternaria rot (*Alternaria alternata*) by enhancing efficacy of antagonistic yeast *R. glutinis* (Qin et al. 2003).

Effect on Antioxidant Enzymes

Stress in plants induces generation of reactive oxygen species (ROS) such as superoxide radicals ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet-}$), hydroperoxyl radical ($HO_2^{\bullet}$), hydrogen peroxide (H_2O_2), and singlet oxygen ($O_2^{\bullet}$) which creates oxidative stress in plants (Elstner 1982; Halliwell and Gutteridge 1988; Asada 1994; Gille and Singler 1995; Monk et al. 1989; Prasad et al. 1999; Panda et al. 2003a, b). These ROS cause damage to the biological macromolecules such as lipids, proteins, and nucleic acids, thus altering the redox homeostasis (Smirnoff 1993; Gille and Singler 1995). To cope up with this oxidative stress, plant has two types of defence system; one includes ROS avoidance genes like alternative oxidase (AOX) while the other includes ROS scavenging genes like superoxide dismutase (SOD), catalase (CAT), ascorbate/glutathione cycle, glutathione peroxidase system, and thioredoxin system (Fig. 4.5) (Buchanan et al. 2000). Application of SA has been reported to increase the antioxidant capacity by inducing expression of AOX. In watermelon, exogenous

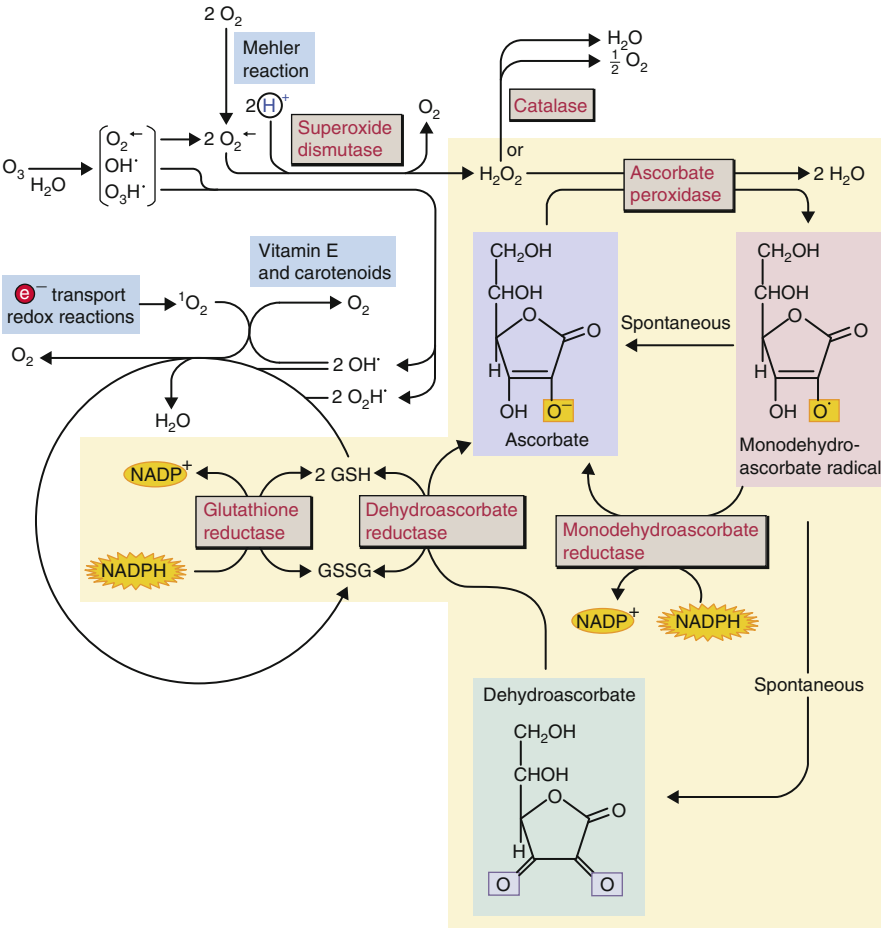


Fig. 4.5 Antioxidant systems in plant (source: Asghari and Aghdam 2010)

application of SA (1.0 mmol L^{-1}) induced the activity of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (G-POD), and glutathione reductase (GR); however, higher concentration of SA inversely depressed the oxidative enzymes activities (Jing-Hua et al. 2008). In strawberry, SA enhanced antioxidant capacity of fruit in a concentration-dependent manner from 0 to 2 mmol L^{-1}, the highest concentration being the most effective treatment. Consequent application of SA at three different stages, viz. vegetative growth stage, fruit development stage, and postharvest stage, also proved effective in enhancing total antioxidant capacity of fruit (Asghari and Babalar 2009). In sweet cherry, postharvest treatment of fruit with SA highly inhibited CAT activity; however, it stimulated the activities of SOD and POD. This treatment also changed the expression of POD isozymes, indicating that SA directly or indirectly activates antioxidant enzymes (Tian et al. 2007). In another study, application of

salicylic acid (SA) or acetyl salicylic acid (ASA) at 1 mM increased total phenolics, anthocyanins, and antioxidant activity of sweet cherry (cv. Cristalina and Prime Giant) during their postharvest storage at cold temperature (Valero et al. 2011). In banana, application of 1000 μM SA reduced activities of CAT and POD in a concentration-dependant manner (Srivastava and Dwivedi 2000); however, Huigang et al. (2009) reported that dipping of banana fruit in 0.8 mmol L^{-1} SA solution for 4 h increased the activities of SOD, CAT, and POD enzymes. In sugar apple fruit, SA increased the activities of antioxidant enzymes SOD, POD, CAT, and APX during their storage (Mo et al. 2008). In vegetables like asparagus and carrot, application of SA increased antioxidant activity during postharvest storage (Wei et al. 2011; Eraslan et al. 2007). In cucumber, treatment of fruit with SA (0.5 mM) increased activities of SOD, CAT, POD, APX, and PAL during storage under chilling stress condition (Cao et al. 2009).

Reduce Browning

Enzymatic browning of fruits and vegetables drastically reduces its nutritional value and consumer appeal. Browning mainly takes place due to oxidation of phenolic compounds by the enzyme polyphenol oxidase (PPO). In plant cells, phenolic compounds are located in the vacuoles, whereas PPO is located in the plastids (Vaughn and Duke 1984). PPO first catalyzes the hydroxylation of monophenols to colorless diphenols, which later converted to brown-colored quinone polymers (Murata et al. 1995). This reaction takes place in the presence of oxygen. Application of SA has been reported to reduce browning incidence in several fruit crops. Dipping of fresh-cut Chinese water chestnut in solution of SA (4.0 mM) highly inhibited browning of fruit by reducing the activities of PPO, POD, and PAL during storage (Peng and Jiang 2006). The bright red color of litchi fruit pericarp rapidly turns brown after harvest within a couple of days, which is a major postharvest problem in litchi. Postharvest application of 0.5 % SA by immersion method has been reported highly effective in reducing pericarp browning of litchi (Kumar et al. 2013). They have mentioned that reduced browning of fruit was due to lower PPO activity, weight loss, and electrolyte leakage in treated fruits compared to control fruit. Similarly in pineapple, preharvest SA spray and/or postharvest SA immersion treatment significantly reduced internal browning incidence and intensity by reducing PPO and PAL activity, during storage at 10 °C (Lu et al. 2011).

Effect on Sugars and Total Soluble Solids

With the onset of fruit ripening, soluble sugar content in the fruit increases by the enzyme sucrose phosphate synthase (SPS) (Hubbard et al. 1991). This enzyme is activated by ethylene that is produced in large quantities with the onset of ripening

in climacteric fruit (Langenkamper et al. 1998). In tomato, SA treatment reduced total soluble solids, total sugars, and reducing and nonreducing sugars, than control. They postulated that SA delays the degradation of starch and other polysaccharides into water soluble sugars. It also slowed down the respiration and metabolic activities, and hence retarded changes of carbohydrates to sugars (Pila et al. 2010). Kiwifruits treated with MeSA 32 ml L^{-1} contained lower TSS than control during postharvest storage at low temperature (Aghdam et al. 2009). MeSA reduced ethylene production rate, thereby reducing activity of SPS enzyme and sucrose synthesis. Application of 1000 μM SA reduced activities of invertase and delayed increase in reducing sugars and decrease in nonreducing sugars in banana (Srivastava and Dwivedi 2000). Similarly, in another study, immersion treatment of banana fruit in 0.8 mmol L^{-1} SA solution for 4 h delayed conversion of starch to soluble sugars (Hui-gang et al. 2009). Furthermore, a large amount of polysaccharides are also present in the cell wall like pectins and cellulose which are broken down due to the activity of cell wall-degrading enzymes. SA was found highly effective in reducing activities of cell wall-degrading enzymes, thereby preventing significant increase in TSS content (Asghari and Aghdam 2010).

Effect on Respiration Rate

SA has been found to be effective in reducing respiration rate of fruit and vegetables. It has been reported that SA induces cyanide resistance respiration in plant cells by affecting antioxidant enzyme activity (Raskin et al. 1989). The reduction of respiration rate by SA is also due to reduced ethylene production and senescence of produce after its application. The rate of respiration is related to ethylene production and senescence; thus any factor increases the ethylene production also promotes increase in respiration rate (Asghari and Aghdam 2010). Thus, lower respiration rate after SA treatment of fruits and vegetables is mainly due to its negative effects on ACC synthase, ACC oxidase, PG, PME, and other antioxidant enzymes that decrease ethylene production and action. Moreover, increase in antioxidant activity of horticultural produce also suppresses the rate of respiration by reducing oxidative stress such as chilling injury. In sugar apple, SA reduced respiration rate by increasing antioxidant enzymes (SOD, POD, CAT, APX) and reducing ethylene production rate and superoxide radicals (Mo et al. 2008). Treatment of pomegranate fruit with ASA and plum with SA reduced respiration rate of fruit by alleviating chilling injury during storage at low temperature (Sayyari et al. 2011; Luo et al. 2011). Srivastava and Dwivedi (2000) reported that SA treatment of banana fruit reduced the rate of respiration and delayed the onset of respiratory climacteric than control fruits in a concentration-dependant manner.

Conclusion

Salicylic acid (SA), a safe phenolic compound, exhibits tremendous potential in reducing postharvest loss of fresh fruits and vegetables. Exogenous application of SA delays fruit ripening and senescence by reducing rate of respiration, ethylene production, and maintaining higher firmness. It also plays an important role in minimizing disease incidence of horticultural produce by inducing local and systemic acquired resistance systems against pathogens. Treatment with SA also modulates activities of antioxidant enzymes, thereby reducing oxidative stress and inducing crop tolerance to chilling injury. Therefore, SA can be effectively used in postharvest management of fresh horticultural produce as an alternative to harmful synthetic chemicals to enhance shelf life and ensure food safety.

References

Aghdam, M. S., Asghari, M. R., Moradbeygi, H., Mohammadkhani, N., Mohayeji, M., & Rezapour-Fard, J. (2012). Effect of postharvest salicylic acid treatment on reducing chilling injury in tomato fruit. *Romanian Biotechnological Letters, 17*(4), 7466–7473.

Aghdam, M. S., Mostofi, Y., Motallebiazar, A., Ghasemneghad, M., & Fattahi Moghaddam, J. (2009, April 8–12). Effects of MeSA vapour treatment on the postharvest quality of Hayward kiwifruit. In *6th International Postharvest Symposium*. 8–12 April, 2009, Antalya, Turkey.

Alibert, G., & Ranjeva, R. (1971). Recharches sur les enzymes catalysant la biosynthese des acides phenoliques chez Quarcus pedunculata (Ehrn): I—formation des series cinnamique et benzoique. *FEBS Letters, 19*, 11–14.

Alibert, G., & Ranjeva, R. (1972). Recharches sur les enzymes catalysant la biosynthesis des acid phenoliques chez Quarcus pedunculata (Ehrn): II—localization intercellulaire de la phenylalanin mmonique-lyase, de la cinnamate 4-hydroxylase, et de la "benzoote synthase". *Biochimica et Biophysica Acta, 279*, 282–289.

Alverez, A. L. (2000). Salicylic acid in machinery of hypersensitive cell death and disease resistance. *Plant Molecular Biology, 44*, 429–442.

Asada, K. (1994). Production and action of active oxygen species in photosynthetic tissues. In C. H. Foyer & P. M. Mullineaux (Eds.), *Causes of photooxidative stress and amelioration of defence system in plants* (pp. 77–104). Boca Ration: CRC Press.

Asghari, M., & Aghdam, M. S. (2010). Impact of salicylic acid on post-harvest physiology of horticultural crops. *Trends in Food Science & Technology, 21*, 502–509.

Asghari, M. R., & Babalar, M. (2009, April 8–12). Use of salicylic acid to increase strawberry fruit total antioxidant activity. In *6th International Postharvest Symposium*. 8–12 April, 2009, Antalya, Turkey.

Babalar, M., Asghari, M., Talaei, A., & Khosroshahi, A. (2007). Effect of pre- and postharvest salicylic acid treatment on ethylene production, fungal decay and overall quality of Selva strawberry fruit. *Food Chemistry, 105*, 449–453.

Balke, N. E., & Schulz, M. (1987). Potential impact of enzymatic glucosidation of allelopathic phenolic compounds. In *Invited Lectures, Sec. 4: Industrial Chemistry, 31st International Congress of Pure and Applied Chemistry* (pp. 17–29). Sofia, Bulgaria: Bulgarian Academy of Sciences.

Barman, K., & Asrey, R. (2014). Salicylic acid pre-treatment alleviates chilling injury, preserves bioactive compounds and enhances shelf life of mango fruit during cold storage. *Journal of Scientific & Industrial Research, 73*, 713–718.

Buchanan, B. B., Gruissem, W., & Jones, R. L. (2000). *Biochemistry and molecular biology of plants*. Maryland, USA: American Society of Plant Physiologists.

Cao, S. F., Hu, Z. C., & Wang, H. O. (2009). Effect of salicylic acid on the activities of anti-oxidant enzymes and phenylalanine ammonia-lyase in cucumber fruit in relation to chilling injury. *Journal of Horticultural Science and Biotechnology, 84*(2), 125–130.

Cao, J., Zeng, K., & Jiang, W. (2006). Enhancement of postharvest disease resistance in Ya Li pear (*Pyrus bretschneideri*) fruit by salicylic acid sprays on the trees during fruit growth. *European Journal of Plant Pathology, 114*(4), 363–370.

Chan, Z. L., & Tian, S. P. (2006). Induction of H_2O_2-metabolizing enzymes and total protein synthesis by antagonistic yeast and salicylic acid in harvested sweet cherry fruit. *Postharvest Biology and Technology, 39*, 314–320.

Chan, Z., Wang, Q., Xu, X., Meng, X., Qin, G., Li, B., et al. (2008). Functions of defense-related proteins and dehydrogenases in resistance response induced by salicylic acid in sweet cherry fruits at different maturity stages. *Proteomics, 8*, 4791–4807.

Chong, J., et al. (2001). Free and conjugated benzoic acid in tobacco plants and cell cultures induced accumulation upon elicitation of defense responses and role as salicylic acid precursors. *Plant Physiology, 125*, 318–328.

Clarke, S. M., Mur, L. A. J., Wood, J. E., & Scott, I. M. (2004). Salicylic acid dependent signaling promotes basal thermotolerance but is not essential for acquired thermotolerance in *Arabidopsis thaliana. Planta, 38*, 432–447.

Dean, J. V., & Delaney, S. P. (2008). Metabolism of salicylic acid in wild-type, ugt74f1 and ugt74f2 glucosyltransferase mutants of *Arabidopsis thaliana. Physiologia Plantarum, 132*, 417–425.

Dean, J. V., & Mills, J. D. (2004). Uptake of salicylic acid 2-O-β-D-glucose into soybean tonoplast vesicles by an ATP-binding cassette transporter-type mechanism. *Physiologia Plantarum, 120*, 603–612.

Dean, J. V., Mohammed, L. A., & Fitzpatrick, T. (2005). The formation, vacuolar localization, and tonoplast transport of salicylic acid glucose conjugates in tobacco cell suspension cultures. *Planta, 221*, 287–296.

Ding, C. K., & Wang, C. Y. (2003). The dual effects of methyl salicylate on ripening and expression of ethylene biosynthetic genes in tomato fruit. *Plant Science, 164*, 589–596.

Ding, C. K., Wang, C. Y., Gross, K. C., & Smith, D. L. (2001). Reduction of chilling injury and transcript accumulation of heat shock protein genes in tomatoes by methyl jasmonate and methyl salicylate. *Plant Science, 161*, 1153–1159.

Durrant, W. E., & Dong, X. (2004). Systemic acquired resistance. *Annual Review of Phytopathology, 42*, 185–209.

Elstner, E. F. (1982). Oxygen activation and oxygen toxicity. *Annual Review of Plant Physiology, 33*, 73–96.

Elwan, M. W. M., & El-Hamahmy, M. A. M. (2009). Improved productivity and quality associated with salicylic acid application in greenhouse pepper. *Scientia Horticulturae, 122*(4), 521–526.

Enyedl, A., Valpanl, N., Silverman, P., & Raskin, I. (1992). Localization, conjugation and function of salicylic acid in tobacco during the hypersensitive reaction to tobacco mosaic virus. *Proceedings of National Academy of Sciences of the United States of America, 89*, 2480–2484.

Eraslan, F., Inal, A., Gunes, A., & Alpaslan, M. (2007). Impact of exogenous salicylic acid on the growth, antioxidant activity and physiology of carrot plants subjected to combined salinity and boron toxicity. *Scientia Horticulturae, 113*(2), 120–128.

Garcion, C., Lohman, A., Lamodière, E., Catinot, J., Buchala, A., et al. (2008). Characterization and biological function of the ISOCHORISMATE SYNTHASE 2 gene of *Arabidopsis thaliana. Plant Physiology, 147*, 1279–1287.

Garcion, C., & Métraux, J. P. (2006). Salicylic acid. *Plant Hormone Signaling, 24*, 229–255.

Gestetner, B., & Conn, E. E. (1974). The 2-hydroxylation of transcimannic acid by chloroplasts from *Melilotus alba* Desr. *Archives of Biochemistry and Biophysics, 163*, 617–624.

Gille, G., & Singler, K. (1995). Oxidative stress in living cells. *Folia Microbiologica, 2*, 131–152.

Halliwell, B., & Gutteridge, J. M. C. (1988). *Free radical in biology and medicine* (p. 1). Oxford: Clarendon.

Harper, J. R., & Balke, N. E. (1981). Characterization of the inhibition of K^+ absorption in oats roots by salicylic acid. *Plant Physiology, 68*, 1349–1353.

Hayat, Q., Hayat, S., Irfan, M., & Ahmad, A. (2010). Effect of exogenous salicylic acid under changing environment: A review. *Environmental and Experimental Botany, 68*, 14–25.

Hubbard, N. L., Pharr, D. M., & Huber, S. C. (1991). Sucrose phosphate synthase and other sucrose metabolizing enzymes in fruits of various species. *Plant Physiology, 82*, 191–196.

Hui-gang, H., Yi-wei, M., Jiang-hui, X., Lu-bin, Z., Hui, G., Jun-feng, L., & De-qiang, G. (2009). Effect of salicylic acid on antioxidation capacity and preservation of post-harvested banana fruits. *Food Science*. 30, 254–259.

Jing-Hua, Y., Yuan, G., Yan-Man, L., Xiao-Hua, Q., & Zhang, M. F. (2008). Salicylic acid-induced enhancement of cold tolerance through activation of antioxidative capacity in watermelon. *Scientia Horticulturae, 118*, 200–205.

Kazemi, M., Aran, M., & Zamani, S. (2011). Effect of calcium chloride and salicylic acid treatments on quality characteristics of kiwifruit (*Actinidia deliciosa* cv. Hayward) during storage. *American Journal of Plant Physiology, 6*(3), 183–189.

Khan, W., Prithviraj, B., & Smith, D. L. (2003). Photosynthetic responses of corn and soybean to foliar application of salicylates. *Journal of Plant Physiology, 160*, 485–492.

Klessig, D. F., & Malamy, J. (1994). The salicylic acid signal in plants. *Plant Molecular Biology, 26*, 1439–1458.

Kumar, D., Mishra, D. S., Chakraborty, B., & Kumar, P. (2013). Pericarp browning and quality management of litchi fruit by antioxidants and salicylic acid during ambient storage. *Journal of Food Science and Technology, 50*(4), 797–802. doi:10.1007/s13197-011-0384-2.

Langenkamper, G., McHale, R., Gardner, R. C., & MacRae, E. (1998). Sucrose phosphate synthase steady-state mRNA increases in ripening kiwifruit. *Plant Molecular Biology, 36*, 857–869.

Leslie, C. A., & Romani, R. J. (1988). Inhibition of ethylene biosynthesis by salicylic acid. *Plant Physiology, 88*, 833–837.

Lu, X., Sun, D., Li, Y., Shi, W., & Sun, G. (2011). Pre- and post-harvest salicylic acid treatments alleviate internal browning and maintain quality of winter pineapple fruit. *Scientia Horticulturae, 130*(1), 97–101.

Luo, Z., Chen, C., & Xie, J. (2011). Effect of salicylic acid treatment on alleviating postharvest chilling injury of 'Qingnai' plum fruit. *Postharvest Biology and Technology, 62*(2), 115–120.

Lyons, J. M. (1973). Chilling injury in plants. *Annual Review in Plant Physiology, 24*, 445–466.

Malamy, J., Carr, J. P., & Klessig, D. F. (1990). Salicylic acid: A likely endogenous signal in the resistance response of tobacco to viral infection. *Science, 250*, 1002–1004.

Malamy, J., & Klessig, D. F. (1992). Salicylic acid and plant disease resistance. *Plant Journal, 2*, 643–654.

Marcelle, R. D. (1991). Relationship between mineral content, lipoxygenase activity, levels of 1-aminocyclopropane-l-carboxylic acid and ethylene emission in apple fruit flesh disks during storage. *Postharvest Biology and Technology, 1*, 101–109.

Meena, B., Marimuthu, T., & Velazhahan, R. (2001). Salicylic acid induces systemic resistance in groundnut against late leaf spot caused by *Cercosporidium personatum. Journal of Mycology and Plant Pathology, 31*, 139–145.

Metraux, J. P., Signer, H., Ryals, J., Ward, E., Wyss-Benz, M., Gaudin, J., et al. (1990). Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. *Science, 250*, 1004–1006.

Mo, Y., Gong, D., Liang, G., Han, R., Xie, J., & Li, W. (2008). Enhanced preservation effects of sugar apple fruits by salicylic acid treatment during postharvest storage. *Journal of the Science of Food and Agriculture, 88*, 2693–2699.

Monk, L. S., Fagersted, K. V., & Crawford, R. M. M. (1989). Oxygen toxicity and superoxide dismutase as an antioxidant in physiological stress. *Plant Physiology, 76*, 456–459.

Morris, K., Mackerness, S. A. H., Page, T., John, C. F., Murphy, A. M., et al. (2000). Salicylic acid has a role in regulating gene expression during leaf senescence. *The Plant Journal, 23*, 677–685.

Murata, M., Noda, I., & Homma, S. (1995). Enzymatic browning of apples on the market: Relationship between browning, polyphenol content, and polyphenol oxidase. *Nipon Shokuhin Kagaku Kogaku Kaishi, 42*, 820–826.

Nair, S., & Singh, Z. (2009). Chilling injury during storage affects respiration rate and fruit quality in Kensington Pride mango fruit. *Acta Horticulturae, 820*, 737–743.

Nair, S., Singh, Z., & Tan, S. C. (2003). Aroma volatiles emissions in relation to chilling injury in 'Kensington Pride' mango fruit. *Journal of Horticultural Science and Biotechnology, 66*, 866–873.

Panda, S. K., Chaudhury, I., & Khan, M. H. (2003a). Heavy metal indunced lipid peroxidation and affects antioxidants in wheat leaves. *Biologia Plantarum, 46*, 289–294.

Panda, S. K., Sinha, L. B., & Khan, M. H. (2003b). Does aluminium phytotoxicity induce oxidative stress in green gram (*Vigna radiata*)? *Bulgarian Journal of Plant Physiology, 29*, 77–86.

Parkin, K. L., Marangoni, A., Jackman, R. L., Yada, R. Y., & Stanley, D. W. (1989). Chilling injury. A review of possible mechanisms. *Journal of Food Biochemistry, 13*, 127–153.

Peng, L., & Jiang, Y. (2006). Exogenous salicylic acid inhibits browning of fresh-cut Chinese water chestnut. *Food Chemistry, 94*, 535–540.

Pieterse, C. M. J., Leon-Reyes, A., Van der Ent, S., & Van Wees, S. C. M. (2009). Networking by small-molecule hormones in plant immunity. *Nature Chemical Biology, 5*, 308–316.

Pila, N., Gol, N. B., & Rao, T. V. R. (2010). Effect of post harvest treatments on physicochemical characteristics and shelf life of tomato (*Lycopersicon esculentum* Mill.) fruits during storage. *American-Eurasian Journal of Agriculture and Environmental Science, 9*(5), 470–479.

Popova, L., Pancheva, T., & Uzunova, A. (1997). Salicylic acid: Properties, biosynthesis and physiological role. *Bulgarian Journal of Plant Physiology, 23*, 85–93.

Prasad, K. V. S. K., Pardha, S. P., & Sharmila, P. (1999). Concerted action of antioxidant enzymes and curtailed growth under zinc toxicity in *Brassica juncea. Environment and Experimental Botany, 42*, 1–10.

Qin, Q. Z., Tian, S. P., Xu, Y., & Wan, Y. K. (2003). Enhancement of biocontrol efficacy of antagonistic yeasts by salicylic acid in sweet cherry fruit. *Physiological and Molecular Plant Pathology, 62*, 147–154.

Rajou, L., Belghazi, M., Huguet, R., Robin, C., Moreau, A., et al. (2006). Proteomic investigation of the effect of salicylic acid on Arabidopsis seed germination and establishment of early defense mechanisms. *Plant Physiology, 141*, 910–923.

Raskin, L. (1992). Role of salicylic acid in plants. *Annual Review of Plant Physiology and Plant Molecular Biology, 43*, 439–463.

Raskin, I., Turner, I. M., & Melander, W. R. (1989). Regulation of heat production in the inflorescences of an Arum lily by endogenous salicylic acid. *PNAS, 86*, 2214–2218.

Rasmussen, J. B., Hammerschmidt, R., & Zook, M. (1991). Systemic induction of salicylic acid accumulation in cucumber after inoculation with *Pseudomonas syringae* pv. syringae. *Plant Physiology, 97*, 1342–1347.

Romani, R. J., Hess, B. M., & Leslie, C. A. (1989). Salicylic acid inhibition of ethylene production by apple discs and other plant tissues. *Journal of Plant Growth Regulator, 8*, 63–69.

Russell, D. W., & Conn, E. E. (1967). The cinnamic acid 4-hydroxylase of pea seedlings. *Archives of Biochemistry and Biophysics, 122*(1), 256–258.

Sayyari, M., Babalare, M., Kalantarie, S., Serranoc, M., & Valero, D. (2009). Effect of salicylic acid treatment on reducing chilling injury in stored pomegranates. *Postharvest Biology and Technology, 53*, 152–154.

Sayyari, M., Castillo, S., Valero, D., Díaz-Mula, H. M., & Serrano, M. (2011). Acetyl salicylic acid alleviates chilling injury and maintains nutritive and bioactive compounds and antioxidant activity during postharvest storage of pomegranates. *Postharvest Biology and Technology, 60*(2), 136–142.

Sevillano, L., Sanchez-Ballesta, M. T., Romojaro, F., & Flores, F. B. (2009). Physiological, hormonal and molecular mechanisms regulating chilling injury in horticultural species. Postharvest technologies applied to reduce its impact. *Journal of the Science and Food and Agriculture, 89*, 555–573.

Shafiee, M., Taghavi, T. S., & Babalar, M. (2010). Addition of salicylic acid to nutrient solution combined with postharvest treatments (hot water, salicylic acid, and calcium dipping) improved postharvest fruit quality of strawberry. Scientia Horticulture, 124, 40–45.

Silverman, P., Seskar, M., Kanter, D., Schweizer, P., Métraux, J. P., et al. (1995). Salicylic acid in rice. Biosynthesis, conjugation, and possible role. *Plant Physiology, 108*, 633–639.

Smirnoff, N. (1993). The role of active oxygen in response of plants to water deficit and desiccation. *New Phytologist, 125*, 27–58.

Smith, J. A., Hammerschmidt, R., & Fulbright, D. W. (1991). Rapid induction of systemic resistance in cucumber by *Pseudomonas syringae* pv. syringae. *Physiological and Molecular Plant Pathology, 38*, 223–235.

Srivastava, M. K., & Dwivedi, U. N. (2000). Delayed ripening of banana fruit by salicylic acid. *Plant Science, 158*, 87–96.

Sticher, L., et al. (1997). Systemic acquired resistance. *Annual Review of Plant Pathology, 35*, 235–270.

Strawn, M. A., Marr, S. K., Inoue, K., Inada, N., Zubieta, C., et al. (2007). Arabidopsis isochorismate synthase functional in pathogen-induced salicylate biosynthesis exhibits properties consistent with a role in diverse stress responses. *The Journal of Biological Chemistry, 282*, 5919–5933.

Tanaka, S., Hayakawa, K., Umetani, Y., & Tabata, M. (1990). Glucosylation of isomeric hydroxybenzoic acids by cell suspension cultures of *Mellotus japonicus. Phytochemistry, 29*, 1555–1558.

Tian, S., Qin, G., Li, B., Wang, Q., & Meng, X. (2007). Effects of salicylic acid on disease resistance and postharvest decay control of fruits. *Stewart Postharvest Review, 6*, 1–7.

Valero, D., Díaz-Mula, H. M., Zapata, P. J., Castillo, S., Guillén, F., Martínez-Romero, D., et al. (2011). Postharvest treatments with salicylic acid, acetylsalicylic acid or oxalic acid delayed ripening and enhanced bioactive compounds and antioxidant capacity in sweet cherry. *Postharvest Biology and Technology, 59*(10), 5483–5489.

Van Loon, L. C., Rep, M., & Pieterse, C. M. J. (2006). Significance of inducible defense-related proteins in infected plants. *Annual Review of Phytopathology, 44*, 135–162.

Vaughn, K. C., & Duke, S. O. (1984). Function of polyphenol oxidase in higher plants. *Physiologia Plantarum, 60*, 106–112.

Verberne, M. C., Verpoorte, R., Bol, J. F., Mercado-Blanco, J., & Linthorst, H. J. M. (2000). Overproduction of salicylic acid in plants by bacterial transgenes enhances pathogen resistance. *Nature Biotechnology, 18*, 779–783.

Vlot, C. A., Dempsey, D. A., & Klessig, D. F. (2009). Salicylic acid, a multifaceted hormone to combat disease. *Annual Review of Phytopathology, 47*, 177–206.

Wang, L., Chena, S., Kong, W., Li, S., & Archbold, D. D. (2006). Salicylic acid pretreatment alleviates chilling injury and affects the antioxidant system and heat shock proteins of peaches during cold storage. *Postharvest Biology and Technology, 41*, 244–251.

Wei, Y., Liu, Z., Su, Y., Liu, D., & Ye, X. (2011). Effect of salicylic acid treatment on postharvest quality, antioxidant activities, and free polyamines of asparagus. *Journal of Food Science, 76*(2), 126–132.

Wildermuth, M. C., Dewdney, J., Wu, G., & Ausubel, F. M. (2001). Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature, 414*, 562–571.

Wills, R. B. H., McGlasson, W. B., Graham, D., & Joyce, D. C. (2007). *Postharvest* (5th ed.). Sydney, NSW: University of New South Wales Press Ltd.

Xu, X., & Tian, S. (2008). Salicylic acid alleviated pathogen-induced oxidative stress in harvested sweet cherry fruit. Postharvest Biology and Technology, 49, 379-385.

Yao, H., & Tian, S. (2005). Effects of pre- and post-harvest application of salicylic acid or methyl jasmonate on inducing disease resistance of sweet cherry fruit in storage. Postharvest Biology and Technology, 35, 253–262.

Yalpani, N., Leen, J., Lawthon, M. A., & Raskin, I. (1993). Pathway of salicylic acid biosynthesis in healthy and virus-inoculated tobacco. *Plant Physiology, 103*, 315–321.

Yalpani, N., Schulz, M., Davies, M. P., & Balke, N. E. (1992). Partial purification of an inducible uridine-5'-diphosphate glucose: Salicylic acid glucosyltransferase from oat roots. *Plant Physiology, 100*, 457–463.

Yu, T., & Zheng, X. D. (2006). Salicylic acid enhances biocontrol efficacy of the antagonist *Cryptococcus laurentii* in apple fruit. *Journal of Plant Growth Regulation, 25*, 166–174.

Zeng, K. F., Cao, J. K., & Jiang, W. B. (2006). Enhancing disease resistance in harvested mango (*Mangifera indica* L. cv. Matisu) fruit by salicylic acid. *Journal of the Science of Food and Agriculture, 86*, 694–698.

Zhang, Y., Chen, K., Zhang, S., & Ferguson, I. (2003). The role of salicylic acid in postharvest ripening of kiwifruit. *Postharvest Biology and Technology, 28*, 67–74.

Chapter 5
Polyamines

Praveen Kumar Mishra, Mohammed Wasim Siddiqui, and Sanjay Sahay

Introduction

Polyamines (PAs), the new group of plant growth regulators, are positively charged nitrogenous compounds derived from amino acids and bound to negatively charged cellular components, such as proteins, DNA, and membrane phospholipids. PAs are polycations with low molecular weight and are found in all living organisms (Raeisi et al. 2013). A number of PAs are present in the plants but only three PAs—viz., putrescine (a diamine), spermidine (a triamine), and spermine (a tetra-amine)—are commercially used in postharvest management of horticultural crops. Putrescine (PUT), spermidine (SPD), and spermine (SPM) are the collective forms of PA. Some other secondary conjugated products are also found in PA (Malmberg et al. 1998). PAs are basic amino acids like arginine, ornithine, and lysine, forming a basic carbon skeleton, whereas methionine contributes a propylamino group to PUT to form SPD and to SPD to form SPM (Bangi 1986). The commercially useful PAs can be found in every plant part, ranging from approximately micromolar to millimolar, together with the enzyme amendable for their metabolism. The amount of PAs and enzymes greatly depend on environmental surroundings, especially during stress (Galston and Sawhney 1990a, b; Asrey et al. 2008).

PAs are involved in several biological and physiological processes, such as growth and development of plants, including flowering, fruit ripening, handling abiotic/biotic stresses, and senescence (Malik and Singh 2004; Adam and Murthy 2013).

P.K. Mishra • S. Sahay
Department of Horticulture (Fruit and Fruit Technology), Bihar Agricultural University, Sabour, Bhagalpur, Bihar 813 210, India

M.W. Siddiqui (✉)
Department of Food Science and Postharvest Technology, Bihar Agricultural University, Sabour, Bhagalpur, Bihar 813 210, India
e-mail: wasim_serene@yahoo.com

M.W. Siddiqui et al. (eds.), *Postharvest Management Approaches for Maintaining Quality of Fresh Produce*, DOI 10.1007/978-3-319-23582-0_5

Polyamines may act as free-radical scavengers and membrane stabilizers (Wang 2010). The use of exogenous PAs (SPM, SPD, and PUT) increases postharvest life, maintains quality, sustains fruit firmness, reduces ethylene synthesis, guards against water loss, delays changes of color, soluble solids and titratable acidity, and protects fruit against chilling and mechanical injury (Malik et al. 2006). This chapter summarizes the available literature on the use and roles of PAs in postharvest management of fruit (Table 5.1).

Occurrence of Polyamines

The naturally occurring PAs (PUT, SPD, and SPM) are collective compounds originating in numerous plant bacteria, viruses, and animal tissues (Beer and Kosuge 1970). They are present in plant systems, either free or in conjugate form and bound with phenolic acids as well as other compounds, such as proteins and nucleic acids. PAs appear in young tissues and function in cellular multiplication, differentiation during organogenesis, flowering, pollination, and early fruit development (Costa et al. 1984). They also stimulate DNA replication, transcription, and translation and are involved in an extensive range of biological processes under several types of biotic and abiotic stresses (Tiburcio et al. 1993; Galston et al. 1997; Bais and Ravishankar 2002).

Sources of Industrial Separation of PAs

Polyamines can be separated industrially from both plant and microbial sources. In the case of plant sources, PAs can be extracted from leaves and stems of corn (*Zea mays* L.), cucumber (*Cucumis sativus* L.), oat (*Avena sativa* L.), and radish (*Raphanus sativus* L.), while *Saccharomyces cerevisiae* and *Candida utilis* are the important microbial sources of PAs (Asrey et al. 2008).

Biosynthesis of Polyamines

The majority of higher plants synthesize PAs in two different pathways (Fig. 5.1). The first step of synthesis starts from the basic amino acid arginine. Putrescine is synthesized purely by two dissimilar pathways, both of them starting from arginine.

In the first pathway, arginine in the presence of the enzyme arginase is converted into ornithine, and putrescine is synthesized by the decarboxylation of ornithine through the enzyme ornithine decarboxylase (ODC). In the second pathway, arginine is transformed into agmatine by the enzyme arginine decarboxylase (ADC);

Table 5.1 Summary of the detrimental effects of PAs related to quality

Crop	Type of PA	Dose(s) used	Effect(s) on quality	References
Mango	PUT	1 mM	Delayed fruit ripening	Malik and Singh (2003)
		2 mM	Enhanced shelf life, maintained fruit firmness	
	SPM and PUT	0.5 mM and 1 mM	Reduced weight loss	Malik et al. (2006)
			Retarded fruit color	
	PUT	1 mM	Reduced softness	
	SPM, SPD and PUT	0.01 mM, 0.5 mM and 1 mM		
	SPM	1 mM	Enhanced shelf life	Malik (2003), Malik et al. (2005)
	SPM	0.01 mM	Reduced respiration rate	
	PUT	1 mM	Delayed fruit ripening	Jawandha et al. (2012)
	PUT	2 mM	Reduced weight loss and spoilage percent	
Strawberry	PUT	1 and 2 mM	Maintained fruit firmness, enhanced shelf life, lowered fungal infection	Khosroshahi et al. (2007)
Apricot	PUT	4 mM	Improved fruit firmness, phenolic content, and antioxidant activity; maintained TSS; decreased respiration rate	Davarynejad et al. (2013)
		1 mM	Increased shelf life; reduced respiration rate, weight loss, bruising regions, ethylene biosynthesis; lowered cellular juice leakage and browning	Perez-Vicente et al. (2002), Martinez-Romero et al. (2001, 2002)
		4 mM	Reduced weight loss	Ghasemnezhad et al. (2010)
Pomegranate	PUT or SPD	1 mM	Maintained fruit firmness, enhanced shelf life, reduced husk scald and weight loss, prevented skin browning	Mirdehghan et al. (2007), Mirdehghan and Rahemi (2002)
'Valencia' orange	PUT+ MJ	5 mM + 10 µmol	Lowered enzymatic activity and chilling injury	Mohammadrezakhani and Pakkish (2014)
			Reduced chilling injury	
	SPD	1.5 mM	Maintained TSS, titratable acidity, flavor index	Raeisi et al. (2013)
		1 mM		

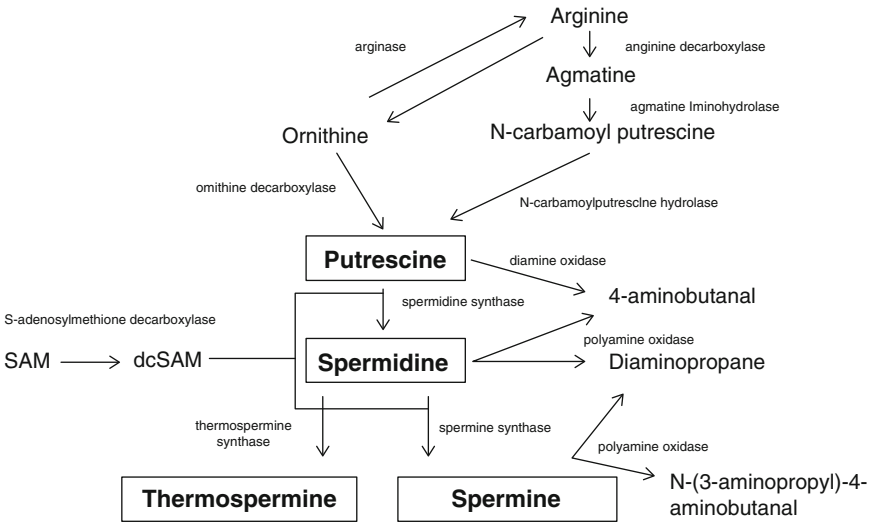


Fig. 5.1 PA biosynthesis and breakdown pathway in plants (Hunter and Burritt 2012)

agmatine is converted in to *N*-carbamoyl putrescine via agmatine iminohydrolase, which is finally converted into putrescine via *N*-carbamoylputrescine amidohydrolase (Alcázar et al. 2006).

Most plants are also richer in arginine than in ornithine, implying that arginine is synthesized. Plants contain both the ADC and the ODC pathways for putrescine biosynthesis. ADC is mostly found linked among the thylakoid membranes of chloroplasts and the nucleus (Bortolotti et al. 2004), while ODC is confined to a small area in the cytosol (Borrell et al. 1995). The biosynthesis of spermidine, spermine, and other polyamines is derived from putrescine (Table 5.2).

Spermidine is synthesized by spermidine synthase (SPDS), along with an aminopropyl group from decarboxylated *S*-adenosylmethionine (dcSAM), which is produced from *S*-adenosylmethionine (SAM) in a reaction that is catalyzed by the enzyme *S*-adenosylmethionine decarboxylase (SAMDC). Spermine is synthesized from the addition of another aminopropyl group to spermidine by spermine synthase (SPMS) (Slocum 1991).

SAMDC greatly affects the biosynthesis of PA and the ripening hormone ethylene because SAM is a precursor for both (Kumar et al. 1996). Putrescine also assists in the synthesis of other PAs, and in some plant groups putrescine can be used to provoke the synthesis of alkaloids with the help of putrescine *N*-methyltransferase or catalyzed by diamine oxidase (DAO). The polyamine oxidases (PAOs) are found in plants and produce H_2O_2. PA oxidases oxidize the secondary amine groups of spermidine and spermine (Medda et al. 1995), while both the enzymes diamine oxidase and polyamine oxidase are closely linked with the cell wall (Slocum 1991).

Table 5.2 Summary of the detrimental effects of PAs related to quality

Crop	Type of PA	Dose(s) used	Effect(s) on quality	References
Plum	PUT	1 mM	Reduced ethylene synthesis, maintained fruit firmness, increased shelf life	Perez-Vicente et al. (2001, 2002), Serrano et al. (2003), Khan et al. (2007)
Kiwi	PUT	1 mM	Enhanced fruit firmness and shelf life, reduced respiration rate, inhibited ethylene production	Petkou et al. (2003)
Peach	PUT+ ultrasound	1 mM + 32 kHz	Maintained TSS, prevented skin browning	Bal (2013)
Apple	PUT	5×10^{-5} M	Higher TSS	Costa and Bagni (1983)
Litchi	PUT, SPM, and SPD	1 mM	Enhanced shelf life, prevented skin browning and ethylene production	Jiang and Chen (1995)

Types of Polyamines

A number of polyamines are found in plants. In addition to the most common PAs, such PUT, SPD, and SPM, several other PAs have been reported in plant systems, including cadaverine; homospermidine; caldopentamine; canavalmine; aminobutyl canavalmine; aminopropyl canavalmine; 1,3-diaminopropane; norspermidine (caldine); and norspermine (thermine) (Edreva 1996).

On the basis of solubility, PAs are available in free and bound forms in the plant. Free forms of PAs are soluble in trichloric acid and perchloric acid, while bound forms are insoluble in these acids. In the free form, PAs are bound electrostatically to negatively charged molecules and conjugated to tiny molecules and proteins (Martin-Tanguy 1997). PAs are most commonly linked to cinnamic acids, and the resulting conjugates are called hydroxycinnamic acid amides (HCAAs). PAs like PUT, SPD, and SPM are water soluble, whereas neutral HCAAs containing aromatic amines such as tyramine, octopamine, and tryptamine are insoluble in water (Facchini et al. 2002).

Time and Method of Application

Several preservation technologies have been suggested to enhance the postharvest life of fresh horticultural produce. In some cases, producers rely on a substitute procedure as well as disinfectants with low-residue thresholds, substantial methods, controlled atmosphere, and biological controls (Eshel et al. 2009). The exogenous application polyamines hampers ethylene evolution and delays fruit ripening, offering enhanced storage life (Perez-Vicente et al. 2002; Torrigiani et al. 2004). PAs work as antisenescents by inhibiting the synthesis of the enzymes necessary for

ethylene production (Ke and Romani 1988). Several studies have noted that externally applied PAs influence fruit quality in terms of fruit firmness, weight loss, ethylene evolution, total soluble solids (TSSs), and titratable acids (Kumar et al. 1996; Khosroshahi et al. 2008).

Effect of PAs on Fruit Quality

Quality can be defined as produce with high nutritive value and good visual appearance and that fulfills the organoleptic criteria (Siddiqui 2015; Barman et al. 2015). Quality products attract the consumer and safeguard nutrients. Advanced storage technologies, like MA storage (Yahia 1998), low temperature storage (Ketsa et al. 1999), and CA storage (Bender et al. 2000), has been studied as ways to lengthen the shelf life of fruit but their effects are unpredictable. The exogenous application of PAs has been reported to slow down the ripening process and significantly extend the shelf life of fruit with better quality. Martinez-Romero et al. (2002) studied the effects of PUT application (1 mM) on apricots (*Prunus armeniaca* L. cv Mauricio), which were then mechanically damaged by a 25-N force and then stored at 10°C for 6 days. The researchers found the PUT-treated fruits, whether damaged or undamaged, significantly maintained quality throughout the storage period, displaying higher firmness, delayed color changes, and reduced weight loss as well as decreased ethylene production, respiration rate, and bruising (from the mechanical damage).

The preharvest and postharvest PUT application inhibited fruit color development in mangoes. For the preharvest application, PUT (1 mM) was sprayed on mango trees 7 days before harvest; for the postharvest application, fruit was dipped in PUT for 6 min. The PUT application enhanced fruit firmness and reduced the sugar content compared to untreated fruit. A preharvest application of 1 mM was effective in delaying fruit ripening at room temperature, whereas a 2-mM application enhanced the storage life of 'Kensington Pride' mangoes (Malik et al. 2003). Bhagwan et al. (2000) and Malik et al. (2003) also stated that the postharvest PUT application conserved sugar in tomatoes (*Lycopersicon esculentum* Mill.) and mangoes (*Mangifera indica* L.) during storage and maintained overall quality when compared with untreated fruit. Serrano et al. (2003) studied the effect of PUT on four plum cultivars ('Golden Japan', 'Black Diamond', 'Black Star', and 'Santa Rosa') for improving shelf life. They reported that a 1-mM application of PUT maintained superior fruit quality during storage, including higher fruit and flesh firmness, lower soluble solids concentration and titratable acidity, and delayed color changes. In addition, higher concentrations of free-form PUT and SPD were also observed in PUT-treated plums, which significantly altered the storage life. The exogenous application of PAs on mangoes at the final fruit set stage retarded fruit skin color development, reduced sugars and TSSs, increased acidity with SPM (16.7%) and with PUT (11%) treatment, improved total carotenoids in the pulp (49%), and significantly reduced ascorbic acid with SPD (24 %) and PUT (20%) treatments (Malik and Singh 2006). Malik et al. (2006) assessed the effect of PAs on

mangoes and observed lower concentrations of SPM (0.5 mM) and higher concentrations of PUT and SPD (1 mM) were more effective in reducing physiological weight loss in mango cv. 'Kensington Pride' during storage. Whereas PUT (1 mM) was more effective in retarding the development of fruit color. The treated fruit showed a lower visual mean fruit color score (2.45) on the first day after storage. The average value of TSS was considerably enhanced with PUT treatment but decreased with SPM-treated fruits.

Prestorage application of polyamines by immersion improves shelf life of pomegranates. Mirdehghan et al. (2007) observed that PUT and SPD (1 mM) significantly reduced firmness during storage, while untreated fruit exhibited softening and delayed ripening. Peel color for pomegranates is the most essential quality, directly controlling consumer attraction as well as aril development. Barman et al. (2011) studied the effects of PUT and carnauba wax pretreatments on pomegranate cold storage. The maximum hue angle (0.347) reading was observed with PUT (2 mM) and carnauba wax (1:10). Fruit with a minimum hue angle were deep tan red and dull, but those with the maximum hue angle were red and shiny. Increased soluble solids and decreased titratable acidity and total sugars were seen in untreated fruit at the end of storage time, but fruit treated with PUT and carnauba wax showed increased levels of total sugars and titratable acidity and decreased amounts of soluble solids content. Raeisi et al. (2013) worked on the improvement of qualitative and quantitative attributes of oranges with postharvest PAs application during storage and observed that SPD (1.5 mM) significantly reduced chilling injury and maintained TSS, titratable acidity, the flavor index (TSS/acid ratio), and weight.

Effect of PA on Respiration Rate

Respiration is a continuous process in living systems. Most of the horticultural products are living entities even after harvest because they continue physiological processes until senescence. Most postharvest losses are caused by respiration, transpiration, and other metabolic activities that convert polysaccharides, or starch, into energy in the presence of oxygen (Siddiqui 2015). Development of a fungal infection, ethylene production, and fruit senescence increase the respiration rate. A reduced respiration rate can slow down the softening and increase the firmness of a fruit as well as improve the quality of the stored products.

Putrescine (1 mM) has been reported to reduce the respiration rate in apricots (Perez-Vicente et al. 2002; Martinez-Romero et al. 2002), pears (Zhao et al. 2007), plums (Chen and Zhu 2011), and kiwi fruits (Petkou et al. 2003) during storage. Mangoes treated with various levels of PUT or SPD (0.01, 0.5, or 1 mM) showed a reduced respiration rate and fruit softening (Malik and Singh 2005). PUT and SPD (1 mM) are also reported to slow down the respiration rate in pomegranates when applied via pressure infiltration or immersion (Mirdehghan et al. 2007). Khan et al. (2008) reported that putrescine-treated 'Angelino' plums stored at low temperature showed delayed respiration rate and improved quality. A PUT plus ultrasound treat-

ment could also delay the ripening process by inhibiting respiration rate in peaches (Bal 2013). Barman et al. (2011) reported that a combined PUT and carnauba wax pretreatment significantly reduces respiration rate (15.97 mL CO_2/kg/h) in pomegranates during cold storage for 60 days.

Effect of PA on Ethylene (C_2H_4) Synthesis

Ethylene is a gaseous plant hormone that dramatically influences the ripening process of fruit to obtain good external appearance and excellent aroma and flavor. The high ethylene concentration greatly affects the postharvest life of the fruit and ultimately leads to senescence. The biosynthesis of PA and ethylene is connected to a common precursor, SAM, but both the molecules have antagonistic effects during fruit ripening and senescence (Pandey et al. 2000). Ethylene production is connected with the biosynthesis of aminocyclopropane-1-carboxylic acid (ACC) and is enhanced in response to mechanical wounding and pathogen contamination. Fruit is divided into two groups on the basis of a climacteric peak and ethylene synthesis throughout ripening (Lelievre et al. 1997; Siddiqui and Dhua 2010). Ethylene biosynthesis can be inhibited by aminooxyacetic acid (AOA), aminoethoxyvinylglycine (AVG), and free-radical scavengers when sprayed at the mature preharvest stage. It extends ripening time but does not completely check fruit ripening (Hobson et al. 1984). Several researchers (Valero et al. 2002; Perez-Vicente et al. 2002; Torrigiani et al. 2004; Khosroshahi et al. 2008; Davarynejad et al. 2013) have reported that PAs act as anti-ethylene agents. The preharvest application of putrescine (1 mM) to mango cv. 'Kensington Pride' has been reported to be more effective due to low ethylene synthesis and delayed fruit ripening at ambient storage (Malik 2003; Malik et al. 2005); however, total free PA was increased in both skin and pulp tissues at the time of ripening through climacteric peak. The skin a has 55.8% higher concentration of PA than does the pulp (Malik and Singh 2004).

In relation to other PAs, SPM (1 mM) has been reported to be the most effective in reducing ethylene production (1.18 nmol/kg/h) in mango cv. 'Kensington Pride' (Malik et al. 2006). This means that among the commercially available PAs, only SPM is the best for mango shelf life enhancement. The optimum balance between polyamines and ethylene can inhibit fruit ripening by checking ethylene synthesis (Lee et al. 1997; Franco-Mora et al. 2005). The PUT (1 mM) treatment under low-pressure infiltration reduces ethylene biosynthesis in apricots (Martinez-Romero et al. 2002). PUT (2 mM) significantly lowers ethylene production in strawberry cv. 'Selva' throughout the storage period (Khosroshahi et al. 2007). Khan et al. (2007) reported that the various concentrations of PUT (1 and 2 mM) significantly reduce ethylene synthesis the 'Angelino' plums. The reduction of ethylene synthesis under PUT treatment shows a logical biosynthesis system between ethylene and polyamine (Pennazio and Roggero 1990).

The exogenous application of PUT (1 mM) also inhibits ethylene production in kiwi cv. 'Hayward'. In case of tomatoes, mutant plants produce high levels of PAs

and lower levels of ethylene, whereas rising level of PUT at the time of ripening also reduces ethylene production in tomato cv. 'Liberty' and enhances shelf life (Dibble et al. 1988; Saftner and Baldi 1990; Li et al. 1992). The combined pretreatment of PUT and carnauba wax on pomegranates retards the ethylene production during cold storage. No ethylene production was seen in pomegranates up to 30 days of storage for fruit treated with PUT or with PUT plus carnauba wax. Untreated fruit and that treated with only carnauba wax showed ethylene evolution from 30 days onward Barman et al. (2011). They also reported no ethylene production was seen in pomegranates up to 30 days of storage for fruit treated with PUT or with PUT plus carnauba wax. Untreated fruit and that treated with only carnauba wax showed ethylene evolution from 30 days onward.

Effect of PA on Weight Loss

Weight loss of fruits and vegetables is one of the essential aspects that determine produce quality. It occurs as a result of moisture loss during storage and leaves fruits shriveled and dry due to high temperature and prolong storage time (Nunes 2008). PA significantly reduces the weight loss of harvested fruit and vegetables during handling and storage. It may be due to several factors such as inhibition of respiration rate, ethylene production, and maintaining the PA levels. Martinez-Romero et al. (2002) assessed the effects of PUT (1 mM) on mechanically damaged apricots during storage at 10°C for 6 days. PUT significantly reduced weight loss with decreased ethylene emission, respiration rate, and bruising zones in both damaged and undamaged fruit. Weight loss of peaches was increased with the extending storage period, and the maximum weight loss was observed in ultrasound-treated fruits (8.7%), followed by PUT (8.2%).

Jawandha et al. (2012) assessed the effect of putrescine (0.0, 1.0, 2.0, and 3.0 mmol/L) on storage life and quality of mango cv. 'Langra'. They found fruit treated with PUT (2.0 mmol/L) gained the best quality in terms of low physiological loss in weight and spoilage percentage, whereas the maximum physiological loss in weight was recorded in untreated mangoes. The prestorage dip treatment with PA reduced weight loss during storage of mango cv. 'Kensington Pride' (Malik and Singh 2005). Malik et al. (2006) stated that the lower concentrations of SPM (0.5 mM) and higher concentrations of PUT and SPD (1 mM) were more effective in reducing physiological weight loss against untreated mango cv. 'Kensington Pride' during storage.

Prestorage application of polyamines significantly reduced weight loss by 13–15% in pomegranates and established the most effective treatment, whereas untreated fruit had a maximum weight loss of ~17% of their initial weight. In addition, among PA treatments no significant differences were obtained in weight loss after 15, 30, and 45 days of storage (Mirdehghan et al. 2007). PUT-treated lemons exhibited lower weight loss than untreated fruit during storage (Valero et al. 1998). Davarynejad et al. 2013 worked on the effect of altered concentration of postharvest

putrescine (1, 2, 3, and 4 mM) on quality attributes of two apricot cultivars ('Lasgerdi' and 'Shahrodi') during storage. The maximum weight loss was found in the control group during storage, followed by a 1-mM PUT treatment group; the minimum was found in the 4-mM PUT group. The qualities of apricots were maintained by the PUT treatment due to its effect on holding up the ripening processes, consolidating cell disposition, and delaying the removal of epicuticular waxes, which play a vital role in water exchange through the skin, and and subsquently PUT reduced the water loss would occur. Khosroshahi et al. (2007) observed the effects of various concentrations of PUT (0.3, 0.5, 1, and 2 mM) on the postharvest life of strawberry cv. 'Selva' at 5°C. No significant weight loss was observed in treated fruits compared to controls and dry treatment at all determination times. The combination of PUT (2 mM) with carnauba wax (1:10) has been reported to significantly lower the weight loss (~10%) in pomegranates, whereas untreated fruit had the highest weight loss (~17%) during the 60-day storage period (Barman et al. 2011). The combined effect of PUT and carnauba wax was more efficient over PUT separately.

Effect of PA on Fruit Firmness

Firmness is one of the quality attributes of fruit that significantly influences the marketing and storage of produce. Firmness of the fruit is due to presence of pectic substances in the cell wall. During the ripening process some enzymatic changes occur, resulting in fruit softening. Among all the degrading enzymes, only polygalacturonase (PG) has a major role in fruit softening; other enzymes like pectin methyl esterase (PME), pectin esterase, and cellulase are also linked with fruit softening (Mithcham and Gross 1991). The cell wall–softening enzymes were found in some fruits like apples, pears, and avocados, and PG is the principle enzyme responsible for hydrolytic cleavage (Fischer and Bennett 1991) and pectin degradation during ripening (Sitrit and Bennett 1998). PAs reduce the softening of fruits by inhibiting cell wall–degrading enzymes, such as pectinesterase, pectin methylesterase, and polygalacturonase (Valero et al. 2002). Postharvest application of PA in fruit has been reported to maintain firmness. PA is directly responsible for declining ethylene synthesis and maintaining fruit firmness (Perez-Vicente et al. 2002; Torrigiani et al. 2004). Fruit firmness improvement via PA application has also been reported in different fruit and vegetable crops, such as apples (Wang et al. 1993), strawberries (Ponappa et al. 1993; Khosroshahi et al. 2007), tomatoes (Law et al. 1991), peaches (Bregoli et al. 2002), plums (Serrano et al. 2003), pomegranates (Mirdehghan et al. 2007), citrus (Walheim 2007), and apricot cultivars (Martinez-Romero et al. 2002; Khosroshahi and Ashari 2008).

The exogenous PA application in apples ('Golden Delicious' and 'McIntosh') maintained firmness. Apples were treated with the low-pressure infiltration (82.7 kPa) method for 4 min with putrescine (1 mM, 10 mM), spermidine (0.25 mM, 1 mM), and spermine (0.25 mM, 1 mM). After 27 weeks of storage the PA-treated

fruit had more firmness than the untreated ones, whereas a minimum amount of spermidine was found to be more responsive in maintaining firmness compared to a maximum amount of putrescine (Kramer et al. 1991). The putrescine (1 mM) treatment of 'Hayward' kiwi fruit resulted in higher flesh firmness.

The exogenous PUT (2 mM) application enhanced the storage life of 'Kensington Pride' mangoes by maintaining high fruit firmness (Malik and Singh 2003). The prestorage dipping treatments of mango cv. 'Kensington Pride' with PA reduces fruit softness during storage, without a significant decline in ethylene synthesis (Malik and Singh 2005). Lower concentrations of SPM (0.01 mM) and higher concentrations of SPD (0.5 mM) and PUT (1 mM) have been reported to be the most effective in reducing softness of mango (*Mangifera indica* L. cv. 'Kensington Pride'). Bal (2013) reported that the PUT and PUT plus ultrasound treatment reduced fruit softening during storage. The highest firmness value was recorded in PUT plus ultrasound–treated fruit (4.7 kg) and then in PUT-treated fruit (4.2 kg) at the finish of storage.

Effect of PA on Total Soluble Solids

Total soluble solids, principal constituents of all fruit, greatly influence their acceptability among consumers. They increase with the maturity and help signal the actual harvesting stage in most horticultural crops. During the storage period, TSS content is continuously increased due to hydrolysis of polysaccharides.

The lower concentrations of SPM (0.01 mM), and higher concentrations of SPD (0.5 mM) and PUT (1 mM) significantly reduce the TSSs of mangoes (Malik et al. 2006). PAs play a key role regarding maintaining the total soluble solids, checking the conversion of starch and various other associated processes during storage. Other researchers reported that PUT-treated fruit exhibits lower TSS content than control groups in low temperature storage (Perez-Vicente et al. 2002; Serrano et al. 2003; Khosroshahi and Ashari 2008; Khan et al. 2008; Bal 2012). Bal (2013) reported that mangoes treated with PUT (1 mM) show lower conversion of TSSs into soluble content and maintain TSS levels throughout storage. Minimum changes in TSS content were observed by Davarynejad et al. (2013) in putrescine-treated (4 mM) apricots. Mangoes treated with putrescine (2.0 mM) retained the best quality in terms of a good blend of total soluble solids during storage (Jawandha et al. 2012).

Effect of PA on Ascorbic Acid

Ascorbic acid is an essential nutrient that is extremely responsive to degradation due to its easier oxidation compared to other nutrients during storage. The reduction in ascorbic acid concentration at the time of storage could be due to the conversion of

dehydroascorbic acid to diketogulonic acid by oxidation (Ishaq et al. 2009). PA application may be attributed to delayed ascorbate oxidase activity. Davarynejad et al. (2013) studied on the effect postharvest putrescine application (1, 2, 3, and 4 mM) on the quality attributes of apricot cv. 'Lasgerdi' and 'Shahrodi' stored at 4°C and 95% relative humidity (RH) for 20 days. The ascorbic acid content decreased considerably during storage in both cultivars, however, the treated fruit showed a significant difference in ascorbic acid concentration. The maximum concentration of ascorbic acid was found in PUT (4 mM) treatment and the lowest in control fruit throughout the storage.

Effect of PA on Total Phenolics

Total phenolics are the vital bioactive compounds in terms of quality attributes. They are highly beneficial to humans by providing resistance against several diseases and strengthening the immune system (Siddiqui et al. 2013, 2014, 2015). Phenolics are the secondary metabolites produced by plants; primary products include carbohydrate and protein. The total phenolics have been reported to decrease quickly during storage, possibly owning to the breakdown of cell structure as part of senescence (Ghasemnezhad et al. 2010). PAs play crucial roles in maintaining the total phenolic content and delaying the senescence process. Davarynejad et al. (2013) studied on the effect of various levels of postharvest putrescine on quality attributes of apricots during storage at 4°C. Total phenolic content was reduced significantly throughout storage in both apricot cultivars ('Lasgerdi' and 'Shahrodi'). The maximum level of total phenolics was obtained in 4-mM PUT treatment followed by 3-mM PUT and 2-nM PUT treatments. At the start of the storage period the total phenolics levels were maximum. They were at a minimum at the end of storage, irrespective of the treatments; however, maximum retention of total phenolics was observed in PUT-treated fruits.

Effect of PA on Antioxidant Activity

Secondary metabolites like flavonoids and phenolic acids and ascorbic acid have antioxidant capacity, and they are largely produced in horticultural crops. Antioxidant activity is an essential nutritional and biological property of the fruit and plays vital roles during stress (biotic and abiotic) conditions to protect and maintain fruit quality during storage. Fruits and vegetables are rich in antioxidant capacity and influence the consumer's health very effectively by providing resistant against various diseases. Therefore, it is necessary to maintain antioxidant activity of the fruit throughout storage period without hampering fruit quality.

When antioxidant activity is reduced, the total phenolics and ascorbic acid contents are also reduced, which indicates that the antioxidant activity is closely

related to total phenolic and ascorbic acid content (Diaz-Mula et al. 2009; Ghasemnezhad et al. 2010). PAs have been reported to maintain significantly the antioxidant capacity during storage. Davarynejad et al. (2013) reported that the application of PUT maintained the antioxidant activity in Iranian apricot (*Prunus armeniaca* L.) cultivars ('Lasgerdi' and 'Shahrodi'). They found that the fruit treated with 4-mM putrescine had the highest antioxidant activity, whereas untreated fruit had the lowest antioxidant activity during storage at 4°C. The treated fruit showed a positive correlation between PUT concentrations and antioxidant activity of the fruit due to a higher retention of total phenolics and ascorbic acid during storage.

Effect of PA on Enzymatic Activity

Enzymatic activities in harvested fruit are changed more drastically due to the several biochemical and metabolic processes. It is more prominent in physiologically active fruits due to some stored food materials. PAs and their biosynthetic enzymes are widely used in several metabolic processes in plants as well as in animals during biotic and abiotic stresses (Kaur et al. 2013). Among all common PAs, spermidine is more effective for lengthening the storage period and reducing damage of fruit (Esna-Ashari and Zokaee-Khosroshahi 2008). PAs, being cationic in nature, can easily mix with anionic compounds like nucleic acids, proteins, phospholipids, and pectic polysaccharides. PAs also conjugate the various types of enzymes that control PA activity as well as decrease pectinesterase activity in the flesh of grapefruit (Leiting and Wicker 1997; Benavides et al. 2000; Tiburcio et al. 1993). The level of PA in plant cells is controlled by its own biosynthesis, translocation, decomposition, and conjugation with several compounds. Moreover, the exogenous application of PAs can delay senescence, decreasing ADC activity as well as that of other essential enzymes responsible for ethylene biosynthesis (Khosroshahi et al. 2007; Roberts et al. 1986; Ke and Romani 1988).

PAs are produced from ornithine, arginine, SAM, and other enzymes involved in amino acid decarboxylation (Malmberg et al. 1998). PAs maintain membrane stability by acting as free-radical scavengers throughout the storage period (Bors et al. 1989) and can be produced by lipoxygenase (LOX) and phospholipase-D (PL-D) (Lester 2000). The relation between pectin and PAs hamper cell wall–degrading enzymes, such as pectinesterase, pectin methyl esterase, and polygalacturonase and maintain fruit firmness by stabilizing cell walls throughout the storage period (Kramer et al. 1991; Valero et al. 2002). PA greatly influences the activity of fruit-softening enzymes in mango cv. 'Kingston Pride' (Malik et al. 2005).

The association between gene expression and enzymatic activities has been reported to be deficient, which might be corrected by translational or posttranscriptional regulation or both as confirmed for ADC (Chang et al. 2000), ODC (Kwak and Lee 2001) and SAMDC (Hanfrey et al. 2002). However, at the time of storage, the PA levels decrease until 6 days after harvest (DAH) followed by a rise at 8 DAH

for peaches. In this condition, ethylene production increased over time, peaked at 6 DAH, and subsequently remained far above the baseline. A sharp loss of fruit firmness at 1 DAH might be due to the prompt ethylene production that can influence the cell wall–modulating enzymes (Bonghi et al. 1998).

PAs have also been reported to be responsible for stress tolerance at low temperatures in most fruit crops. In case of orange cv. 'Valencia' treated with putrescine (5 mM) a significantly low level of lipid peroxidation and peroxide hydrogen in peel and pulp has been observed under chilling stress. The low temperature interrupts the balance of active oxygen species metabolism such as hydrogen peroxide accumulation and demolition of scavenging enzymes such as peroxidise. The activities of antioxidant enzymes and chilling tolerance significantly influence the storage life of the harvested horticultural crops (Mohammadrezakhani and Pakkish 2014). PA inhibits the transcription, synthesis, and activity of ACC synthase in tomatoes, and reduces ACC and ACC-oxidase activity (Li et al. 1992). PA significantly inhibits the ethylene biosynthesis in avocados (Winer and Apelbaum 1986) and pears (Toumadje and Richardson 1988) by restricting ACC-synthase activity.

Effect of PA on Gene Expression

PAs have been found to manipulate the gene expression with active participation of several biosynthetic enzymes such as ADC, ODC, SAMDC, SPDS, and SPMS. In early studies, the SAMDC gene of yeast was transferred into tomato plants, which enhanced putrescine levels in transgenic tomatoes at the time ripening, thereby increasing the shelf life of the fruit (Mehta et al. 2002). Liu et al. (2006) demonstrated the role of PA in preharvest and postharvest fruit development to quantify the gene coding expression in peach cv. 'Akatsuki'. In the beginning of the work, researchers used reverse transcription polymerase chain reactions (RT-PCRs) with degenerate primers to intensify cDNA produced from the RNA of peach flower buds to get one-sided fragments of the genes encoding PA biosynthetic enzymes like ADC, ODC, SAMDC, SPDS, and SPMS. Each and every PCR produced was cloned and sequenced. The clones were named *pPpADC* (partial *Prunus persica* ADC), *pPpODC*, *pPpSAMDC*, *pPpSPDS,* and *pPpSPMS* under Accession Nos.AB194102, AB-194103, AB194104, AB194105, and AB194106, respectively. Among all the biosynthetic genes, *pPpODC* expression was near to baseline in postharvest fruit and the same as in preharvest fruit. Whereas the expression prototypes of *pPpADC*, *pPpSAMDC*, *pPpSPDS,* and *pPpSPMS* were decreased 48 DAF at pit hardening in peaches among all genes. The uppermost expression was found at 2 DAH, and the expression subsequently decreased (*pPpADC*, *pPpSAMDC,* and *pPpSPMS*) or stayed comparatively constant (*pPpSPDS*) at the end of the experiment. The reduced gene expression was unremarkable because PA concentrations decreased, and enzymatic activities were not perceived during storage. On the other hand, apart from ODC, expression of other genes was comparatively constant, especially for those of SAMDC, SPDS, and SPMS. This expression of genes might be a characteristic

feature of a stress response rather than a senescence response at the time of peach storage. The sequential trends in enzymatic activities did not accurately correspond with changes in gene expression patterns. Likewise, Ziosi et al. (2003) established that gene expression patterns of peaches were not simultaneous with enzymatic activities but were corrected by free polyamine concentrations. The association between gene expression and enzymatic activity was deficient, and it might be corrected by translational or posttranscriptional regulation or both, which was confirmed for ADC (Chang et al. 2000), ODC (Kwak and Lee 2001), and SAMDC (Hanfrey et al. 2002).

Effect of PA on Chilling Injury

Chilling injury (CI) is one of the abiotic stresses that occur due to extreme low temperature. Particularly, tropical and subtropical fruits are more susceptible to low temperatures during storage than are temperate fruits. This is the major drawback of postharvest handling of the fruits like mangoes, bananas, and guavas, as a comparatively high temperature (11–13°C) is essential during storage (Kader and Arpaia 2002). PA biosynthesis has been accounted significantly to modify chilling injury as well as other stresses (Malik et al. 2003). CI is a serious problem during storage of subtropical fruits at low temperatures such as 2–5°C (Ritenour et al. 2004). Low temperature interrupts the balance of active oxygen species metabolism, for instance hydrogen peroxide, and is key to their accumulation and the demolition of scavenging enzymes such as peroxidases. On the other hand, the accumulation of hydrogen peroxide also provokes lipid peroxidation and disturbs the membrane structure. The damaging of tissues and the mixing of enzymes and substrates greatly affect the fruit flavor and texture and reduce shelf life and economic value due to increasing membrane permeability, lipid peroxidation, and hydrogen peroxide content (Lee et al. 2001). The activities of antioxidant enzymes and the improvement of chilling tolerance are directly associated with each other and influence the storage life fresh perishables effectively. Oranges are sensitive to chilling injury however, a range of 2–7°C is safe for storage (Marcilla et al. 2006). The chilling injury symptoms come earlier on the peel than in the pulp in case of oranges due to damage to surface cells and injury stresses to the underlying tissues (Hung et al. 2007). Application of PAs (spermine, spermidine, and putrescine) at various levels (0, 0.5, 1, and 1.5 mM) significantly reduces chilling injury in sweet lemons during storage at 3 and 10°C. The PA-treated fruit exhibits no CI symptoms at 3°C, while untreated fruit suffer from chilling, which becomes visible with brown-spotted skin necrosis after 2 weeks (Amin and Rahemi 2007). CI increases the cell membrane damage of the organelles, and the production of hydrogen peroxide enhances lipid peroxidation, ultimately causing solute leaking. A substantial rise in CI might be due to an increase of malondialdehyde (MDA) and hydrogen peroxide content. PAs can prevent CI by maintaining membrane structure and reducing the gathering of reactive oxygen species (ROS). ROS consist of

superoxide radicals (O^-), hydroxyl radicals (OH^-), and hydrogen peroxide (H_2O_2), which are formed as byproducts through membrane-related electron transport activity and by numerous metabolic pathways (Shah et al. 2001). ROS in the membrane are mainly influenced by lipid bioconstituents; ROS affect the integrity of membrane structure and promote cell death by the main lipid biomolecules, polyunsaturated fatty acids (PUFAs) (Esterbauer et al. 1991). ROS also alters the redox potential of adjoining cells, where it provokes an antioxidative reaction by acting as a signal of oxidative stress (Sairam and Srivastava 2000). Several substances like mannitol, sodium formate, and sodium benzoate, which are synthesized in various plant parts, protect plant cells from these free radicals (Shen et al. 1997). PUT (5 mM) treatment has been reported to reduce chilling injury and improve the chilling resistance of orange cv. 'Valencia' (Mohammadrezakhani and Pakkish 2014). The polyamines at low temperatures affect antioxidant activities and reduce chilling injury in fruits such as apples and may be associated with resistance to chilling stress (Yoshikawa et al. 2007).

Mirdehghan et al. (2007) observed the effect prestorage application of polyamines by pressure infiltration, which improves shelf life of pomegranates and abates CI browning. They observed untreated fruit to attain the maximum skin browning (~55% of browned peel surface), whereas the application of PUT and SPD (1 mM) significantly reduced the skin browning (~30%) at the end of storage. The increase in skin browning is accompanied by enhanced electrolyte leakage. The researchers also reported that the color of the skin was decreased in untreated pomegranates whereas PA-treated fruit stayed significantly unchanged during storage. Seyf et al. (2008) worked on the application of polyamines and benzyladenine on nutritional value during storage of pomegranates and found that spermine and spermidine induce cold acclimation, maintaining the membrane fluidity at low temperatures and enhancing the endogenous levels of polyamines; this ultimately results in decreased electrolyte leakage and skin browning in treated fruit.

Barman et al. (2011) studied the effect of PUT and carnauba wax pretreatments. PUT (2 mM) separately or PUT (2 mM) plus carnauba wax (1:10) appreciably reduces CI and skin browning in pomegranates during cold storage, whereas untreated and carnauba wax–treated fruits possess maximum CI. The PUT plus carnauba wax–treated fruits had a 65% reduced incidence of chilling injury in relation to control fruit. Raeisi et al. (2013) reported that increased levels of spermidine significantly reduce chilling injury. They found that spermidine (1.5 mM) was the most effective treatment for reducing chilling injury on 'Valencia' orange var. 'Olinda' during low temperature storage.

Effect of PA on Mechanical Damage

Mechanical damage or abrasion is an important aspect that significantly affects all horticultural crops because of their smooth surfaces. It is caused by pressure between fruits and machinery at the time of harvesting, packaging, and handling

and in several other ways, such as loading and unloading compression, collision, and shaking in normal packing during transportation (Ahmad and Siddiqui 2015). Fruit bruising is directly associated with intensity of the collision force (Garcia et al. 1995), and mechanically damaged fruit shows noticeable deterioration of mesocarp and endocarp during storage. Some other factors like tissue anatomy, cell-to-cell union, cell turgor pressure, and cell wall force also contribute to the bruising response (Hayes et al. 1994; Martinez-Romero et al. 2002). Mechanical damage varies among fruit, depending on the cultivar's bruising susceptibility; the methods of harvesting, packaging, and shipping; and the storage circumstances. Due to these factors, the most important physiological responses that occur in damaged fruits are juice leakage, flesh browning, and weight loss resulting in reduced consumer acceptability (Asrey et al. 2008).

PAs significantly reduce the intensity of mechanical damage by making fruits firmer, allowing storage for relatively longer periods. The exogenous application of PA enhances fruit firmness with less fruit deformation throughout storage due to elevated endogenous levels of PAs and ultimately increased shelf life of apples (Kramer et al. 1991), peaches, and lemons (Valero et al. 1998). Maturity of the fruit can also alter the mechanical damage because both immature and more mature fruit have reduced storage life, reduced flavor, and a negative effect on consumers. The more mature fruits have significantly reduced shelf life and are more susceptible to mechanical damage (Miller 1992), whereas the susceptibility to mechanical damage can be minimised mature unripe fruits (Vergano et al. 1995). Martinez-Romero et al. (2001) reported that apricots treated with PUT (1 mM) via pressure infiltration show better firmness retention. In the study, the treatment was given after 24 h, and the fruit was mechanically damaged with a 25-N force in three equatorial fruit zones and stored at 10°C and 90% RH for 6 days. The PUT-treated fruit had less susceptibility to mechanical damage; moreover, the bruising volume, area, and fruit deformation percentage were lower in putrescine-treated fruit. This is due solely to the elevated firmness by the subordinate tissue disruption, cellular juice leakage, and browning, which could be a result of the enzymatic oxidation of phenolic substrates by polyphenoloxidase, which produces quinines in the presence of oxygen.

The exogenous application of PUT (1 mM) on mechanically damaged plums significantly lowers the fruit flesh deformation. an accumulation of both putrescine and spermidine (cell-wall conjugated) in treated plums was noted in the initial 2 weeks of storage; these elevated levels of bound PAs could be connected to the higher fruit firmness. Damaged control plums had significantly improved levels of free spermidine during the first 7 days of storage. The occurence of free spermidine might be considered a physiological marker category of stress (Perez-Vicente et al. 2001).

Perez-Vicente et al. (2002) assessed the effect of exogenous PUT (1 mM) application during the postharvest storage of mechanically damaged (50-N force) plum cv. 'Black Star' at 10°C to find out the force:deformation ratio and fruit flesh deformation. PUT has been reported to be directly involved in the reduction of mechanical damage as well as in the increase in firmness of fruit flesh. The exogenous application of PA has also been reported to reduce mechanical damage for both climacteric and nonclimacteric fruit, and it has potential at commercial level to

control the qualitative attributes of harvested fruit, with increased shelf life (Valero et al. 2002). Lower levels of ABA have been reported in both damaged and non-damaged putrescine-treated apricots as compared to control apricots. It might be due to the antisenescent properties of PAs in a variety of organ and plant tissues (Galston and Sawhney 1987). On the other hand, mechanically damaged fruit produces more ethylene due to bruising injury. The postharvest application of PAs inhibits ethylene production in various fruit crops (Martinez-Romero et al. 2004).

Effect of PA as Antibrowning Agent

Skin browning is a major problem in most of harvested fruits and vegetables and greatly affects the storage life and market value of the produce. Consumers require fresh fruits and vegetables that are free from surface blemishes. The exogenous application of 1 mM PUT, SPM, and SPD reduces alterations connected with senescence, such as ethylene production, browning, peroxide level, and cell leakage in litchis stored at 5°C due to an increased intensity of PA (Jiang and Chen 1995).

Mechanically damaged fruits are highly susceptible to browning because of several enzymatic reactions and increased fruit metabolism. PAs play a significant role in the reduction of browning during storage of produce. The main physiological effects that occur in damaged fruit are an increase in fruit metabolism, leakage of juice, flesh browning, and weight loss (Miller 1992). The exogenous application of PUT (1 mM) develops resistant in plums to mechanical damage during handling and packaging and prevents them from browning at low temperature (Perez-Vicente et al. 2002). Mirdehghan and Rahemi 2002 observed that the exogenous application of PAs on the storage life of pomegranates prevented skin browning of the fruit along with a reduced transpiration rate. Prestorage application of putrescine or spermidine either by pressure or by immersion led to significant reduction in the skin browning of pomegranates compared to controls (Mirdehghan et al. 2007). Bal (2013) reported that the browning symptoms first appear in nontreated peaches, whereas the fruits treated with PUT (1 mM) alone and in combination with ultrasound (32 kHz at 60 W/L for 10 min in 4 L distilled water) show very fewer symptoms of leatheriness and browning after 3 weeks of storage. A significant reduction in browning has also been reported in oranges treated with PUT (5 mM) and methyl jasmonate (MJ) (10 μM) compared to controls up to 4 months of storage Mohammadrezakhani and Pakkish (2014).

Effect of PA on Shelf Life

PA significantly increases the postharvest shelf life by affecting several physiological processes during storage and maintaining and improving the quality of fresh fruits and vegetables, such as apples (Kramer et al. 1991), strawberries (Ponappa

et al. 1993), plums (Ren et al. 1995), peaches (Martinez-Romero et al. 2000), and mangoes (Malik and Singh 2003, 2005). The application of PUT, SPM, and SPD (1 mM) enhances the shelf life of litchis by delaying ethylene production, browning, and cell leakage, and reducing peroxide levels (Jiang and Chen 1995).

Similarly, Martinez-Romero et al. (2002) reported that postharvest PUT (1 mM) improves the shelf life of nondamaged and intentionally damaged apricots by delaying ethylene emission and respiration rate and decreasing bruising zones caused by the mechanical damage. The exogenous application of PUT (2 mM) enhances the shelf life of mango cv. 'Kensington Pride' (Malik and Singh 2003). The exogenous application of PA has been reported for climacteric and nonclimacteric fruits, reducing mechanical damage and chilling injury, delaying color changes, and enhancing shelf life (Serrano et al. 1996; Perez-Vicente et al. 2002).

The exogenous application of PUT (1 mM) on 'Hayward' kiwi fruit was found to increase shelf life, decrease ethylene production, slow down respiration rate, and elevate flesh firmness. The exogenous application of PAs lengthened the shelf life as well as the quality attributes in pomegranates (Mirdehghan et al. 2007), and the relationship between ethylene and PAs was found to have a suppressive effect on fruit ripening and senescence (Katoh et al. 1987). Malik et al. 2006 also reported the exogenous application of polyamines improved the shelf life of mangoes without having deleterious effects on fruit quality.

Consequently PA treatment has a great potential to check commercial qualitative attributes with improved shelf life of harvested perishable products. PAs and ethylene have antagonistic effects on fruit ripening and senescence. Therefore, equilibrium between them is necessary to improve storage life and delay the ripening process of fruit. Generally, the PA concentration is reduced at the time of tissue senescence with hastened ethylene production (Valero et al. 2002; Rommero et al. 2007). The exogenous application of polyamine prolongs shelf life and delays senescence of 'Stark Red Gold' nectarines by hampering the ethylene production (Torrigiani et al. 2004). PA directly effects ethylene production because of the common precursor SAM, and it reduces C_2H_4 production by post-transcriptional alteration, which inhibits the enzymes of ethylene biosynthesis (Pandey et al. 2000).

Effect of PA on Postharvest Diseases

Strawberry (cv. 'Selva') treated with PUT (0.3, 0.5, 1, and 2 mM) for 5 min showed fewer fungal infection symptoms than untreated fruit and the fruit maintained a very good appearance when stored at 5°C (Khosroshahi et al. 2007). PA metabolism significantly distorts and counters the biotic stress in plants interacting with fungal pathogens. PA conjugated phenolic compounds, and hydroxycinnamic acid amides build up in cells due to the interaction between plants and a variety of pathogens (Walters 2003). All these results indicate that exogenous application of PUT may have an antipathogenic effect in strawberries. *Botrytis* (gray mold) is the most

important fungus that has been noticed in strawberries and is known to reduce storage life. Other fungi that are capable of infecting strawberries include *Rhizopus*, *Penicillium,* and *Alternaria*.

The distinctive symptom of husk scald in pomegranates appears as an intense brown-black discoloration starting from the blossom end (De Filippi et al. 2006), which increases and restricts on the skin. The dipping of pomegranates in PA mitigates typical symptoms of chilling injury and reduces the severity of husk scald (Mirdehghan et al. 2007).

Effect of PA on Quality and Other Treatments

PAs are used as shelf life enhancers of horticultural crops and are applied either endogenously or exogenously. They help maintain the freshness of the produce for a longer time. Individual PA treatment has a positive response in maintaining produce quality during storage, but conjugation of PA with other treatments shows better effects (Faust and Wang 1993).

'Tommy Atkins' mangoes treated with UV-C for 20 min had a lower amount of PA compared to fruit treated for 10 min. This could be connected to decay and softening (Gonzalez-Aguilar et al. 2001). Gonzalez-Aguilar et al. (2004) also reported that UV-C exposures for 3–5 min increases putrescine levels and results in higher accumulation of spermidine and spermine in peaches. These peak levels of PAs are due only to the response to the UV-C irradiation, and it could be beneficial for escalating the resistance of fruit tissue to deterioration and preventing chilling injury.

Several other treatments like high temperature stress were found to increase putrescine levels and improve physiological disorders in citrus (McDonald 1989). Hot water dips increased putrescine absorption and reduced decay and CI symptoms in citrus (Gonzalez-Aguilar et al. 1997). UV-C treatment through most selected doses commonly formed higher levels of free and conjugated polyamines, particularly putrescine, in comparison to fruit without treatments. The exogenous application of PUT or SPD either by pressure infiltration or immersion on pomegranates at chilling temperatures influences the fruit quality during storage. The effectiveness of PAs for CI was increased by pressure infiltration or immersion on pomegranates (Mirdehghan et al. 2007).

Ultrasound is the most recent nonthermal method for increasing shelf life of fresh fruit during storage. It is safe, nontoxic, and eco-friendly (Aday et al. 2012). Ultrasound treatment in liquid is used to eradicate bacteria, inactivate viruses, and various other microbials that harm the cell wall, thus extending the fruit and vegetable storage period (Ji et al. 2012). It has several beneficial effects in the processing industries, for example cleaning surfaces, drying and filtration, microorganism and enzyme inactivation, enzyme extraction, protein and antioxidant compounds, cell disruption, and heat transfer acceleration (Knorr et al. 2004). Ultrasound treatments on postharvest life of horticultural crops have been reported to lengthen the shelf

life and to sustain quality in strawberries (Cao et al. 2010) and litchis (Chen et al. 2012). The joint effect of PUT and ultrasound treatment controls chilling injury and decaying. It can also delay the ripening process by inhibiting respiration rate in peaches and can easily be used as an alternative of painstaking postharvest treatments to get better peach quality and storability (Bal 2013).

Application of polyamines along with benzyladenine maintains the nutritional value and increases the shelf life of pomegranates during storage, inducing cold acclimation and maintaining membrane fluidity (Seyf et al. 2008). PUT (2 mM) individually or jointly with carnauba wax (1:10) significantly retards ethylene production and respiration rate; reduces weight loss, chilling injury, and skin browning; improves fruit firmness; and maintains fruit quality of pomegranates during storage (Barman et al. 2011). Postharvest application of PUT (5 mM) in combination with methyl jasmonate (10 μmol) reduces catabolic enzymatic activities and alleviates chilling injury in orange cv. 'Valencia' (Mohammadrezakhani and Pakkish 2014) during storage.

Conclusion and Future Direction

Polyamines are a group of plant growth regulators with low molecular polycationic nitrogenous compounds that are present everywhere in living systems. It is a collective form of putrescine, spermidine, spermine, cadaverine, homospermidine, caldopentamine, canavalmine, norspermidine, and norspermine. The well-known polyamines like PUT, SPD, and SPM are present naturally in fruit and respond to numerous postharvest processes. The higher endogenous levels of free PAs—mainly PUT, SPD, and SPM—at the beginning of fruit development might help in cell division. Moreover, higher level of PAs during ripening helps to increase the storage life of fruits. Overall, exogenously PA-treated fruits, vegetables, and some flowers show prolonged shelf life with improved quality. PAs have been reported to make a significant contribution in delaying ethylene production and respiration rate, reducing physiological weight loss, retarding color changes, inducing mechanical resistance, and maintaining fruit firmness and antioxidant capacity as well as enhancing fruit quality for distant marketing with longer duration. The activated oxygen free radicals cause oxidative damage to all membranes and hasten senescence but PAs act as free-radical scavengers and membrane stabilizers.

Thus postharvest treatment of fruits with PAs (PUT, SPD, and SPM) is effective for delaying the ripening processes and therefore could be used commercially to extend the life of fruit with acceptable fruit quality for local and distant markets. Some contradictory reports urge further research for better understanding the mechanism and postharvest application of PAs among all the horticultural crops at the time of ripening and storage to know the exact effect of PAs on biosynthesis of aroma, volatile compounds, and sensory attributes of climacteric and nonclimacteric fruit at the molecular level.

References

Adam, S., & Murthy, S. D. S. (2013). Role of polyamines and their effect on photosynthesis in plants. *Research Journal of Pharmaceutical, Biological and Chemical Sciences, 4*(3), 596–605.

Aday, M. S., Temizkan, R., Buyukcan, M. B., & Caner, C. (2012). An innovative technique for extending shelf life of strawberry: Ultrasound. *LWT-Food Science Technology, 52*, 93–101.

Ahmad, M. S., & Siddiqui, M. W. (2015). *Postharvest quality assurance of fruits: Practical approaches for developing countries*. New York: Springer.

Alcázar, R., Marco, F., Cuevas, J. C., Patron, M., Ferrando, A., Carrasco, P., et al. (2006). Involvement of polyamines in plant response to abiotic stress. *Biotechnology Letters, 28*, 1867–1876.

Amin, H., & Rahemi, M. (2007). Investigation of effect of polyamines on chilling control in lemon. *5th Congress of Iranian for Horticultural Science*. Shiraz.

Asrey, R., Sasikala, C., Barman, K., & Koley, T. K. (2008). Advances in post harvest treatments of fruits—A review. *Annals of Horticulture, 1*(1), 1–10.

Bais, H. P., & Ravishankar, G. A. (2002). Role of polyamines in the ontogeny of plants and their biotechnological applications. *Plant Cell, Tissue and Organ Culture, 69*, 1–34.

Bal, E. (2012). Effect of postharvest putrescine and salicylic acid treatments on cold storage duration and quality of sweet cherries. *Suleyman Demirel University Journal of the Faculty of Agriculture, 7*, 23–31.

Bal, E. (2013). Effects of exogenous polyamine and ultrasound treatment to improve peach storability. *Chilean Journal of Agricultural Research, 73*(4), 435–440.

Bangi, N. (1986). The function of metabolism of polyamines in plant. *Acta Horticulturae, 179*, 95–103.

Barman, K., Ahmad, M.S., Siddiqui, M.W. (2015). Factors Affecting the Quality of Fruits and Vegetables: Recent Understandings. In: Postharvest Biology and Technology of Horticultural Crops: Principles and Practices for Quality Maintenance (Ed. M.W. Siddiqui). Apple Academic Press, Inc., New Jersey, USA. pp. 1–50.

Barman, K., Asrey, R., & Pal, R. K. (2011). Putrescine and carnauba wax pretreatments alleviate chilling injury, enhance shelf life and preserve pomegranate quality during cold storage. *Scientia Horticulturae, 130*, 795–800.

Beer, S. V., & Kosuge, G. (1970). Spermidine and spermine—Polyamine components of turnip yellow mosaic virus. *Virology, 40*, 930–938.

Benavides, M. P., Gallego, S. M., Comba, M. E., & Tomaro, M. L. (2000). Relationship between polyamines and paraquat toxicity in sunflower leaf discs. *Plant Growth Regulation, 31*(3), 215–224.

Bender, R. J., Brecht, J. K., Sargent, S. A., Huber, D. J., Subhadrabandhu, S., & Pichakum, A. (2000). Low temperature controlled atmosphere storage for tree-ripe mangoes (Mangifera indica L.). *Acta Horticulturae, 509*, 447–458.

Bhagwan, A., Reddy, Y. N., & Rao, P. V. (2000). Postharvest application of polyamines to improve the shelf-life of tomato fruit. *Indian Journal of Horticulture, 57*, 133–138.

Bonghi, C., Ferrarese, L., Ruperti, B., Tonutti, P., & Ramina, A. (1998). Endo-β-1, 4-glucanases are involved in peach fruit growth and ripening, and regulated by ethylene. *Physiologia Plantarum, 102*, 346–352.

Borrell, A., Culianezmacia, F. A., Altabella, T., Besford, R. T., Flores, D., & Tiburcio, A. F. (1995). Arginine decarboxylase is localized in chloroplasts. *Plant Physiology, 109*, 771–776.

Bors, N., Langebartels, C., Michel, H., & Sanderman, H. J. (1989). Polyamines as radical scavengers and potents against ozone damage. *Phytochemistry, 28*, 1589–1595.

Bortolotti, C., Cordeiro, A., Alcazar, R., Borrell, A., Culianez-Macia, F. A., Tiburcio, A. F., et al. (2004). Localization of arginine decarboxylase in tobacco plants. *Physiologia Plantarum, 120*, 84–92.

Bregoli, A. M., Scaramagli, S., Costa, G., Sabatini, E., Ziosi, V., Biondi, S., et al. (2002). Peach (*Prunus persica* L.) fruit ripening: Aminoethoxyvinylglycine (AVG) and exogenous polyamines affect ethylene emission and flesh firmness. *Physiologia Plantarum, 114*, 472–481.

Cao, S., Hu, Z., & Pang, B. (2010). Optimization of postharvest ultrasonic treatment of strawberry fruit. *Postharvest Biology and Technology, 55*, 150–153.

Chang, K. S., Lee, S. H., Hwang, S. B., & Park, K. Y. (2000). Characterization and translational regulation of the arginine decarboxylase gene in carnation (*Dianthus caryophyllus* L.). *The Plant Journal, 24*, 45–56.

Chen, Y., Jiang, Y., Yang, S., Yang, E., Yang, B., & Prasad, K. N. (2012). Effects of ultrasonic treatment on pericarp browning of postharvest litchi fruit. *Journal of Food Biochemistry, 36*, 613–620.

Chen, Z., & Zhu, C. (2011). Combined effects of aqueous chlorine dioxide and ultrasonic treatments on postharvest storage quality of plum fruit (*Prunus salicina* L.). *Postharvest Biology and Technology, 61*, 117–123.

Costa, G., & Bagni, N. (1983). Effect of polyamines on fruit set of apple. *HortScience, 18*, 59–61.

Costa, G., Baraldi, R., & Bagbi, N. (1984). Influence of putrescine on fruit-set of apple. *Acta Horticulturae, 149*, 189–195.

Davarynejad, G., Zarei, M., Ardakani, E., & Nasrabadi, M. E. (2013). Influence of putrescine application on storability, postharvest quality and antioxidant activity of two Iranian apricot (*Prunus armeniaca* L.) cultivars. *Notulae Scientia Biologicae, 5*(2), 212–219.

De Filippi, B. G., Whitaker, B. D., Hess-Pierce, B. M., & Kader, A. A. (2006). Development and control of scald on wonderful pomegranates during long-term storage. *Postharvest Biology and Technology, 41*, 234–243.

Diaz-Mula, H. M., Zapata, P. J., Guillen, F., Martínez-Romero, D., Cas-tillo, S., Serrano, M., et al. (2009). Changes in hydrophilic and lipophilic antioxidant activity and related bioactive compounds during postharvest storage of yellow and purple plum cultivars. *Postharvest Biology and Technology, 51*, 354–363.

Dibble, A. R. G., Davies, P. J., & Mutschler, M. A. (1988). Polyamine content of long-keeping Alcobaca tomato fruit. *Plant Physiology, 86*, 338–340.

Edreva, A. (1996). Polyamines in plants: Review. *Bulgarian Journal of Plant Physiology, 22*(1–2), 73–101.

Eshel, D., Regev, R., Orenstein, J., Droby, S., & Gan-Mor, S. (2009). Combining physical, chemical and biological methods for synergistic control of postharvest diseases: A case study of Black Root Rot of carrot. *Postharvest Biology and Technology, 54*, 48–52.

Esna-Ashari, M., & Zokaee-Khosroshahi, M. (2008). *Polyamines and horticultural science* (p. 293). Hamedan: Publication of Bu-ali Sina University.

Esterbauer, H., Schaur, J. R., & Zollner, H. (1991). Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes. *Free Radical Biology and Medicine, 11*, 81–128.

Facchini, P. J., Hagel, J., & Zulak, K. G. (2002). Hydroxycinnamic acid amide metabolism: Physiology and biochemistry. *Canadian Journal of Botany, 80*, 577–589.

Faust, M., & Wang, S. Y. (1993). Polyamines in horticultural important plants. *Horticultural Review, 14*, 333–356.

Fischer, R. L., & Bennett, A. B. (1991). Role of cell wall hydrolysis in fruit ripening. *Plant Molecular Biology, 42*, 675–703.

Franco-Mora, O., Tanabe, K., Itai, A., Tamura, F., & Itamura, H. (2005). Relationship between endogenous free polyamine content and ethylene evolution during fruit growth and ripening of Japanese pear (*Pyrus pyrifolia* Nakai). *Journal of the Japanese Society for Horticultural Science, 74*, 221–227.

Galston, A. W., & Sawhney, R. K. (1987). Polyamines and senescence in plants. In W. W. Thomson, E. A. Nothnagel, & R. C. Huffaker (Eds.), *Plant senescence: Its biochemistry and physiology* (pp. 167–181). Rockville, MD: American Society of Plant Physiologists.

Galston, A. W., & Sawhney, R. K. (1990a). Polyamines in plant physiology. *Plant Physiology, 94*, 606–610.

Galston, A. W., & Sawhney, R. K. (1990b). Polyamine and plant physiology. *Plant Physiology, 94*, 406–410.

Galston, A. W., Sawhney, R. K., Altabella, T., & Tiburcio, A. F. (1997). Plant polyamines in reproductive activity and response to abiotic stress. *Botanica Acta, 110*, 197–207.

Garcia, J. L., Rufz-Altisent, M., & Barreiro, P. (1995). Factors influencing mechanical properties and bruise susceptibility of apples and pears. *Journal of Agricultural Engineering and Research, 61*, 11–18.

Ghasemnezhad, M., Shiri, M. A., & Sanavi, M. (2010). Effect of chitosan coatings on some quality indices of apricot (Prunus armeniaca L.) during cold storage. *Caspian Journal of Environmental Sciences, 8*(1), 2533.

Gonzalez-Aguilar, G. A., Wang, C. Y., Buta, J. G., & Krizek, D. T. (2001). Use of UV-C irradiation to prevent decay and maintain postharvest quality of ripe 'Tommy Atkins' mangoes. *International Journal of Food Science and Technology, 36*, 767–773.

Gonzalez-Aguilar, G., Wang, C. Y., & Buta, G. J. (2004). UV-C irradiation reduces breakdown and chilling injury of peaches during cold storage. *Journal of the Science of Food and Agriculture, 84*, 415–422.

Gonzalez-Aguilar, G. A., Zacaras, L., Mulas, M., & Lafu-ente, M. T. (1997). Temperature and duration of water dips influence chilling injury, decay and polyamine content in 'Fortune' mandarins. *Postharvest Biology and Technology, 12*, 61–69.

Hanfrey, C., Franceschetti, M., Mayer, M. J., Illingworth, C., & Michael, A. J. (2002). Abrogation of upstream open reading frame-mediated translational control of a plant *S*-adenosylmethionine decarboxylase results in polyamine disruption and growth perturbations. *The Journal of Biological Chemistry, 277*, 44131–44139.

Hayes, J. A., Blaikie, F. H., Downs, C. G., & Sealey, D. F. (1994). Textural and physiological changes during ripening. *Scientia Horticulturae, 58*, 1–15.

Hobson, G. E., Nichol, S. R., Davies, J. N., & Atkey, P. T. (1984). The inhibition of tomato fruit ripening by silver. *Journal of Plant Physiology, 116*, 21–29.

Hung, S. H., Wang, C. C., Ivanov, S. V., Alexieva, V., & Yu, C. W. (2007). Repetition of hydrogen peroxide treatment induces a chilling tolerance comparable to cold acclimation in mung bean. *Journal of the American Society for Horticultural Science, 132*(6), 770–776.

Hunter, D. C., & Burritt, D. J. (2012). Polyamines of plant origin - An important dietary consideration for human health. In: Venketeshwer R, editor. Phytochemicals as nutraceuticals - Global approaches to their role in nutrition and health. Rijeka, Croatia: InTech. pp. 225–244.

Ishaq, S., Rathore, H. A., Majeed, S., Awan, S., & Zulfiqar-Ali-Shah, S. (2009). The studies on the physico-chemical and organoleptic characteristics of apricot (Prunus armeniaca L.) produced in Rawalakot, Azad Jammu and Kashmir during storage. *Pakistan Journal of Nutrition, 8*(6), 856–860.

Jawandha, S. K., Gill, M. S., Singh, N. P., Gill, P. P. S., & Singh, N. (2012). Effect of post-harvest treatments of putrescine on storage of Mango cv. Langra. *African Journal of Agricultural Research, 7*(48), 6432–6436.

Ji, L., Pang, J., Li, S., Xiong, B., & Cai, L. G. (2012). Application of new physical storage technology in fruit and vegetable industry. *African Journal of Biotechnology, 11*, 6718–6722.

Jiang, Y. M., & Chen, F. (1995). A study on polyamine change and browning of fruit during cold storage of litchi (Litchi chinensis Sonn). *Postharvest Biology and Technology, 5*, 245–250.

Kader, A. A., & Arpaia, M. L. (2002). Postharvest handling systems: Subtropical fruit. In A. Kader (Ed.), *Postharvest technology of horticultural crops* (pp. 233–240). Oakland, CA: Regents of the University of California, DNAR.

Katoh, Y., Hasegawa, T., Suzuki, T., & Fujji, T. (1987). Effect of 1-aminocyclopropane 1-carboxylic acid production on the changes in the polyamine levels in Hiproly barley callus after auxin withdrawal. *Agricultural and Biological Chemistry, 51*, 2457–2463.

Kaur, B., Jawandha, S. K., Singh, H., & Thakur, A. (2013). Effect of putrescine and calcium on colour changes of stored peach fruits. *International Journal of Agriculture, Environment & Biotechnology, 6*(2), 301–304.

Ke, D., & Romani, R. J. (1988). Effects of spermidine on ethylene production and the senescence of suspension-cultured pear fruit cells. *Plant Physiology and Biochemistry, 26*, 109–116.

Ketsa, S., Chidtragool, S., Klein, J. D., & Lurie, S. (1999). Firmness, pectin components and cell wall hydrolases of mango fruit following low-temperature stress. *The Journal of Horticultural Science and Biotechnology, 74*, 685–689.

Khan, A. S., Singh, Z., Abbasi, N. A., & Swinny, E. E. (2008). Pre or post-harvest applications of putrescine and low temperature storage affect fruit ripening and quality of 'Angelino' plum. *Journal of the Science of Food and Agriculture, 88*, 1686–1695.

Khan, A. S., Singh, Z., & Abbasi, N. A. (2007). Pre-storage putrescine application suppresses ethylene biosynthesis and retards fruit softening during low temperature storage in 'Angelino' plum. *Postharvest Biology and Technology, 46*, 36–46.

Khosroshahi, M. R. Z., & Ashari, E. M. (2008). Effect of putrescine application on post-harvest life and physiology of strawberry, apricot, peach and sweet cherry fruits. *Journal of Science and Technology of Agriculture and Natural Resources, 45*, 219–230.

Khosroshahi, M. R. Z., Ashari, E. M., & Fattahi, M. (2008). Effect of exogenous putrescine on postharvest of sweet cherry fruit, cultivar 'Surati-e Hamedan'. *Journal of Applied Horticulture, 10*, 154–157.

Khosroshahi, M. R. Z., Esna-Ashari, M., & Ershadi, A. (2007). Effect of exogenous putrescine on post-harvest life of strawberry (Fragaria ananassa Duch.) fruit, cultivar Selva. *Scientia Horticulturae, 114*, 27–32.

Knorr, D., Zenker, M., Heinz, V., & Lee, D. (2004). Applications and potential of ultrasonics in food processing. *Trends in Food Science and Technology, 15*, 261–266.

Kramer, G. F., Wang, C. Y., & Conway, W. S. (1991). Inhibition of softening by polyamine application in 'Golden Delicious' and 'Mcintosh' apples. *Journal of the American Society for Horicultural Science, 116*(5), 813–817.

Kumar, A., Taylor, M. A., Arif, S. A., & Davies, H. V. (1996). Potato plants expressing antisense and sense SAMDC transgenes show altered levels of polyamines and ethylene: Antisense plants display abnormal phenotypes. *The Plant Journal, 9*, 147–158.

Kwak, S. H., & Lee, S. H. (2001). The regulation of ornithine decarboxylase gene expression by sucrose and small upstream open reading frame in tomato (*Lycopersicon esculentum* Mill). *Plant and Cell Physiology, 42*, 314–323.

Law, D. M., Davies, P. J., & Mutschler, M. A. (1991). Polyamine-induced prolongation of storage in tomato fruits. *Plant Growth Regulation, 10*, 283–290.

Lee, D. H., Kim, Y. S., & Lee, C. B. (2001). The inductive response of the antioxidant enzymes by salt stress in the rice (*Oryza sativa* L.). *Journal of Plant Physiology, 158*, 737–745.

Lee, M. M., Lee, S. H., & Park, K. Y. (1997). Effects of spermine on ethylene biosynthesis in cut carnation (*Dianthus caryophyllus* L.) during senescence. *Journal of Plant Physiology, 151*, 68–73.

Leiting, V. A., & Wicker, L. (1997). Inorganic cations and polyamines moderate pectinesterase activity. *Journal of Food Science, 62*(2), 253–255.

Lelievre, J. M., Latche, A., Jones, B., Bouzayen, M., & Pech, J. C. (1997). Ethylene and fruit ripening. *Physiologia Plantarum, 101*, 727–739.

Lester, G. E. (2000). Polyamines and their cellular anti-senescence properties in honey dew muskmelon fruit. *Plant Science, 160*, 105–112.

Li, N., Parsons, B. L., Liu, D., & Mattoo, A. K. (1992). Accumulation of wound inducible ACC synthase transcript in tomato fruits is inhibited by salicylic acid and polyamines. *Plant Molecular Biology, 48*, 477–487.

Liu, J., Nada, K., Pang, X., Honda, C., Kitashiba, H., & Moriguchi, T. (2006). Role of polyamines in peach fruit development and storage. *Tree Physiology, 26*, 791–798.

Maharaj, R. (1995). *The effect of ultraviolet radiation (UV-C) on the postharvest storage behaviour of tomato (Lycopersicon esculentum Mill, cv. Capello)*. PhD dissertation. University Laval, Quebec, Canada.

Malik, A. U. (2003). *Fruitlet abscission and fruit ripening of mango in relation to polyamines*. PhD thesis, Curtin University of Technology, Perth Western Australia.

Malik, A. U., & Singh, Z. (2004). Endogenous free polyamines of mangos in relation to development and ripening. *Journal of the American Society for Horticultural Science, 129*(3), 280–286.

Malik, A. U., & Singh, Z. (2005). Pre-storage application of polyamines improves shelf-life and fruit quality of mango. *Journal of Horticultural Science and Biotechnology, 80*, 363–369.

Malik, A. U., & Singh, Z. (2006). Improved fruit retention, yield and fruit quality in mango with exogenous application of polyamines. *Scientia Horticulturae, 110*, 167–174.

Malik, A. U., Singh, Z., & Dhaliwal, S. S. (2003). Exogenous application of putrescine affects mango fruit quality and shelf life. *Acta Horticulturae, 628*, 121–127.

Malik, A. U., Singh, Z., & Khan, A. S. (2005). Role of polyamines in fruit development, ripening, chilling injury, storage and quality of mango and other fruits: A review. In A. U. Malik et al. (Eds.), *Proceedings: International Conference on Mango and Date Palm: Culture and Export* (pp. 181–187). Faisalabad: University of Agriculture.

Malik, A. U., Tan, S. C., & Singh, Z. (2006). Exogenous application of polyamines improves shelf life and fruit quality of mango. *Acta Horticulturae, 699*, 291–296.

Malmberg, R. L., Watson, M. B., Galloway, G. L., Yu, W., & Yu, W. (1998). Molecular genetics analyses of plant polyamines. *Critical Reviews in Plant Sciences, 17*, 199–224.

Marcilla, A., Zarzo, M., & Rio, M. A. (2006). Effect of storage temperature on the flavour of citrus fruit. *Spanish Journal of Agricultural Research, 4*(4), 336–344.

Martinez-Romero, D., Serrano, M., Carbonell, A., Burgos, L., Riquelme, F., & Valero, D. (2002). Effects of postharvest putrescine treatment on extending shelf life and reducing mechanical damage in apricot. *Journal of Food Science, 67*(5), 1706–1712.

Martinez-Romero, D., Serrano, M., Carbonell, A., Castillo, S., Riquelme, F., & Valero, D. (2004). Mechanical damage during fruit post-harvest handling: Technical and physiological implications. In R. Dris & S. M. Jain (Eds.), *Production practises and quality assessment of food crops* (Quality handling and evaluation, Vol. 3, pp. 233–252). Dordrecht: Kluwer Academic Publishers.

Martinez-Romero, D., Valero, D., Riquelme, F., Zuzunaga, F., Serrano, F., Burlo, F., & Carbonell, A. (2001). Infiltration of putrescine into apricots helps handling and storage. *Acta Horticulturae*, 189–192.

Martinez-Romero, D., Valero, D., Serrano, M., Burlo, F., Carbonell, A., Burgos, L., et al. (2000). Exogenous polyamines and gibberellic acid effects on peach (Prunus persica L) storability improvement. *Journal of Food Science, 65*, 288–294.

Martin-Tanguy, J. (1997). Conjugated polyamines and reproductive development: Biochemical, molecular and physiological approaches. *Physiologia Plantarum, 100*, 675–688.

McDonald, R. E. (1989). Temperature conditioning affects polyamines of lemon fruits stored at chilling temperatures. *HortScience, 24*, 475–477.

Medda, R., Padiglia, A., & Floris, G. (1995). Plant copper-amine oxidases. *Phytochemistry, 39*, 1–9.

Mehta, R. A., Cassol, T., Li, N., Ali, N., Handa, A. K., & Mattoo, A. K. (2002). Engineered polyamine accumulation in tomato enhances phytonutrient content, juice quality and vine life. *Nature Biotechnology, 20*, 613–618.

Miller, A. R. (1992). Physiology, biochemistry and detection of bruising (mechanical stress) in fruits and vegetables. *Postharvest News and Information, 3*, 53–58.

Mirdehghan, S. H., & Rahemi, M. (2002). Reduction of chilling injury in the pomegranate (*Punica granatum*) fruit by intermittent warming. *Iranian Journal of Agricultural Science, 33*(1), 75–80.

Mirdehghan, S. H., Rahemi, M., Castillo, S., Martinez, R. D., Serrano, M., & Valero, D. (2007). Pre-storage application of polyamines by pressure or immersion improves shelf life of pomegranate stored at chilling temperature by increasing endogenous polyamine levels. *Postharvest Biology and Technology, 44*, 26–33.

Mithcham, E. J., & Gross, K. C. (1991). Ripening and cell wall synthesis in normal and mutant tomato fruit. *Phytochemistry, 30*, 1777–1780.

Mohammadrezakhani, S., & Pakkish, Z. (2014). Chilling injury induces lipid peroxidation and alters the hydrogen peroxide content in peel and pulp of "Valencia" orange fruit under low temperature storage conditions. *Acta Advances in Agricultural Sciences, 2*(5), 10–26.

Nunes, M. C. N. (2008). Color atlas of postharvest quality of fruits and vegetables. In M. C. N. Nunes (Ed.), (463 p.). Wiley-Blackwell Publishing, Ames, IA, USA

Pandey, S., Ranade, S. A., Nagar, P. K., & Kumar, N. (2000). Role of polyamines and ethylene as modulators of plant senescence. *Journal of Biosciences, 25*(3), 291–299.

Pennazio, S., & Roggero, P. (1990). Exogenous polyamines stimulate ethylene synthesis by soybean leaf tissue. *Annals of Botany, 65*, 45–50.

Perez-Vicente, A., Martínez-Romero, D., Carbonell, A., Burlo, F., Serrano, M., Valero, D., et al. (2001). Role of exogenous putrescine on the metabolism of conjugated polyamines in mechanically damaged plum during storage. *Acta Horticulturae, 553*, 193–194.

Perez-Vicente, A., Martínez-Romero, D., Carbonell, A., Serrano, M., Riquelme, F., & Guillen, F. (2002). Role of polyamines in extending shelf life and the reduction of mechanical damage during plum (*Prunus salicina* Lindl.) storage. *Postharvest Biology and Technology, 25*, 25–32.

Petkou, I., Pritsa, T., & Sfakiotakis, E. (2003). Effect of dipping and pressure infiltration of putrescine on the propylene induced autocatalytic ethylene production and ripening of 'Hayward' kiwi fruit. *Acta Horticulturae, 610*, 261–266.

Ponappa, T., Scheerens, J. C., & Miller, A. R. (1993). Vacuum infiltration of polyamines increases firmness of strawberry slices under various storage conditions. *Journal of Food Science, 58*(2), 361–364.

Raeisi, M., Samani, R. B., & Honarvar, M. (2013). Application of exogenous spermidine treatment for reducing of chilling on fruit quality and quantity of Valencia orange var. olinda. *International Journal of Farming and Allied Sciences, 2*(S), 1292–1297.

Ren, X., Ma, F., Wang, F., Ren, X. L., Ma, F. W., & Wang, F. (1995). Effect of spermidine on ethylene and respiration of plum. *Plant Physiology Communications, 31*, 186–188.

Ritenour, M. A., Dou, H., & McCollum, G. T. (2003). Chilling injury of grapefruit and its control. Horticultural Science Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. https://edis.ifas.ufl.edu/pdffiles/HS/HS19100.pdf.

Roberts, D. R., Dumbroff, E. B., & Thompson, J. E. (1986). Exogenous polyamines alter membrane fluidity in bean leaves—A basis for potential misinterpretation of their physiological role. *Planta, 167*, 395–401.

Rommero, D. M., Bailen, G., Serrano, M., Guillen, F., Valverdi, J. M., Zapata, P., et al. (2007). Tools to maintain postharvest fruit and vegetable quality through the inhibition of ethylene action: A review. *Critical Reviews in Food Science and Nutrition, 47*, 543–560.

Saftner, R. A., & Baldi, B. G. (1990). Polyamine levels and tomato fruit development: Possible interaction with ethylene. *Plant Physiology, 92*, 547–550.

Sairam, R. K., & Srivastava, G. C. (2000). Induction of oxidative stress and antioxidant activity by hydrogen peroxide treatment in tolerant and susceptible wheat genotypes. *Biologia Plantarum, 43*, 381–386.

Serrano, M., Martinez-Madrid, M. C., Martinez, G., Riquelme, F., Pretel, M. T., & Romojaro, F. (1996). Review: Role of polyamines in chilling injury of fruit and vegetables. *Food Science and Technology International, 2*, 195–199.

Serrano, M., Martinez-Romero, D., Guillen, F., & Valero, D. (2003). Effects of exogenous putrescine on improving shelf life of four plum cultivar. *Postharvest Biology and Technology, 30*, 259–271.

Seyf, S. N., Abotalebi, A., & Zakerin, A. (2008). Investigation of effect of polyamine and benzyladenine on maintenance of nutritional properties of pomegranate (Punica granatum L.) during storage period. *18th National Congress on Food Technology*, Mashhad.

Shah, K., Kumar, R. G., Verma, S., & Dubey, R. S. (2001). Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. *Plant Science, 161*, 1135–1144.

Shen, B., Jenson, R. G., & Bohnert, H. J. (1997). Mannitol protects against oxidation by hydroxyl radicals. *Plant Physiology, 115*, 527–532.

Siddiqui, M. W. (2015). *Postharvest biology and technology of horticultural crops: Principles and practices for quality maintenance* (p. 572). Boca Raton, FL, USA: CRC Press.

Siddiqui, M. W., Ayala-Zavala, J. F., & Dhua, R. S. (2015). Genotypic variation in tomatoes affecting processing and antioxidant attributes. *Critical Reviews in Food Science and Nutrition, 55*(13), 1819–1835. doi:10.1080/10408398.2012.710278.

Siddiqui, M. W., Chakraborty, I., Mishra, P., & Hazra, P. (2014). Bioactive attributes of tomatoes possessing dg, ogc, and rin genes. *Food & Function, 5*, 936–943. doi:10.1039/c3fo60520e.

Siddiqui, M. W., & Dhua, R. S. (2010). Eating artificially ripened fruits is harmful. *Current Science, 99*(12), 1664–1668.

Siddiqui, M. W., Momin, C. M., Acharya, P., Kabir, J., Debnath, M. K., & Dhua, R. S. (2013). Dynamics of changes in bioactive molecules and antioxidant potential of Capsicum chinense Jacq. cv. Habanero at nine maturity stages. *Acta Physiologiae Plantarum, 35*(4), 1141–1148. doi:10.1007/s11738-012-1152-2.

Sitrit, Y., & Bennett, A. B. (1998). Regulation of tomato fruit polygalacturonase mRNA accumulation by ethylene: A re-examination. *Plant Physiology, 116*, 1145–1150.

Slocum, R. D. (1991). Polyamine biosynthesis in plants. In H. E. Flores & R. D. Slocum (Eds.), *Biochemistry and Physiology of Polyamines in Plants* (pp. 23–40). Boca Raton: CRC Press.

Smith, T. A. (1985). Polyamines. *Annual Review of Plant Physiology, 36*, 117–143.

Tiburcio, A. F., Campos, J. L., Figueras, X., & Besford, R. T. (1993). Recent advances in the understanding of polyamine functions during plant development. *Plant Growth Regulation, 12*, 331–340.

Torrigiani, P., Bregoli, A. M., Ziosi, V., Scaramagli, S., Ciriaci, T., & Rasori, A. (2004). Preharvest polyamine and aminoethoxyvinylglycine (AVG) applications modulate fruit ripening in Stark Red Gold nectarines (*Prunus persica* L. Batsch). *Postharvest Biology and Technology, 33*, 293–308.

Toumadje, A., & Richardson, D. G. (1988). Endogenouse polyamine concentrations during development, storage and ripening of pear fruits. *Phytochemistry, 27*, 335–338.

Valero, D., Martinez, D., Riquelme, F., & Serrano, M. (1998). Polyamine response to external mechanical brushing in two mandarin cultivars. *HortScience, 33*(7), 120–1223.

Valero, D., Martinez-Romero, D., & Serrano, M. (2002). The role of polyamines in the improvement of the shelf life of fruit. *Trends in Food Science & Technology, 13*(6–7), 228–234.

Vergano, P. J., Testin, R. F., Newall, J. R. W. C., & Trezza, T. (1995). Damage loss cost for peach impact bruising. *Journal of Food Quality, 18*, 265–278.

Walheim, L. (2007). *Citrus: Complete guide to selecting & growing more than 100 varieties for California, Arizona Texas, the Gulf Coast and Florida* (p. 160). Translator: Daneshgar, K. publication of Marze Danesh.

Walters, D. R. (2003). Polyamines and plant disease. *Phytochemistry, 64*, 97–107.

Wang, C. Y. (2010). Alleviation of chilling injury in tropical and subtropical fruits. In M. Souza & R. Drew (Eds.), *IIIrd IS on Trop. and Subtrop. Fruits. Acta Hort* (p. 864).

Wang, C. Y., Conway, W. S., Abbott, J. A., Kramer, G. F., & Sams, C. E. (1993). Postharvest infiltration of polyamines and calcium influences ethylene production and texture changes in 'Golden Delicious' apples. *Journal of the American Society for Horticultural Science, 118*, 801–806.

Winer, L., & Apelbaum, A. (1986). Involvement of polyamines in the development and ripening of avocado fruits. *Journal of Plant Physiology, 126*, 223–233.

Yahia, E. M. (1998). Modified and controlled atmospheres for tropical fruits. *Horticultural Reviews, 22*, 123–183.

Yoshikawa, H., Honda, C., & Konda, S. (2007). Effect of low-temperature stress on abscisic acid, jasmonates, and polyamine in apples. *Plant Growth Regulation, 52*, 199–206.

Zhao, Y., Feng, Z., & Li, X. (2007). Effect of ultrasonic and MA packaging method on quality and some physiological changes of fragrant pear. *Journal of Xinjiang Agricultural University, 30*, 61–63.

Ziosi, V., Scaramagli, S., Bregoli, A. M., Biondi, S., & Torrigiani, P. (2003). Peach (*Prunus persica* L.) fruit growth and ripening: Transcript levels and activity of polyamine biosynthetic enzymes in the mesocarp. *Journal of Plant Physiology, 160*, 1109–1115.

Chapter 6
Methyl Jasmonate

Shiping Tian and Zhanquan Zhang

Methyl jasmonate (MeJA) (Fig. 6.1), the derivative of Jasmonic acid (JA), has been found to occur naturally in a wide range of higher plants. The biosynthesis of MeJA starts with linolenic acid and proceeds through a number of stages involving lipoxidation, cyclization, and β-oxidation (Creelman and Mullet 1997). MeJA has been considered as an elicitor or signaling agent involved in many physiological and biochemical processes and widely used in the field of postharvest preservation of fruits and vegetables (Aghdam and Bodbodak 2013). According to the recent reports, the positive effects of MeJA include the following: (1) inducing the resistance of fruits against fungal pathogens; (2) inducing the resistance of fruits to low temperature stress; (3) keeping the quality of harvested fruits; (4) improving the biocontrol efficiency of antagonistic yeasts; (5) holding direct antimicrobial activity against some fungal pathogens.

S. Tian (✉)
Key Laboratory of Plant Resources, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

Institute of Botany, The Chinese Academy of Sciences, No. 20 Nanxincun, Xiangshan, Haidian District, Beijing 100093, China
e-mail: tsp@ibcas.ac.cn

Z. Zhang
Key Laboratory of Plant Resources, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

M.W. Siddiqui et al. (eds.), *Postharvest Management Approaches for Maintaining Quality of Fresh Produce*, DOI 10.1007/978-3-319-23582-0_6

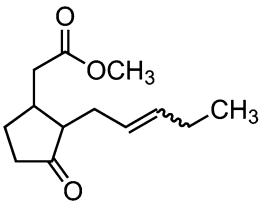


Fig. 6.1 Chemical structure of methyl jasmonate

MeJA Induces the Resistance of Fruits Against Fungal Pathogens

Horticultural crops often encounter various pathogens in postharvest storage periods. Postharvest diseases are usually controlled by the application of synthetic fungicides. However, due to problems related to the toxicity of fungicide, development of fungicide resistance by pathogens, and potential harmful effects on the environment and human health, alternatives to synthetic chemicals have been proposed. Defense responses to the attack of pathogens enable the horticultural crops to survive. Resistance responses refer to traits that inhibit or limit attack, while tolerance mechanisms reduce or offset consequences for disease damage. Induction of disease resistance in horticultural crops is a vital strategy to reduce damage of diseases, because it uses the defense mechanisms of the plant itself and has a broad-spectrum disease resistance (Tian and Chan 2004; Walters et al. 2005). The agents that are usually used to induce disease resistance include biotic (antagonistic yeasts) and abiotic factors (physical treatments: V-radiation, hot water brushing, UV-C light; chemical compounds: borate, silicon; hormone regulators: salicylic acid, jasmonic acid, methyl jasmonate, brassinosteroid) and so on (Han et al. 2006; Li et al. 2012; Meng et al. 2010; Zhu et al. 2010). MeJA is considered as a pivotal compound in the defense response of plant against pathogen infection. Several lines of evidence suggested that MeJA holds the ability to induce the disease resistance in various fruits (Yao and Tian 2005a). MeJA accumulates in wounded plants and in plants or cell cultures treated with elicitors of pathogen defense (Creelman et al. 1992). Exogenous MeJA has been reported to reduce postharvest decay and induce disease resistance to multiple pathogens in various horticultural crops (Buta and Moline 1998; Droby et al. 1999; Ding et al. 2002; González-Aguilar et al. 2003; Guo et al. 2014). Pre- and postharvest treatment of sweet cherry fruit with MeJA effectively inhibited the postharvest disease, but the effect of preharvest treatment was better than that of the postharvest treatment (Yao and Tian 2005a). Treating the peach fruit with 200 μmol L^{-1} MeJA alone or combined with antagonistic yeast significantly inhibits brown rot and blue mould caused by *Monilinia fructicola* and *Penicillium expansum* (Yao and Tian 2005b). In recent times, we investigated the effect of exogenous MeJA on gray mould rot caused by *Botrytis cinerea* in tomato fruit and its possible mechanism (Zhu and Tian 2012). We found that MeJA at a concentration of 10 mM effectively inhibited lesion diameter of gray mould rot in tomato fruit, and proved

that resistant response of MeJA-treated tomato fruit to *B. cinerea* was attributed to an accumulation of H_2O_2, concomitant with enhanced Cu-Zn superoxide dismutase (Cu-Zn SOD) gene expression and decreased catalase (CAT) transcript level during the early stage of storage (Fig. 6.2). Our results indicated that mechanisms involved in the induction of the fruit resistance to *B. cinerea* might be related to reactive oxygen species (ROS) metabolism.

The modes of the action of MeJA to induce disease resistance are summarized in Fig. 6.3. First defense response against pathogens induced by MeJA was promoting the capacities of antioxidant defenses and alleviating oxidative stress. It has been reported that fungal pathogen infection causes oxidative stress by inducing the generation and accumulation of ROS in plants, such as H_2O_2 and O_2^- (Campo et al. 2004). ROS leads to oxidative damages by oxidizing nucleic acids, proteins, lipids, or carbohydrates, affecting the integrity of cell membranes and inactivating key cellular functions (Tian et al. 2013). The constitutive levels of antioxidant defenses may be insufficient to control the oxidative burst. Therefore, activation of antioxidant strategies is necessary for the maintenance of cell redox homeostasis. Accumulating studies have reported that the mechanisms involved in the induction of the disease resistance might be associated with elicitation of antioxidative reaction which controls the homeostatic levels of ROS. Reported that MeJA suppressed fungal decay and prolonged postharvest life of strawberries by increasing antioxidant capacity. Our results proved that MeJA significantly induced the activity of peroxidase (POD) in sweet cherry and peach fruit (Yao and Tian 2005a, b), stimulated catalase (CAT) and ascorbate peroxidase (APX) gene expression, and enhanced ascorbate (ASC) and glutathione (GSH) content, which is beneficial for scavenging excess ROS and alleviating oxidative damage of proteins (Zhu and Tian 2012).

Another mechanism involved in the MeJA-induced disease resistance might be the expression of pathogenesis-related (PR) proteins. Jin et al. (2009) reported that MeJA was effective in reducing decay and might enhance disease resistance in

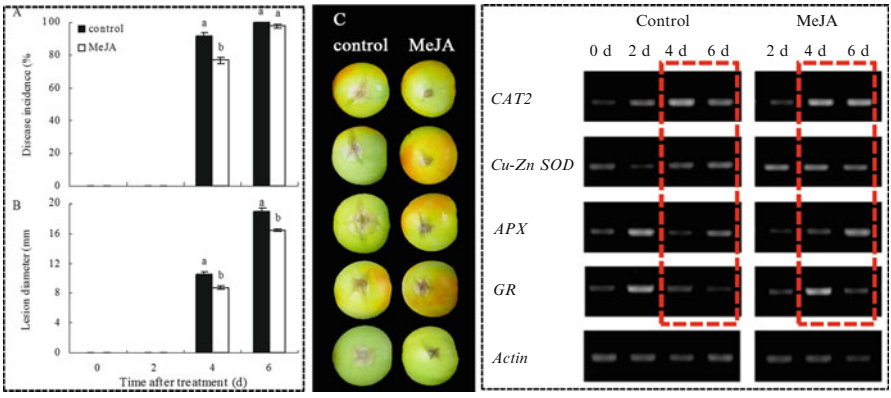


Fig. 6.2 Effect of MeJA on gray mould incidence and lesion diameter in tomato fruit and semi-quantitative RT-PCR analysis for the gene expression of *CAT2*, *Cu-Zn SOD*, *APX*, and *GR* in *B. cinerea* inoculated tomato fruit treated with or without MeJA

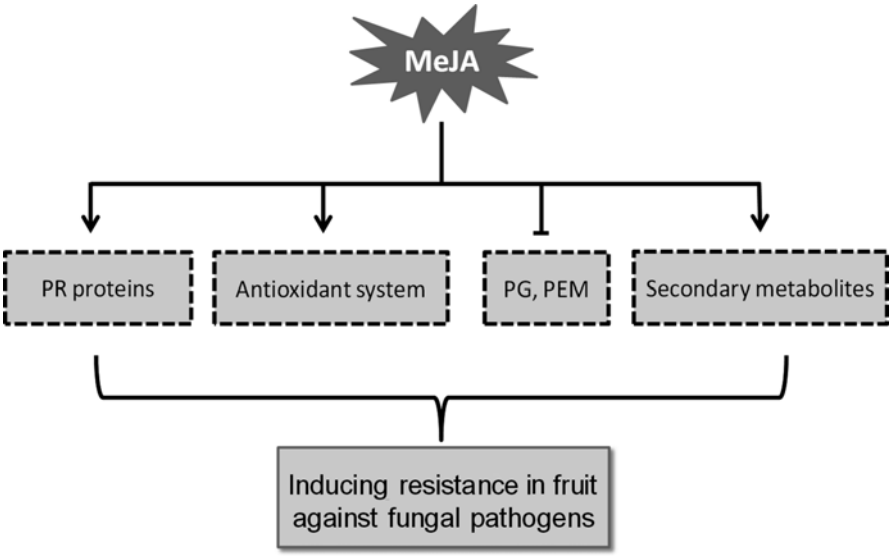


Fig. 6.3 The mechanisms involved in the MeJA-induced disease resistance. ↓: induce, ⊥: suppress

peach fruit by increasing activities of defense enzymes, including chitinase, β-1,3-glucanase (GLU), phenylalanine ammonia-lyase (PAL), and polyphenol oxidase (PPO). Wang et al. (2009) pointed out that MeJA at 10 μmol L^{-1} was effective in reducing fruit decay of Chinese bayberries by the induction of PAL activity. Ding et al. (2002) considered that low concentrations of (0.01 mM) methyl jasmonate decreased the incidence of decay during low temperature storage of tomato fruit, increased the accumulation of PR-2b transcripts encoding intracellular GLU, and enhanced the mRNA levels of PR-2a and PR-3b encoding extracellular GLU and intracellular chitinase (CHI). However, the defense responses induced by MeJA against postharvest pathogens varied from different organisms. Wang et al. (2015) proposed that MeJA triggered a priming mechanism in sweet cherry fruit. They found that the fruit pretreated with MeJA and then challenged with *P. expansum* has shown to have higher capacity of defense responses. And these augmented responses included enhanced activities of CHI and GLU and increased gene expression levels of calmodulin (*CaM*), *GLU*, *PAL*, nonexpressor of pathogenesis-related genes 1 (*NPR1-like*), and thaumatin-like (*THAU*). In addition, MeJA can inhibit the increase of activities of polygalacturonase (PG) and pectin methylesterase (PME), which degrade the cell wall components (Wang et al. 2015).

Most plants produce a broad range of secondary metabolites that are toxic to pathogens, either as part of their normal program of growth and development or in response to biotic stress, enable plants to build chemical barriers. Phenolics, secondary metabolites that encompass multiple classes structurally diverse of natural products which

arise from the shikimate- phenylpropanoids- flavonoids pathways, are essential in plant diseases resistance. Jin et al. (2009) found that the level of total phenolics in MeJA-treated peach fruit was higher than that in control fruit. Higher levels of total phenolics, flavonoids, and anthocyanins as well as individual phenolic compounds were observed in MeJA-treated Chinese bayberries with lower decay rate (Wang et al. 2009). Moreover, polyamines have been reported to be associated with plant resistance (Walters 2003). In loquat fruit, 10 μmol L^{-1} MeJA treatment significantly inhibited anthracnose rot and reduced decay incidence by manifesting higher contents of polyamines including putrescine, spermidine, and spermine (Cao et al. 2014).

MeJA Enhances Resistance of Fruits to Low Temperature Stress

Chilling injury (CI) is one of the important physiological disorders in postharvest storage periods, resulting in a decrease in the overall quality and marketability of many tropical and subtropical fruits. Peach fruits are sensitive to low temperature and CI often occur at 5 °C (Zhang and Tian 2010). We found that MeJA at 0.1 mM concentration significantly enhanced resistance of peach fruit to low temperature stress (Fig. 6.4). Much work has also shown that MeJA can increase fruit resistance to chilling stress and has been widely used to alleviate CI in a wide range of fruits, including mango (González-Aguilar, et al. 2000; Han et al. 2006), loquat (Cao et al. 2009a; Cai et al. 2011), tomato (Ding et al. 2001; Fung et al. 2006; Zhang et al. 2009), plum (Martínez-Esplá et al. 2014), and guava (González-Aguilar et al. 2004). However, the mechanism by which MeJA enhances tolerance of fruit to low temperature stress is summarized in Fig. 6.5.

Increasing evidence suggests that the cell membrane is the primary site for chilling stress; the membrane disruption and loss of membrane integrity are common features accompanying CI symptoms (Saltveit and Morris 1990; Marangoni et al. 1996; Li et al. 2012). The development of CI is associated with increases in electrolyte leakage and membrane lipid peroxidation products, such as malondialdehyde (MDA). Jin et al. (2013) reported that postharvest MeJA treatment can inhibit ion leakage and MDA content and protect membrane damage, thereby enhancing tolerance of peach fruit to CJ. Considered that the role of MeJA in alleviating CI of peach fruits was associated with its potential ability to protect cell membrane by inhibiting membrane lipid peroxidation and enhancing POD activity.

The chilling tolerance has been proved to be related to fatty acid composition in membrane lipids. Cao et al. (2009b) found that MeJA treatment alleviated CI in loquat fruit by reducing lipoxygenase (LOX) activity and maintaining high unsaturated/saturated fatty acid ratio. They suggested that the increase of membrane unsaturation degree may be involved in the induction of resistance towards chilling stresses by MeJA.

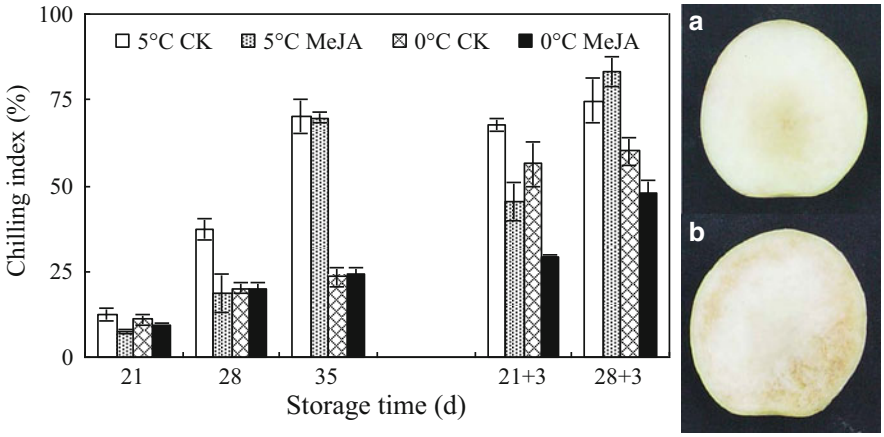


Fig. 6.4 Effect of MeJA on chilling injury in peach fruit kept at different low temperatures. (**a**) MeJA at 0.1 mM; (**b**) control; "*n*+3" means storage day+3 days of shelf life at 25 °C

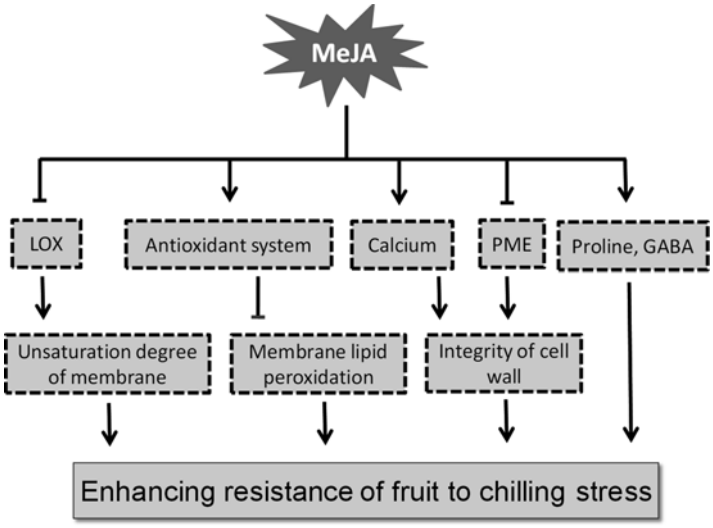


Fig. 6.5 The mechanisms involved in the MeJA-induced chilling resistance. ↓: induce, ⊥: suppress

Oxidative stress from overproduction and accumulation of ROS is a major reason to the development of CI (Apel and Hirt 2004; Shewfelt and Del Rosario 2000; Suzuki and Mittler 2006). The antioxidant systems, including enzymatic and nonenzymatic components, play a significant role in scavenging ROS and alleviating CI in various fruits (Møller 2001; Jimenez et al. 2002). Emerging evidence suggests that CI alleviating in the fruit treated with MeJA may be attributed to enhancing the activity

of antioxidant enzymes, such as SOD, CAT, APX, dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), and glutathione reductase (GR) (Zheng et al. 2008). Cao et al. (2009b) observed that MeJA markedly delayed the increases in $O_2^{-}\cdot$ production rate and H_2O_2 content and induced the activities of SOD, CAT, and APX in loquat fruit under chilling stress. In nonenzymatic scavenging system, antioxidant compounds including ascorbate (AsA) and glutathione (GSH) also play an important role in maintaining cellular redox status and antioxidant protection (Noctor and Foyer 1998). MeJA treatment can induce DHAR, MDHAR, and GR activities, resulting in high levels of AsA and GSH, which is contributed to the tolerance against the oxidative stress associated with CI (Cai et al. 2011).

Several papers have shown that enhancing arginine pathway and accumulation of proline and γ-aminobutyric acid (GABA) are involved in stress resistance mechanisms to chilling stress (Zhang et al. 2010a, 2011; Shang et al. 2011). MeJA treatment increased proline and GABA contents by means of increasing δ^1-pyrroline-5-carboxylate synthetase (P5CS) and ornithine δ-aminotransferase (OAT) activity and decreasing proline dehydrogenase (PDH) activity, thus enhancing tolerance of loquat fruit to chilling stress (Cao et al. 2012).

The mechanisms underlying MeJA action in CI might be activating the production of heat shock proteins, which contribute to abiotic stress tolerance by maintaining cell membranes integrity (Ding et al. 2001). Meanwhile, MeJA treatment also affected the degradation of cell wall, perhaps by regulation of cell wall modifying enzymes and inhibiting lignin accumulation (Meng et al. 2009; Cao et al. 2010).

Effects of MeJA on the Quality of Harvested Fruits

MeJA, as a plant growth regulator, also affects fruit quality, which includes texture, color, flavor, aroma, and antioxidant properties. The external application of MeJA showed multiple effects on physiological processes, affecting the postharvest quality of fruit. Wang and Buta (2003) found that a postharvest application of MeJA maintained higher levels of sugars and organic acids in fresh-cut kiwifruit. Beneficial effect of MeJA on the preservation of postharvest quality has been also reported in various fruits and vegetables, including papaya fruit (González-Aguilar et al. 2003), loquat fruit (Cao et al. 2009a), and radishes (Wang 1998).

Fruit color results from a class of pigments including chlorophyll, carotenoid, flavonoid, and anthocyanins. The chemical composition and concentration of pigments in fruit, which greatly determine the appearance quality of fruit, are affected by genetic variations, geographic location, environmental factors, and cultural strategies. Several studies have shown that preharvest sprays of MeJA could affect the development of apple fruit, leading to improved fruit color through accumulation of flavonoids and anthocyanins in fruit skin without adversely affecting quality at harvest (Shafiq et al. 2013). Meanwhile, postharvest immersion of MeJA emulsion enhanced the anthocyanin synthesis of "Fuji" apple, and the chlorogenic acid, most cyaniding, quercetin, and phloretin glycosides increased with MeJA treatment

concentration (Rudell et al. 2002). Postharvest treatment of mango fruit with MeJA stimulates yellow and red color development during storage at 20 °C (González-Aguilar et al. 2001). Exogenous MeJA applications improved skin color by accelerating chlorophyll degradation and promoting β-carotene synthesis in "Golden Delicious" apple (Perez et al. 1993) and in tomato fruit (Saniewski and Czapski 1983). Among the family of jasmonates, MeJA promoted color change more effectively than JA with increasing concentration range from 0.1 mmol L^{-1} to 10 mmol L^{-1} (Fan et al. 1998). Moreover, response to MeJA vapor treatment depends on fruit developmental stage, with the maximum effect occurring as fruit began to produce ethylene (Fan et al. 1998).

Aroma volatile compounds are one of the important factors to determine fruit quality. MeJA can inhibit production of many volatile alcohols and esters in "Golden Delicious" and "Fuji" apple fruits (Olias et al. 1992; Fan and Mattheis 1999). Exogenous application of MeJA promoted ripening-related aroma compounds in an early-maturing summer apple variety, Summerred (Fan et al. 1997). The impact of MeJA application on aroma volatile production might be cultivar dependent. According to the results descried by Kondo et al. (2005), the combination of ethephon with MeJA reduced volatile production by "Delicious" compared with ethephon only, but this treatment combination stimulated volatile production by "Golden Delicious" with the exception of esters. Current knowledge indicates that aroma production increases with ripening and is associated with ethylene production (Song and Bangerth 1996; Lalel et al. 2003). Hence, we propose that the effect of MeJA on volatile production might be related to internal ethylene concentration. Fan et al. (1997) considered that responses to MeJA treatment depended on the stage of fruit development, and MeJA stimulated ethylene, ester, alcohol, and acetic acid production in pre-climacteric fruits, while little or no response was elicited from post-climacteric fruits. Consequently, the effect of MeJA on aroma volatiles in fruit may be related to ethylene and depend on the growth stage of the fruit when treated with MeJA.

Fruits are rich in natural antioxidants and possess remarkably high antioxidant activity. The biological effects of these antioxidant components may provide protection against numerous chronic diseases, including cancer, cardiovascular, ocular, and neurological diseases. And this has stimulated the interest to find an effective method for maintaining or even improving antioxidant activity during postharvest storage. Several papers have shown that MeJA has a potential application for improving functional properties of harvested fruit via enhancing antioxidant activity (Chanjirakul et al. 2006, 2007). Cao et al. (2009b) found that MeJA-treated loquat fruit exhibited higher levels of total phenolics and total flavonoids and maintained higher antioxidant activity as compared to control fruit. Meanwhile, there was a significant positive linear relationship between total phenolic content and antioxidant activity (2009b). MeJA applications significantly increased the contents of total phenolics, chlorogenic acid, and antioxidant values in plum fruit during cold storage (Karaman et al. 2013). Wang and Zheng (2005) investigated the effect of preharvest MeJA application on flavonoid content and antioxidant capacity in raspberry and suggested that MeJA significantly enhanced the content of flavonoids and the antioxidant capacities in the fruit.

MeJA Improves the Biocontrol Efficiency of Antagonistic Yeasts

Antagonistic yeasts have been shown to effectively control a lot of postharvest diseases of various fruits (Fan and Tian 2001; Janisiewicz and Korsten 2002). The rapid propagation of antagonistic yeasts on the fruit surface and wounds allows them to compete with the pathogen for space and nutrients (Chan and Tian 2005; Sharma et al. 2009). Moreover, environmental stresses, such as oxidative stress, heat stress, and antimicrobial compounds, during the application of biocontrol agents can decrease the viability of yeast cells (Li and Tian 2006). In order to improve the survival rate of antagonistic yeasts, combining biocontrol yeasts with some chemical compounds, such as calcium (Tian et al. 2002), salicylic acid (Qin et al. 2003), sodium bicarbonate, and chitosan (Meng and Tian 2009), has been proved to be an effective way in enhancing their biocontrol ability against pathogenic fungi. We found that MeJA at 200 μmol L^{-1} can effectively enhance the colonization of *Cryptococcus laurentii* in peach fruits kept at both 25 °C and 0 °C (Yao and Tian 2005b). Population of *C. laurentii* combined with MeJA increased by more than 2 times compared to *C. laurentii* without MeJA at 25 °C. At 0 °C, the growth rate of *C. laurentii* population combined with MeJA is about 40 % higher than that without MeJA (Fig. 6.6). Guo et al. (2014) reported that MeJA at 10, 100, and 200 μmol L^{-1} had no significant effect on the growth of *C. laurentii* in vitro, while a high concentration (400 μmol L^{-1}) inhibited its growth. Moreover, they found that in the fruit peel wounds the population of *C. laurentii* with and without MeJA increased approximately 22.3-fold and 13.3-fold at 24 h, respectively, compared with that at 0 h at 20 °C (Guo et al. 2014). Application of MeJA can also effectively increase the population and biocontrol efficiency of *Rhodotorula glutinis* (Zhang et al. 2009), *Pichia membranaefaciens* (Wang et al. 2011), and *Metschnikowia pulcherrima* (Ebrahimi et al. 2013). These results suggested that MeJA enhanced the biocontrol efficacy of antagonistic yeasts via facilitating the growth of yeasts in fruits and then enhancing space and nutrient competition against pathogens. The mechanisms by which MeJA enhances the biocontrol efficacy of yeast are complex and may be attributed to the influence of MeJA on antagonist, pathogen, and fruits.

MeJA Inhibits the Growth of Some Fungal Pathogens

In addition to the ability of inducing the host resistant, the fungicide characteristic of MeJA has also attracted people's attention. Several studies indicate that MeJA has direct antimicrobial activity against some fungal pathogens in vitro. For different pathogens, MeJA exhibited different antimicrobial ability. Zhu and Tian (2012) reported that MeJA at 10 mM significantly inhibited spore germination and germ tube elongation of *B. cinerea* by 75.5 % and 86.6 %, respectively. Besides, they found that MeJA could destroy the plasma membrane integrity of *B. cinerea* spores,

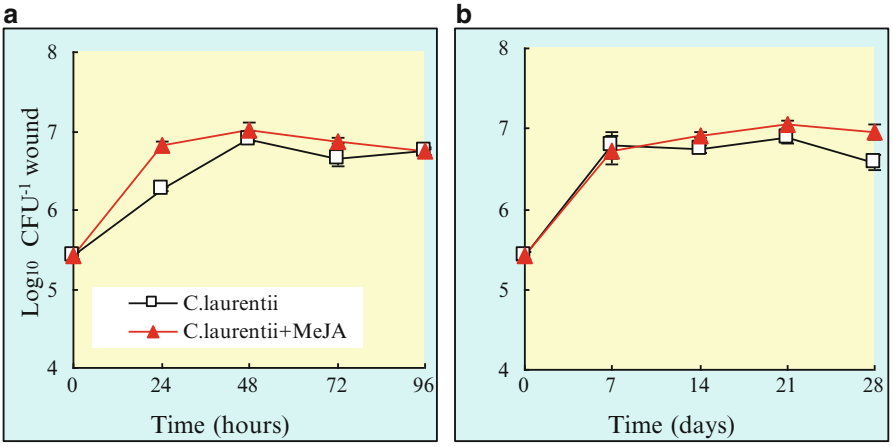


Fig. 6.6 Effect of MeJA on growth of *C. laurentii* in the wound of peach fruit kept at 25 °C (**a**) and 0 °C (**b**)

suggesting MeJA has direct fungitoxic property against the *B. cinerea*. In addition, MeJA at a concentration of 200 μmol L^{-1} effectively inhibited mycelial growth of *Penicillium expansum* and the inhibitory rate can reach to 84.3 % and 89.6 % at 3 days after treatment at 25 °C and 20 days after treatment at 0 °C, respectively. But the inhibitory rate of MeJA on mycelial growth of *M. fructicola* was 6.5 % at 3 days after treatment at 25 °C (Fig. 6.7), and spore germination and germ tube length of *M. fructicola* were also slightly inhibited by MeJA (Table 6.1). In vitro, 10 μmol L^{-1} MeJA could significantly inhibit spore germination, germ tube elongation, and mycelial growth of *C. acutatum*, which causes anthracnose rot in loquat fruit (Cao et al. 2008). Nevertheless, some results demonstrated that MeJA had no direct impact on the growth of *Penicillium digitatum*, *Colletotrichum coccodes*, and *Magnaporthe grisea* (Droby et al. 1999; Tzortzakis 2007; Zhang et al. 2010b), suggesting that the efficacy of MeJA at a certain range of concentrations greatly depends on different kinds of pathogens.

In conclusion, MeJA plays a versatile role in the field of postharvest preservation. First, using MeJA alone or combined with biocontrol agents can effectively reduce the loss caused by pathogen infection by (1) inducing the resistant of fruits against fungal pathogens, (2) inhibiting the growth of fungous pathogens directly, and (3) promoting the propagation of biocontrol agents. Second, application of MeJA significantly induces resistance of fruit to chilling stress via inducing antioxidant activity and maintaining the membrane integrity and cell wall integrity of fruits. Third, pre- or postharvest application of MeJA can well keep fruit quality, including color, flavor, aroma, and antioxidant properties. Considerable progress has been made in understanding the function of MeJA, as a promising technique to control postharvest diseases and to maintain fruit quality.

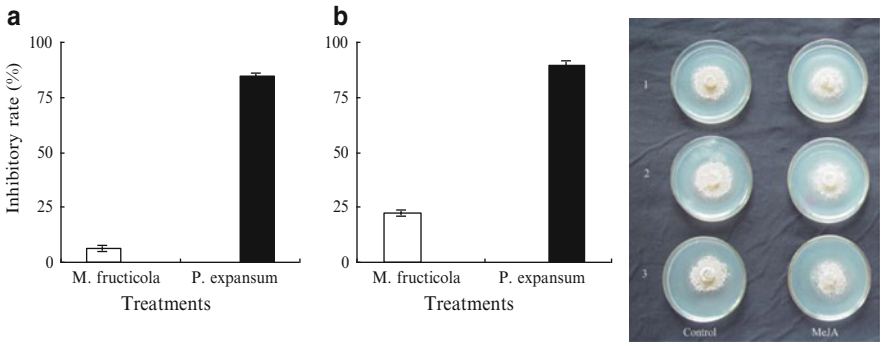


Fig. 6.7 Effect of MeJA with 200 μmol L^{-1} on mycelial growth of *M. fructicola* (*white bar*) and *P. expansum* (*black bar*) on PDA after 3 days at 25 °C (**a**) and 20 days at 0 °C (**b**), respectively

Table 6.1 Effect of MeJA on spore germination and germ tube length of *M. fructicola* (25 °C, 12 h)

Treatments	Spore germination (%)	Germ tube length (μm)
Control	91.39 ± 7.47 a	99.13 ± 18.32 a
MeJA	88.8 ± 2.46 a	77.60 ± 17.02 b

References

Aghdam, M. S., & Bodbodak, S. (2013). Physiological and biochemical mechanisms regulating chilling tolerance in fruits and vegetables under postharvest salicylates and jasmonates treatments. *Scientia Horticulturae, 156*, 73–85.

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

Buta, I. T., & Moline, H. E. (1998). Methyl jasmonate extends shelf life and reduces microbial contamination of fresh-cut celery and peppers. *Journal of Agricultural and Food Chemistry, 46*, 1253–1256.

Cai, Y., Cao, S., Yang, Z., & Zheng, Y. (2011). MeJA regulates enzymes involved in ascorbic acid and glutathione metabolism and improves chilling tolerance in loquat fruit. *Postharvest Biology and Technology, 59*, 324–326.

Campo, S., Carrascal, M., Coca, M., Abian, J., & Segundo, B. S. (2004). The defense response of germinating maize embryos against fungal infection: A proteomics approach. *Proteomics, 4*, 383–396.

Cao, S., Cai, Y., Yang, Z., Joyce, D. C., & Zheng, Y. (2014). Effect of MeJA treatment on polyamine, energy status and anthracnose rot of loquat fruit. *Food Chemistry, 145*, 86–89.

Cao, S., Yang, Z., Cai, Y., & Zheng, Y. (2012). MeJA induces chilling tolerance in loquat fruit by regulating proline and γ-aminobutyric acid contents. *Food Chemistry, 133*, 1466–1470.

Cao, S., Zheng, Y., Wang, K., Rui, H., & Tang, S. (2010). Effect of methyl jasmonate on cell wall modification of loquat fruit in relation to chilling injury after harvest. *Food Chemistry, 118*, 641–647.

Cao, S., Zheng, Y., Yang, Z., Tang, S., Jin, P., Wang, K., et al. (2008). Effect of methyl jasmonate on the inhibition of *Colletotrichum acutatum* infection in loquat fruit and the possible mechanisms. *Postharvest Biology and Technology, 49*, 301–307.

Cao, S., Zheng, Y., Yang, Z., Rui, H., & Wang, K. (2009a). Effect of methyl jasmonate on quality and antioxidant activity of postharvest loquat fruit. *Journal of the Science of Food and Agriculture, 89*, 2064–2070.

Cao, S., Zheng, Y., Wang, K., Jin, P., & Rui, H. (2009b). Methyl jasmonate reduces chilling injury and enhances antioxidant enzyme activity in postharvest loquat fruit. *Food Chemistry, 115*, 1458–1463.

Chan, Z. L., & Tian, S. P. (2005). Interaction of antagonistic yeasts against postharvest pathogens of apple fruit and possible mode of action. *Postharvest Biology and Technology, 36*, 215–223.

Chanjirakul, K., Wang, S. Y., Wang, C. Y., & Siriphanich, J. (2006). Effect of natural volatile compounds on antioxidant capacity and antioxidant enzymes in raspberries. *Postharvest Biology and Technology, 40*, 106–115.

Chanjirakul, K., Wang, S. Y., Wang, C. Y., & Siriphanich, J. (2007). Natural volatile treatments increase free-radical scavenging capacity of straw berries and black berries. *Journal of the Science of Food and Agriculture, 87*, 1463–1472.

Creelman, R., & Mullet, J. E. (1997). Biosynthesis and action of jasmonates in plants. *Annual Review of Plant Physiology and Plant Molecular Biology, 48*, 355–381.

Creelman, R. A., Tierney, M. L., & Mullet, J. E. (1992). Jasmonic acid/methyl jasmonate accumulate in wounded soybean hypocotyls and modulate wound gene expression. *Proceedings of the National Academy of Sciences of the USA, 89*, 4938–4941.

Ding, C. K., Wang, C. Y., Gross, K. C., & Smith, D. L. (2001). Reduction of chilling injury and transcript accumulation of heat shock proteins in tomato fruit by methyl jasmonate and methyl salicylate. *Plant Science, 161*, 1153–1159.

Ding, C. K., Wang, C. Y., Gross, K. C., & Smith, D. L. (2002). Jasmonate and salicylate induce the expression of pathogenesis-related-protein genes and increase resistance to chilling injury in tomato fruit. *Planta, 214*, 895–901.

Droby, S., Porat, R., Cohen, L., Weiss, B., Shapio, B., Philosoph-Hadas, S., et al. (1999). Suppressing green mold decay in grapefruit with postharvest jasmonate application. *Journal of the American Society for Horticultural Science, 124*, 184–188.

Ebrahimi, L., Etebarian, H. R., Aminian, H., & Sahebani, N. (2013). Effect of *Metschnikowia pulcherrima* and methyl jasmonate on apple blue mold disease and the possible mechanisms involved. *Phytoparasitica, 41*, 515–519.

Fan, X., & Mattheis, J. P. (1999). Impact of 1-methylcyclopropene and methyl jasmonate on apple volatile production. *Journal of Agricultural and Food Chemistry, 47*, 2847–2853.

Fan, X., Mattheis, J. P., Fellman, J. K., & Patterson, M. E. (1997). Effect of methyl jasmonate on ethylene and volatile production by Summerred apples depends on fruit developmental stage. *Journal of Agricultural and Food Chemistry, 45*, 208–211.

Fan, X., Mattheis, J. P., & Fellman, J. K. (1998). Responses of apple to postharvest jasmonate treatments. *Journal of the American Society for Horticultural Science, 123*, 421–425.

Fan, Q., & Tian, S. P. (2001). Postharvest biological control of grey mold and blue mold on apple by *Cryptococcus albidus* (Saito) Skinner. *Postharvest Biology and Technology, 21*, 341–350.

Fung, R. W. M., Wang, C. Y., Smith, D. L., Gross, K. C., Tao, Y., & Tian, M. (2006). Characterization of alternative oxidase (AOX) gene expression in response to methyl salicylate and methyl jasmonate pre-treatment and low temperature in tomatoes. *Journal of Plant Physiology, 163*, 1049–1060.

González-Aguilar, G. A., Buta, J. G., & Wang, C. Y. (2001). Methyl jasmonate reduces chilling injury symptoms and enhances colour development of 'Kent' mangoes. *Journal of the Science of Food and Agriculture, 81*, 1244–1249.

González-Aguilar, G. A., Buta, J. G., & Wang, C. Y. (2003). Methyl jasmonate and modified atmosphere packaging (MAP) reduce decay and maintain postharvest quality of papaya 'Sunrise'. *Postharvest Biology and Technology, 28*, 361–370.

González-Aguilar, G. A., Fortiz, J., & Wang, C. Y. (2000). Methyl jasmonate reduces chilling injury and maintains postharvest quality of mango fruit. *Journal of Agriculture and Food Chemistry, 48*, 515–519.

González-Aguilar, G. A., Tiznado-Hernandez, M. E., Zavaleta-Gatica, R., & Martınez-Téllez, M. A. (2004). Methyl jasmonate treatments reduce chilling injury and activate the defense response of guava fruits. *Biochemical and Biophysical Research Communications, 313*, 694–701.

Guo, J., Fang, W., Lu, H., Zhu, R., Lu, L., Zheng, X., et al. (2014). Inhibition of green mold disease in mandarins by preventive applications of methyl jasmonate and antagonistic yeast *Cryptococcus laurentii. Postharvest Biology and Technology, 88*, 72–78.

Han, J., Tian, S. P., Meng, X. H., & Ding, Z. S. (2006). Response of physiological metabolism and cell structures of mango fruit to exogenous methyl salicylate under low temperature stress. *Physiologia Plantarum, 128*, 125–133.

Janisiewicz, W. J., & Korsten, L. (2002). Biological control of postharvest diseases of fruit. *Annual Review of Phytopathology, 40*, 411–441.

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

Jin, P., Tang, S., Zheng, Y., Rui, H., & Wang, C. Y. (2009). Enhancing disease resistance in peach fruit with methyl jasmonate. *Journal of Science and Food Agriculture, 89*, 802–808.

Jin, P., Zhu, H., Wang, J., Chen, J., Wang, X., & Zheng, Y. (2013). Effect of methyl jasmonate on energy metabolism in peach fruit during chilling stress. *Journal of the Science of Food and Agriculture, 93*, 1827–1832.

Karaman, S., Ozturk, B., Genc, N., & Celik, S. M. (2013). Effect of preharvest application of methyl jasmonate on fruit quality of plum (*Prunus salicina* Lindell cv. “Fortune”) at harvest and during cold storage. *Journal of Food Processing and Preservation, 37*, 1049–1059.

Kondo, S., Setha, S., Rudell, D. R., Buchanan, D. A., & Mattheis, J. P. (2005). Aroma volatile biosynthesis in apples affected by 1-MCP and methyl jasmonate. *Postharvest Biology and Technology, 36*, 61–68.

Lalel, H. J. D., Singh, Z., & Tan, S. C. (2003). The role of ethylene in mango fruit aroma volatiles biosynthesis. *Journal of Horticultural Science and Biotechnology, 78*, 485–496.

Li, B. Q., & Tian, S. P. (2006). Effects of trehalose on stress tolerance and biocontrol efficacy of *Cryptococcus laurentii. Journal of Applied Microbiology, 100*, 854–861.

Li, B. Q., Zhang, C. F., Cao, B. H., Wang, W. H., Qin, G. Z., & Tian, S. P. (2012). Brassinosteroids enhance cold stress tolerance in fruit by regulating plasma membrane proteins and lipids. *Amino Acids, 43*, 2469–2480.

Marangoni, A. G., Palma, T., & Stanley, D. W. (1996). Membrane effects in postharvest physiology. *Postharvest Biology and Technology, 7*, 193–217.

Martínez-Esplá, A., Zapata, P. J., Castillo, S., Guillén, F., Martínez-Romero, D., Valero, D., et al. (2014). Preharvest application of methyl jasmonate (MeJA) in two plum cultivars. 1. Improvement of fruit growth and quality attributes at harvest. *Postharvest Biology and Technology, 98*, 98–105.

Meng, X. H., Han, J., Wang, Q., & Tian, S. P. (2009). Changes in physiology and quality of peach fruit treated by methyl jasmonate under low temperature stress. *Food Chemistry, 106*, 501–508.

Meng, X. H., Qin, G. Z., & Tian, S. P. (2010). Influences of preharvest spraying *Cryptococcus laurentii* combined with postharvest chitosan coating on postharvest diseases and quality of table grapes in storage. *LWT - Food Science and Technology, 43*, 596–601.

Meng, X. H., & Tian, S. P. (2009). Effects of preharvest application of antagonistic yeast combined with chitosan on decay and quality of harvested table grape fruit. *Journal of the Science of Food and Agriculture, 89*, 1838–1842.

Møller, I. M. (2001). Plant mitochondria and oxidative stress: Electron transport, NADPH turnover, and metabolism of reactive oxygen species. *Annual Review of Plant Biology, 52*, 561–591.

Noctor, G., & Foyer, C. H. (1998). Ascorbate and glutathione: Keeping active oxygen under control. *Annual Review of Plant Biology, 49*, 249–279.

Olias, J. M., Sanz, L. C., Rios, J. J., & Perez, A. G. (1992). Inhibitory effect of methyl jasmonate on the volatile ester-forming enzyme system in Golden Delicious apples. *Journal of Agricultural and Food Chemistry, 40*, 266–270.

Perez, A. G., Sanz, C., Richardson, D. G., & Olias, J. M. (1993). Methyl jasmonate vapor promotes β-carotene synthesis and chlorophyll degradation in Golden Delicious apple peel. *Journal of Plant Growth Regulation, 12*, 163–167.

Qin, Q. Z., Tian, S. P., Xu, Y., & Wan, Y. K. (2003). Enhancement of biocontrol efficacy of antagonistic yeasts by salicylic acid in sweet cherry fruit. *Physiological and Molecular Plant Pathology, 62*, 147–154.

Rudell, D. R., Mattheis, J. P., Fan, X., & Fellman, J. K. (2002). Methyl jasmonate enhances anthocyanin accumulation and modifies production of phenolics and pigments in 'Fuji' apples. *Journal of the American Society for Horticultural Science, 127*, 435–441.

Saltveit, M. E., & Morris, L. L. (1990). Overview on chilling injury of horticultural crops. In C. Y. Wang (Ed.), *Chilling injury of horticultural crops* (pp. 3–15). Boca Raton: CRC Press.

Saniewski, M., & Czapski, J. (1983). The effect of methyl jasmonate on lycopene and β-carotene accumulation in ripening red tomatoes. *Experientia, 39*, 1373–1374.

Shafiq, M., Singh, Z., & Khan, A. S. (2013). Time of methyl jasmonate application influences the development of 'Cripps Pink' apple fruit colour. *Journal of the Science of Food and Agriculture, 93*, 611–618.

Shang, H., Cao, S., Yang, Z., Cai, Y., & Zheng, Y. (2011). Effect of exogenous gamma-aminobutyric acid treatment on proline accumulation and chilling injury in peach fruit after long-term cold storage. *Journal of Agricultural and Food Chemistry, 59*, 1264–1268.

Sharma, R. R., Singh, D., & Singh, R. (2009). Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. *Biological Control, 50*, 205–221.

Shewfelt, R. L., & Del Rosario, B. A. (2000). The role of lipid peroxidation in storage disorders of fresh fruit and vegetables. *HortScience, 35*, 575–579.

Song, J., & Bangerth, F. (1996). The effect of harvest date on aroma compound production from 'Golden Delicious' apple fruit and relationship to respiration and ethylene production. *Postharvest Biology and Technology, 8*, 259–269.

Suzuki, N., & Mittler, R. (2006). Reactive oxygen species and temperature stresses: A delicate balance between signaling and destruction. *Physiologia Plantarum, 126*, 45–51.

Tian, S. P., & Chan, Z. L. (2004). Potential of induced resistance in postharvest diseases control of fruits and vegetables. *Acta Phytopathologica Sinica, 34*, 385–394.

Tian, S. P., Fan, Q., Xu, Y., & Jiang, A. L. (2002). Effects of calcium on biocontrol activity of yeast antagonists against postharvest fungal pathogen. *Plant Pathology, 51*, 352–358.

Tian, S. P., Qin, G. Z., & Li, B. Q. (2013). Reactive oxygen species involved in regulating fruit senescence and fungal pathogenicity. *Plant Molecular Biology, 82*, 593–602.

Tzortzakis, N. G. (2007). Methyl jasmonate-induced suppression of anthracnose rot in tomato fruit. *Crop Protection, 26*, 1507–1513.

Walters, D. R. (2003). Resistance to plant pathogens: Possible roles for free polyamines and polyamine catabolism. *New Phytologist, 159*, 109–115.

Walters, D., Walsh, D., Newton, A., & Lyon, G. (2005). Induced resistance for plant disease control: Maximizing the efficacy of resistance elicitors. *Phytopathology, 95*, 1368–1373.

Wang, C. Y. (1998). Methyl jasmonate inhibits postharvest sprouting and improves storage quality of radishes. *Postharvest Biological and Technology, 14*, 179–183.

Wang, C. Y., & Buta, J. G. (2003). Maintaining quality of fresh-cut kiwifruit with volatile compounds. *Postharvest Biological and Technology, 28*, 181–186.

Wang, K., Jin, P., Cao, S., Shang, H., Yang, Z., & Zheng, Y. (2009). Methyl jasmonate reduces decay and enhances antioxidant capacity in Chinese bayberries. *Journal of Agriculture and Food Chemistry, 57*, 5809–5815.

Wang, L., Jin, P., Wang, J., Jiang, L. L., Shan, T. M., & Zheng, Y. H. (2015). Methyl jasmonate primed defense responses against *Penicillium expansum* in sweet cherry fruit. *Plant Molecular Biology Reporter*. doi:10.1007/s11105-014-0844-8.

Wang, S. Y., & Zheng, W. (2005). Preharvest application of methyl jasmonate increases fruit quality and antioxidant capacity in raspberries. *International Journal of Food Science and Technology, 40*, 187–195.

Wang, K. T., Zheng, Y. H., Shang, H. T., Jin, P., & Rui, H. J. (2011). Study on improved biocontrol of *Pichia membranifaciens* for control of green mold decay on Chinese bayberry fruit by methyl jasmonate. *Food and Fermentation Industries, 37*, 11–16.

Yao, H. J., & Tian, S. P. (2005a). Effects of pre- and postharvest application of SA or MeJA on inducing disease resistance of sweet cherry fruit in storage. *Postharvest Biology and Technology, 35*, 253–262.

Yao, H. J., & Tian, S. P. (2005b). Effects of a biocontrol agent and methyl jasmonate on postharvest diseases of peach fruit and the possible mechanisms involved. *Journal of Applied Microbiology, 98*, 941–950.

Zhang, H. Y., Ma, L. C., Turner, M., Xu, H. X., Dong, Y., & Jiang, S. (2009). Methyl jasmonate enhances biocontrol efficacy of *Rhodotorula glutinis* to postharvest blue mold decay of pears. *Food Chemistry, 117*, 621–626.

Zhang, X., Shen, L., Li, F., Meng, D., & Sheng, J. (2011). Methyl salicylate-induced arginine catabolism is associated with up-regulation of polyamine and nitric oxide levels and improves chilling tolerance in cherry tomato fruit. *Journal of Agricultural and Food Chemistry, 59*, 9351–9357.

Zhang, C. F., & Tian, S. P. (2010). Peach fruit acquired the tolerance to low temperature stress by accumulation of linolenic acid and N-acylphosphatidylethanolamine in plasma membrane. *Food Chemistry, 120*, 864–872.

Zhang, X. H., Shen, L., Li, F. J., Zhang, Y. X., Meng, D. M., & Sheng, J. P. (2010a). Up-regulating arginase contributes to amelioration of chilling stress and the antioxidant system in cherry tomato fruits. *Journal of Science of Food and Agriculture, 90*, 2195–2202.

Zhang, Z. H., Nie, Y. F., He, L., Li, Y. F., & Wang, Z. Z. (2010b). Resistance-related defense enzymes and endogenous salicylic acid induced by exogenous methyl jasmonate in rice against blast disease. *Acta Phytopathologica Sinica, 40*, 395–403.

Zheng, Y. H., Raymond, W. M. F., Wang, S. Y., & Wang, C. Y. (2008). Transcript levels of antioxidative genes and oxygen radical scavenging enzyme activities in chilled zucchini squash in response to super atmospheric oxygen. *Postharvest Biology and Technology, 47*, 151–158.

Zhu, Z., & Tian, S. P. (2012). Resistant responses of tomato fruit treated by exogenous methyl jasmonate to *Botrytis cinerea* infection. *Scientia Horticulturea, 142*, 38–43.

Zhu, Z., Zhang, Z. Q., Qin, G. Z., & Tian, S. P. (2010). Effects of brassinosteroids on ripening and disease resistance of jujube fruit in storage. *Postharvest Biology and Technology, 56*, 50–55.

Chapter 7
Essential Oils

M.R. Moreira, M.V. Alvarez, and A.G. Ponce

Introduction

The recent exploitation of natural products to control biological spoilage and extend the storage life of perishables has received more and more attention (Tripathi and Dubey 2004). These natural compounds are generally recognized as safe (GRAS) for environment and human health, so interest in their use in the goal for sustainable agriculture has increased. Thus, many research studies were developed proving, in many cases, that plant essential oils and extracts have a role as food preservatives (Hammer et al. 1999; Ayala-Zavala et al. 2008c). Natural antimicrobial compounds are a re-emerging alternative to fresh and minimally processed fruit and vegetable preservation (González-Aguilar et al. 2010). The antimicrobial power of plants and herb extracts has been recognized for centuries, and mainly used as natural medicine. Plant volatiles have been widely used as food-flavoring agents, and many are generally GRAS.

Essential oils (EOs), also called volatile oils, are aromatic oily liquids obtained from plant materials (flowers, herbs, buds, leaves, fruits, twigs, bark, seeds, wood, and roots). EOs can be obtained by extraction, fermentation, or expression, but steam distillation is the most commonly used method. Essential oils are very complex natural mixtures which contain about 20–60 components at quite different concentrations. They are characterized by two or three major components at fairly high concentrations (20–70 %) compared to other components present in trace amounts (González-Aguilar et al. 2013). However, it has been shown that minor components

M.R. Moreira • M.V. Alvarez • A.G. Ponce (✉)
Grupo de Investigación en Ingeniería en Alimentos, Universidad Nacional de Mar del Plata, Buenos Aires, Argentina

Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Avenida Rivadavia, Argentina
e-mail: agponce@mdp.edu.ar

M.W. Siddiqui et al. (eds.), *Postharvest Management Approaches for Maintaining Quality of Fresh Produce*, DOI 10.1007/978-3-319-23582-0_7

play a critical role in the antimicrobial properties of the essential oil, possibly showing synergistic effects along with other compounds (Chang et al. 2008).

The components include different groups of distinct biosynthetical origin depending on the plant source (Bowles 2003; Pichersky et al. 2006). The main chemical group is composed of terpenes and terpenoids and the other of aromatic and aliphatic constituents, all characterized by low molecular weight. The inherent aroma and antimicrobial activity of EOs are related commonly to the chemical configuration of the components, to the proportions in which they are present, and to interactions between them, affecting their bioactive properties (Fisher and Phillips 2008). Considering the complex mixture of EO constituents it is difficult to attribute the antimicrobial mode of action to one specific mechanism, being reported several targets being reported in the microbial cell. It seems that they may cause deterioration of cell wall, damage to cytoplasmic membrane, damage to membrane proteins, leakage of cell contents, coagulation of cytoplasm, depletion of proton motive active sites, inactivation of essential enzymes, and disturbance of genetic material functionality (Burt 2004; Ayala-Zavala et al. 2008b; Gutierrez et al. 2008).

Derived from phenylpropane, the phenolic compounds occur less frequently than the terpenes. The biosynthetic pathways concerning terpenes and phenylpropanic derivatives generally are separated in plants but may coexist in some, with one major pathway taking over (see cinnamon oil with cinnamaldehyde as major and eugenol as minor constituents, also clove oil, fennel, etc.). The phenolic compounds found in essential oils normally have a carbon side chain; thymol, eugenol, and carvacrol are included in this group. These components have great antiseptic, antibacterial, and disinfectant qualities and also have greatly stimulating therapeutic properties. Evidence suggests that phenol induces progressive loss of intracellular constituents from treated bacteria and produces generalized membrane damage with intracellular coagulation occurring at higher concentrations.

The mechanisms thought to be responsible for the phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins. The site(s) and number of hydroxyl groups on the phenol group are thought to be related with the relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity (Troncoso-Rojas and Tiznado-Hernández 2007). Due to the nature of phenols, essential oils with high phenol content should be used in low concentrations and for short periods of time, since they can lead to toxicity if used over long periods of time. The principal plant sources for these compounds are anise, cinnamon, clove, fennel, nutmeg, parsley, sassafras, star anise, tarragon, and some botanical families (*Apiaceae*, *Lamiaceae*, *Myrtaceae*, *Rutaceae*) (Speranza and Corbo 2010).

Sulfur-rich compounds are another kind of natural volatiles that had been shown a strong antimicrobial activity. They are present in several plants like onion, garlic, and others. The active constituents of garlic and onion are sulfur-rich compounds that are rapidly absorbed and metabolized. Allicin is considered to be the most important biologically active compound in garlic. Chemical analysis of garlic

showed that 54.5 % of the total sulfides were the sum of diallyl monosulfide, diallyl disulfide, diallyl trisulfide, and diallyl tetrasulfide (Troncoso-Rojas and Tiznado-Hernández 2007).

Antimicrobial Activity of Essential Oils: In Vitro Versus In Vivo Studies

To evaluate the antimicrobial activity of botanical biopreservatives numerous studies have been done in vitro. However, establishing the technical feasibility of their use as natural sanitizing agents in postharvest processing it is necessary to evaluate their effects on actual crops since the organic system complexity may introduce factors that may in vitro result inapplicable. Moreira et al. (2005) reported that clove (*Syzygium aromaticum*) and tea tree (*Melaleuca alternifolia*) essential oils showed inhibitory effects on *Escherichia coli* growth using the Agar Diffusion Method for in vitro assays. Also, other in vitro studies have demonstrated antibacterial activity of essential oils against *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli* O157:H7, *Shigella dysenteria*, *Bacillus cereus,* and *Staphylococcus aureus* (Hammer et al. 1999; Elgayyar et al. 2001; Friedman et al. 2002; Burt 2004; Dadalioglu and Evrendilek 2004). However, the information of their effect in actual food systems is still insufficient.

The greater availability of nutrients in foods compared to laboratory media may enable bacteria to repair damaged cells faster (Gill et al. 2002; Moreira et al. 2007). Higher concentrations of EOs are needed to achieve the same effects in foods systems, and as a consequence sensory quality and acceptability of the products may be affected (Gutierrez et al. 2009). The studies on actual foods show that some botanicals have the potential to be effective biopreservatives, although product development to optimize foods functionality and flavor will be challenging; more studies are needed on the topic of botanical applications to optimize their use and provide the fundamentals needed by the food industry (Draughon 2004). The effectiveness of essential oils application in food systems is the result of multiple factor associations such as the food composition or the storage temperatures (Gill et al. 2002; Burt 2004; Ponce et al. 2004a, 2011). The presence of surfactants and organic substances that interact with active sites of the antimicrobial substances also plays an important role (Hammer et al. 1999). In addition, techniques used to apply the essential oils on foods constitute an important factor affecting their antibacterial and antioxidant activity (Pandit and Shelef 1994).

Two important variables appear when essential oils are applied either in vivo or in vitro; one is the contact time for oils to exert their effect and the other is the concentration required to achieve the same degree of inhibition and these two variables would be interacting. The lower water content of foods compared to laboratory media may hamper the progress of antibacterial agents to the target site in the bacteria cell (Selim 2011). Furthermore, in in vitro assays, microorganisms and essential oils come into close contact, while in vivo the food matrix has cell membranes that act as physical barriers interfering oil and microorganism contact.

Moreira et al. (2005), working with clove EO, reported that the maximum *Escherichia coli* O157:H7 reductions (3.5 log) required 15 min of contact time when the oil was applied at the minimum inhibitory concentration (MIC) (2.5 μL/mL) by the in vitro assays. However, longer contact times did not result in higher *E. coli* reductions. In another study, these authors tested the effectiveness of clove EO to control *E. coli* O157:H7 on inoculated blanched spinach (in vivo assays). It was observed that only clove EO applied at 3 MICs (7.5 μL/mL) on blanched spinach produced significant inhibition of *E. coli* within the first 5 min of application (Moreira et al. 2007). *E. coli* reductions observed by in vivo assays were significantly lower than in vitro. It has generally been found that a greater concentration of essential oils is needed to achieve the same effect in actual foods compared to in vitro assays (Moreira et al. 2007; Ponce et al. 2011).

The oil concentrations required to produce a certain level of inhibition in food systems could be questionable due to the organoleptic impact. In this way, Moreira et al. (2007) reported that blanched spinach treated with the highest concentrations (3MIC) of clove and tea tree oils presented a strong off-flavor. Thus, the oils concentrations required to reach an effective *E. coli* O157:H7 control produced in food systems undesirable organoleptic characteristics. Therefore, synergistic effects would have to be exploited to maximize the antibacterial activity of essential oils and to minimize the concentrations required to achieve a particular antibacterial effect without adversely affecting the sensorial acceptability.

Essential Oils Applied as Biopreservatives in Fresh Produce

When designing the application of an antimicrobial to a food, some critical aspects that may affect the effectiveness of the additive should be taken into account. These factors relate to the type of antimicrobial (physicochemical properties), food type (composition), processing operations to which it was subjected, conditions in which it will be stored, and target microorganism or microbial population. The effective application of natural antimicrobials to fruits is not severely affected by interactions due to its low protein and lipids content although the possible effects of interactions with carbohydrates should be taken into account. Moreover, vegetables also have a low content of proteins and lipids, but they have higher pH and water activity than fruits, and so higher concentrations of antimicrobials may be required for their preservation (Davidson et al. 2013). Also, in many fruits, lowered pH may act together with the antimicrobial in inhibiting the growth of microorganisms (Davidson et al. 2013). It is also important to note that the effectiveness of the antimicrobials, specifically flavor compounds and essential oils, may be reduced not only by interactions with the food matrix but also because of their high volatility (Ponce et al. 2004a; Ayala-Zavala et al. 2008a). The forms of application of essential oils commonly used for fruit and vegetable preservation include solutions or emulsions, applied by dipping or spraying the product.

Investigations were carried out by Ponce et al. (2011) to assess the efficiency of three essential oils, clove, tea tree, and rosemary, as natural preservatives during the postharvest of lettuce leaves. The effect of different concentrations (1 and 0.5 MIC) of plant essential oils applied in three forms (spray, immersion, and capsules) was studied on lettuce leaves. The MIC values used in this study were 2.5, 5.0, and 6.0 μL/mL for clove, tea tree, and rosemary, respectively. The evolution of different native microbial populations was evaluated during refrigerated storage. The application forms of the preservatives were an important factor in determining the effectiveness of the essential oils. Clove and tea tree essential oils at 1 MIC and applied embedded in lactose capsules showed a significant inhibition on the growth of mesophilic, psicrotrophic, and coliforms populations, while rosemary was not effective in any of the three technological application forms. Essential oils (at 0.5 MIC) applied by spray, immersion, and embedded in lactose capsules exerted lower inhibitory effects, with respect to 1 MIC, on the different microbial populations present on lettuce leaves. At the end of the storage (7 days), lettuce samples treated with tea tree, clove, and rosemary (at 1 and 0.5 MIC) by spray were the only organoleptically acceptable (Ponce et al. 2011). In a previous study developed by Ponce et al. (2004a), clove, tea tree, and eucalyptus EO solutions (0.5–1.3 μL/mL) were sprayed on organic Swiss chard leaves. It was observed that these treatments significantly reduced microbial counts along refrigerated storage. However, they were not effective in extending the shelf life of the Swiss chard leaves from a sensory point of view (Ponce et al. 2004a).

Moreover, the use of essential oils applied during fruit and vegetable production as natural preharvest sanitizers is a promising alternative for the control of potential microbiological hazards in fresh produce. Goñi et al. (2013) applied solutions of tea tree essential oil (2.7 μL/mL) and clove essential oil (1.5 μL/mL) on late development stages of Butterhead lettuce (14, 10, 7, 3, and 0 days before harvest). These authors demonstrated the efficacy of tea tree oil to reduce microbial counts at harvest and after 5 days of refrigerated postharvest storage; the application of clove was not effective in reducing postharvest microbial counts. It was reported that sensory attributes of lettuce as overall visual quality, leaf color, texture, brightness, and browning were not affected by essential oil treatment (Goñi et al. 2013). Essential oil application in preharvest stages may help to reduce the negative effect they have in the quality attributes when applied at postharvest, as was reported by Ponce et al. (2004b). However, the application of essential oils at preharvest on fruits and vegetables requires more research work in order to develop natural treatments to preserve quality and ensure safety of the products.

On the other hand, new technologies have been developed and continue to be under study with the aim of solving the problems associated with the effective implementation of essential oils. In the face of the need to use concentrations that are sufficient to inhibit microbial growth but that at the same time allow the preservation of the sensory appeal of the products, technological developments focus on the incorporation of essential oils into edible films and coatings and also encapsulation of these agents in a food-grade material. Figure 7.1 summarizes the

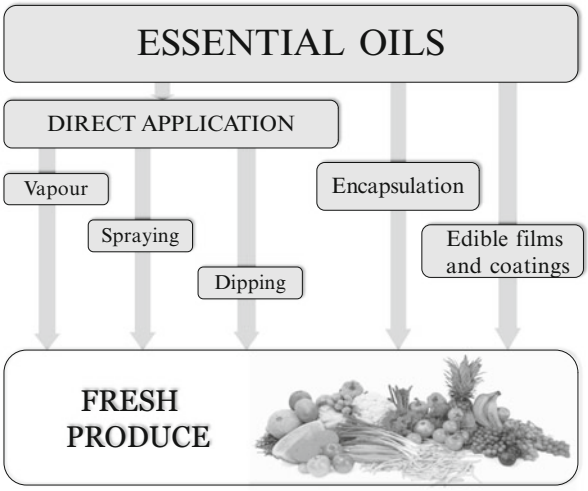


Fig. 7.1 Systems to incorporate essential oils on fresh and processed produce

systems used to incorporate natural antimicrobials as essential oils on fresh and processed produce.

Edible films and coatings are applied on many fruits and vegetables to control moisture transfer, gas exchange, or to prevent oxidation processes. However, the new generation of edible films and coatings is being especially designed as a carrier of active ingredients as antimicrobials, antioxidants, vitamins, and nutraceuticals. These agents are incorporated with the aim of assuring safety, preventing microbiological and sensory spoilage, or enhancing nutritional attributes of the product (Rojas-Graü et al. 2009). The application of antimicrobial coatings presents several advantages over the direct application of natural antimicrobials as essential oils on food. These coatings can be developed to reduce the diffusion rate from the coated surface inwards. In this way, the activity of the antimicrobial agent is maintained in the surface of the food. Thus, smaller amounts of the antimicrobial come into contact with the food compared to other application methods such as immersion or spray (Min and Krochta 2005). This results in a smaller impact in the sensory attributes of fruits and vegetables when they are treated with antimicrobial coatings.

Ayala-Zavala et al. (2013) developed pectin films enriched with cinnamon (*Cinnamomum zeylanicum* L.) essential oil that were applied on fresh-cut peaches to preserve their microbiological quality along with refrigerated shelf life. Enriched pectin film showed the highest antimicrobial activity at a cinnamon leaf oil concentration of 36.1 g/L. It was able to reduce mesophilic bacteria growth, maintain overall quality, and increase shelf life of fresh-cut peaches. In addition, the development of coatings from polymers that exhibit antimicrobial activity is very promising: such is the case of chitosan. Chitosan films can be used as a means to incorporate natural antimicrobials such as essential oils (Alvarez et al. 2013; Pranoto et al. 2005). With the aim to assure safety and extend shelf life of minimally processed

broccoli, Alvarez et al. (2013) studied the antimicrobial properties of chitosan coatings combined with tea tree essential oil and other natural antimicrobials rich in phenolic compounds. They found that the enrichment of chitosan coatings with these natural agents enhanced antimicrobial action when compared with pure chitosan treatment. These treatments significantly reduced the growth of natural flora and pathogens (*E. coli* O157:H7 and *Listeria monocytogenes*) artificially inoculated on broccoli during refrigerated storage.

Antioxidant Properties of Essential Oils Applied in Fresh Produce

Many essential oils and their components can act also as antioxidant agents at the same time protecting the product against the proliferation of spoilage or pathogen microorganisms. Thus, the application of these agents to fresh and processed produce improves antioxidant content of the product, could avoid oxidative processes catalyzed by enzymes, and can also prevent losses of natural antioxidants. Ayala-Zavala et al. (2013) reported that the treatment of fresh cut peaches with an edible pectin film enriched with cinnamon leaf essential oil was effective to increase total phenolic content and antioxidant capacity of the product (using DPPH and TEAC methods). Furthermore, Wang et al. (2008) evaluated the effectiveness of several naturally occurring EOs in reducing decay and increasing antioxidant levels and activities in "Duke" blueberries (*Vaccinium corymbosum*). Carvacrol, anethole, and perillaldehyde were able to promote total anthocyanins and total phenolics and also enhanced antioxidant activity in fruit tissues (ORAC capacity and hydroxyl radical scavenging activity).

Peroxidase (POD) is a plant enzyme associated with off-flavors and browning in fruits and vegetables (Ponce et al. 2004b). Inactivation of deteriorative enzymes is considered necessary to minimize the possibility of decay. Antioxidant properties of several plant extracts (as enzyme inhibitors) were studied on vegetable products. Ponce et al. (2004b) demonstrated the efficacy of clove, rosemary, lemon, melissa, and tea tree essential oils as reducing agents of peroxidase activity in leafy vegetables (swiss chard, spinach, lettuce, butter lettuce, and cabbage). Essential oils were used at the MICs determined in previous in vitro antimicrobial assays. Clove essential oil showed better results than the others.

Essential Oils as Quorum-Sensing Inhibitors of Food-Related Bacteria

Fresh produce spoilage is a complex process. It is caused by the various biochemical changes naturally occurring in foods and due to microbial activities. Microbial spoilage is the most common cause of spoilage (Gram et al. 2002). In recent years, the

detection of quorum-sensing signals in spoiled food products, including vegetables, has added a new dimension to study the process of food spoilage. Quorum sensing (QS) or cell-to-cell communication is employed by a diverse group of bacteria including those commonly associated with food to communicate with each other by producing signaling molecules called autoinducers (Truchado et al. 2009). Through the mechanisms of QS, bacteria are able to express specific genes in response to population density. It is known that many bacteria depend on QS to establish a pathogenic interaction with the host and the formation of biofilms; moreover, it is possible that the bacterial spoilage of some food products is influenced by QS-regulated phenotypes (Jamuna Bai and Rai 2011). The discovery of QS might have opened a new line of action against microorganisms.

The ideal QS inhibitors have been defined as chemically stable and highly effective low-molecular-mass molecules, which exhibit a high degree of specificity for the QS regulator without toxic side effects (Rasmussen and Givskov 2006). In the last years, some studies have identified QS inhibitors in a variety of medicinal and dietary plants (Gao et al. 2003; Adonizio et al. 2006; Vattem et al. 2007; Brahma et al. 2009) and phytochemicals have been presented as new anti-pathogenic agents to control microbial infections via the inhibition of QS (Al-Hussaini and Mahasneh 2009; Brackman et al. 2009; Vikram et al. 2010).

QS has been extensively studied in bacterial pathogenicity (Smith et al. 2004), and now it is implicated that this process could be involved in bacterial food spoilage. Several proteolytic, lipolytic, chitinolytic, and pectinolytic activities associated with the deterioration of foods are regulated by QS. The pectinolytic activity of *Pseudomonadaceae* or *Enterobacteriaceae* (mostly *Erwinia* spp.) growing to high-cell densities (10^8 to 10^9 CFU/g) in fruits and vegetables causes off-tastes, off-odors, and/or texture breakdown resulting in their spoilage (Liao 1989). *Erwinia* and *Pseudomonas* produce various pectinolytic enzymes, namely pectin lyases, pectate lyase, poly-galacturonase, and pectin methyl esterases, which are responsible for the spoilage of ready-to-eat vegetables, and also produce a broad range of AHLs (acylated homoserine lactones) as signaling molecules (Rasch et al. 2005). This suggests the involvement of AHL-based quorum-sensing systems in the spoilage of vegetables and fruits.

Essential oils used in traditional medicine are one of the most promising areas in the search for new biologically active compounds (Hullati and Rai 2004; Jamuna Bai et al. 2011). Essential oils derived from medicinal and food plants are known for their application in traditional medicine, in the food industry, and in a number of therapeutic uses (Bakkali et al. 2008). However, the plant essential oils, rich diverse source of bioactive compounds, have not yet been subjected to systematic scrutiny for their anti-QS activity.

In a recent study developed by Alvarez et al. (2012), QS inhibitory activity of two essential oils (tea tree and rosemary) and other plant extracts was evaluated by in vitro assays using the bacteria model *Chromobacterium violaceum*. These essential oils were the most effective to inhibit the violacein pigment production by *C. violaceum* applied at low concentrations (0.5 μL/mL), demonstrating their potential as QS inhibitors. In addition, Pellegrini et al. (2014) studied the antimicrobial and

anti-QS activity of several EOs obtained from Argentinian species. *Minthostachys mollis* EO showed significant QS inhibition properties (using *C. violaceum* model) at a sublethal concentration (0.2 μL/mL) and also a high bactericidal activity against various indicator strains. This EO resulted as a good candidate for the development of anti-QS treatment with a potential application in the control of bacterial diseases mediated by QS (Pellegrini et al. 2014).

Moreover, oregano essential oil (OEO) has proved to be useful as food antimicrobial; however, its food applications can be compromised by the volatile character of its active constituents. Therefore, formulation of edible films containing OEO can be an alternative to improve its food usages. Thus, in a recent study, Alvarez et al. (2014) evaluated QS inhibitory activity of oregano essential oil (*Lippia graveolens* Kunt) and pectin-OEO films by in vitro assays using *C. violaceum* model. OEO and pectin-OEO films showed a significant anti-QS activity expressed as inhibition of violacein production by *C. violaceum*, even at the lowest tested concentrations (0.0156 mg/mL and 15.7 mg/mL, respectively). These results demonstrated the potential of pectin films enriched with OEO as QS inhibitors (Alvarez et al. 2014).

The natural occurrence of QS inhibitors is an important consideration for the assessment of their toxicological status and may facilitate their use in food as preservatives and hence pave way for the application of novel preservation techniques to control food spoilage and potentially hazardous foodborne bacterial contamination.

Conclusions and Future Trends

Preservation and safety of fresh and processed fruits and vegetables are topics that concern authorities, food producers, and scientists due to the growing consumer demand for these foods. Given the limitations in the use of synthetic disinfectants, it is important to continue the search for natural and safe preservation alternatives. In the last two decades, significant progress has been made, but further research is necessary to develop new technologies such as the use of natural preservatives (essential oils and other plant extracts), optimizing their application methods to reach higher efficiency. The use of essential oils on fruits and vegetables without negatively affecting the sensory properties is still a challenge for researchers. To inhibit proliferation or eliminate pathogenic bacteria, the required concentrations of these agents are very high, and at such concentrations they can affect the sensory qualities of foods. Hence, combining these treatments with physical barriers, such as ultrasound and high pressures, may be a viable strategy to ensure the safety, nutritional, and sensory qualities of fresh produce. Furthermore, not enough research has been done on the potential synergistic combinations of natural agents to reduce microbial growth in fruits and vegetables; using more than one natural agent can reduce the concentrations needed, thereby minimizing the effect on organoleptic characteristics of the treated fresh produce.

References

Adonizio, A. L., Downum, K., Bennett, B. C., & Mathee, K. (2006). Anti-quorum sensing activity of medicinal plants in southern Florida. *Journal of Ethnopharmacology, 105*(3), 427–435.

Al-Hussaini, R., & Mahasneh, A. M. (2009). Microbial growth and quorum sensing antagonist activities of herbal plants extracts. *Molecules, 14*, 3425–3435.

Alvarez, M. V., Moreira, M. R., & Ponce, A. (2012). Antiquorum sensing and antimicrobial activity of natural agents with potential use in food. *Journal of Food Safety, 32*(3), 379–387.

Alvarez, M. V., Ortega-Ramirez, L. A., Gutierrez-Pacheco, M. M., Bernal-Mercado, A. T., Rodriguez-Garcia, I., Gonzalez-Aguilar, G. A., et al. (2014). Oregano essential oil-pectin edible films as anti-quorum sensing and food antimicrobial agents. *Frontiers in Microbiology, 5*, 699.

Alvarez, M. V., Ponce, A. G., & Moreira, M. R. (2013). Antimicrobial efficiency of chitosan coating enriched with bioactive compounds to improve the safety of fresh cut broccoli. *LWT-Food Science and Technology, 20*, 78–87.

Ayala-Zavala, J. F., Del Toro-Sánchez, L., Alvarez-Parrilla, E., & González-Aguilar, G. A. (2008a). High relative humidity in-package of fresh-cut fruits and vegetables: Advantage or disadvantage considering microbiological problems and antimicrobial delivering systems? *Journal of Food Science, 73*, 41–47.

Ayala-Zavala, J. F., Oms-Oliu, G., Odriozola-Serrano, I., González-Aguilar, G. A., Álvarez-Parrilla, E., & Martín-Belloso, O. (2008b). Bio-preservation of fresh-cut tomatoes using natural antimicrobials. *European Food Research and Technology, 226*, 1047–1055.

Ayala-Zavala, J. F., Silva-Espinoza, B. A., Cruz-Valenzuela, M. R., Leyva, J. M., Ortega-Ramírez, L. A., Carrazco-Lugo, D. K., et al. (2013). Pectin-cinnamon leaf oil coatings add antioxidant and antibacterial properties to fresh-cut peach. *Flavour and Fragrance Journal, 28*, 39–45.

Ayala-Zavala, J. F., Soto-Valdez, H., González-León, A., Álvarez-Parrilla, E., Martín-Belloso, O., & González-Aguilar, G. A. (2008c). Microencapsulation of cinnamon leaf (*Cinnamomum zeylanicum*) and garlic (*Allium sativum*) oils in β-cyclodextrin. *Journal of Inclusion Phenomena and Macrocyclic Chemistry, 60*, 359–368.

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils—A review. *Food and Chemical Toxicology, 46*(2), 446–475.

Bowles, E. J. (2003). *Chemistry of aromatherapeutic oils*. Australia: Allen and Unwin. ISBN 174114051X.

Brackman, G., Hillaert, U., Van Calenbergh, S., Nelis, H. J., & Coenye, T. (2009). Use of quorum sensing inhibitors to interfere with biofilm formation and development in *Burkholderia multivorans* and *Burkholderia cenocepacia. Research in Microbiology, 160*, 144–151.

Brahma, N. S., Singh, B. R., Singh, R. L., Prakash, D., Sarma, B. K., & Singh, H. B. (2009). Antioxidant and anti-quorum sensing activities of green pod of *Acacia nilotica* L. *Food and Chemical Toxicology, 47*, 778–786.

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods—A review. *International Journal of Food Microbiology, 94*, 223–253.

Chang, H. T., Cheng, Y. H., Wu, C. L., Chang, S. T., Chang, T. T., & Su, Y. C. (2008). Antifungal activity of essential oil and its constituents from *Calocedrus macrolepis* var. *formosana* florin leaf against plant pathogenic fungi. *Bioresource Technology, 99*, 6266–6270.

Dadalioglu, I., & Evrendilek, G. A. (2004). Chemical compositions and antibacterial effects of essential oils of Turkish oregano (*Origanum minutiflorum*), bay laurel (*Laurus nobilis*), Spanish lavender (*Lavandula stoechas* L.), and fennel (*Foeniculum vulgare*) on common foodborne pathogens. *Journal of Agricultural and Food Chemistry, 52*(26), 8255–8260.

Davidson, P. M., Critzer, F. J., & Taylor, T. M. (2013). Naturally occurring antimicrobials for minimally processed foods. *Annual Review of Food Science and Technology, 4*, 163–190.

Draughon, F. A. (2004). Use of botanicals as biopreservatives in foods. *Food Technology, 58*(2), 20–29.

Elgayyar, M., Draughon, F. A., Golden, D. A., & Mount, J. R. (2001). Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. *Journal of Food Protection, 64*(7), 1019–1024.

Fisher, K., & Phillips, C. (2008). Potential antimicrobial uses of essential oils in food: Is citrus the answer? *Trends in Food Science and Technology, 19*(3), 156–164.

Friedman, M., Henika, P. R., & Mandrell, R. E. (2002). Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica. Journal of Food Protection, 65*(10), 1545–1560.

Gao, M., Teplitski, M., Robinson, J. B., & Bauer, W. D. (2003). Production of substances by *Medicago truncatula* that affect bacterial quorum sensing. *Molecular Plant-Microbe Interactions, 16*, 827–834.

Gill, A. O., Delaquis, P., Russo, P., & Holley, R. A. (2002). Evaluation of antilisterial action of cilantro oil on vacuum packed ham. *International Journal of Food Microbiology, 73*(1), 83–92.

Goñi, M. G., Tomadoni, B., Moreira, M. R., & Roura, S. I. (2013). Application of tea tree and clove essential oil on late development stages of Butterhead lettuce: Impact on microbiological quality. *LWT-Food Science and Technology, 54*(1), 107–113.

González-Aguilar, G. A., Ansorena, M. R., Viacava, G. E., Roura, S. I., & Ayala-Zavala, J. F. (2013). Plant essential oils as antifungal treatments on the postharvest of fruit and vegetables. In M. Razzaghi-Abyaneh & M. Rai (Eds.), *Antifungal metabolites from plants* (pp. 429–446). Berlin Heidelberg: Springer.

González-Aguilar, G. A., Ayala-Zavala, J. F., Olivas, G. I., de la Rosa, L. A., & Álvarez-Parrilla, E. (2010). Preserving quality of fresh-cut products using safe technologies. *Journal für Verbraucherschutz und Lebensmittelsicherheit, 5*(1), 65–72.

Gram, L., Ravn, L., Rasch, M., Bruhn, J., Christensen, A., & Givskov, M. (2002). Food spoilage interactions between food spoilage bacteria. *International Journal of Food Microbiology, 78*, 79–97.

Gutierrez, J., Barry-Ryan, C., & Bourke, P. (2008). The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *International Journal of Food Microbiology, 124*, 91–97.

Gutierrez, J., Bourke, P., Lonchamp, J., & Barry-Ryan, C. (2009). Impact of plant essential oils on microbiological, organoleptic and quality markers of minimally processed vegetables. *Innovative Food Science and Emerging Technologies, 10*(2), 195–202.

Hammer, K. A., Carson, C. F., & Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology, 86*(6), 985–990.

Hullati, K. K., & Rai, V. R. (2004). Antimicrobial activity of *Memecylon malabaricum. Fitoterapia, 75*, 409–411.

Jamuna Bai, A., & Rai, V. R. (2011). Bacterial quorum sensing and food industry. *Comprehensive Reviews in Food Science and Food Safety, 10*(3), 183–193.

Jamuna Bai, A., Rai, V. R., & Pradeepa, V. S. (2011). Evaluation of the antimicrobial activity of three medicinal plants of South India. *Malaysian Journal of Microbiology, 7*, 14–18.

Liao, C. H. (1989). Analysis of pectate lyases produced by soft rot bacteria associated with spoilage of vegetables. *Applied and Environmental Microbiology, 55*, 1677–1683.

Min, S., & Krochta, J. M. (2005). Inhibition of *Penicillium commune* by edible whey protein films incorporating lactoferrin, lacto-ferrin hydrolysate, and lactoperoxidase systems. *Journal of Food Science, 70*, 87–94.

Moreira, M. R., Ponce, A. G., del Valle, C. E., & Roura, S. I. (2005). Inhibitory parameters of essential oils to reduce a foodborne pathogen. *LWT-Food Science and Technology, 38*, 565–570.

Moreira, M. R., Ponce, A. G., Del Valle, C. E., & Roura, S. I. (2007). Effects of clove and tea tree oils on *Escherichia coli* O157:H7 in blanched spinach and minced cooked beef. *Journal Of Food Processing and Preservation, 31*(4), 379–391.

Pandit, V. A., & Shelef, L. A. (1994). Sensitivity of *Listeria monocytogenes* to rosemary (*Rosmarinus officinalis* L.). *Food Microbiology, 11*(1), 57–63.

Pellegrini, M. C., Alvarez, M. V., Ponce, A. G., Cugnata, N. M., De Piano, F. G., & Fuselli, S. R. (2014). Anti-quorum sensing and antimicrobial activity of aromatic species from South America. *Journal of Essential Oil Research, 26*(6), 458–465.

Pichersky, E., Noel, J. P., & Dudareva, N. (2006). Biosynthesis of plant volatiles: Nature's diversity and ingenuity. *Science, 311*(5762), 808–811.

Ponce, A. G., Del Valle, C. E., & Roura, S. I. (2004a). Shelf life of leafy vegetables treated with natural essential oils. *Journal of Food Science, 69*(2), fms50–fms56.

Ponce, A. G., Del Valle, C. E., & Roura, S. I. (2004b). Natural essential oils as reducing agents of peroxidase activity in leafy vegetables. *LWT-Food Science and Technology, 37*, 199–204.

Ponce, A. G., Roura, S. I., & Moreira, M. D. R. (2011). Essential oils as biopreservatives: Different methods for the technological application in lettuce leaves. *Journal of Food Science, 76*(1), M34–M40.

Pranoto, Y., Rakshit, S. K., & Salokhe, V. M. (2005). Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. *LWT-Food Science and Technology, 38*, 859–865.

Rasch, M., Andersen, J., Nielsen, K., Flodgaard, L., Christensen, H., Givskov, M., et al. (2005). Involvement of bacterial quorum-sensing signals in spoilage of bean sprouts. *Applied and Environmental Microbiology, 71*, 3321–3330.

Rasmussen, T. B., & Givskov, M. (2006). Quorum-sensing inhibitors as anti-pathogenic drugs. *International Journal of Medical Microbiology, 296*(2), 149–161.

Rojas-Graü, M. A., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). Edible coatings to incorporate active ingredients to fresh-cut fruits: A review. *Trends in Food Science and Technology, 20*, 438–447.

Selim, S. (2011). Antimicrobial activity of essential oils against Vancomycin-Resistant enterococci (VRE) and *Escherichia coli* O157:H7 in feta soft cheese and minced beef meat. *Brazilian Journal of Microbiology, 42*(1), 187–196.

Smith, J. L., Fratamico, P. M., & Novak, J. S. (2004). Quorum sensing: A primer for food microbiologists. *Journal of Food Protection, 67*, 1053–1070.

Speranza, B., & Corbo, M. R. (2010). Essential oils for preserving perishable foods: Possibilities and limitations. In A. Bevilacqua, M. R. Corbo, & M. Sinigaglia (Eds.), *Application of alternative food-preservation technologies to enhance food safety & stability* (pp. 35–57). China: Bentham Science Publishers.

Tripathi, P., & Dubey, N. K. (2004). Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. *Postharvest Biology and Technology, 32*, 235–245.

Troncoso-Rojas, R., & Tiznado-Hernández, M. E. (2007). Natural compounds to control fungal postharvest diseases. In R. Troncoso-Rojas, M. E. Tiznado-Hernández, & A. González-León (Eds.), *Recent advances in alternative postharvest technologies to control fungal diseases in fruits and vegetables* (pp. 127–156). Trivandrum, Kerala: Research Signpost.

Truchado, P., López-Gálvez, F., Gil, M., Tomás-Barberán, F., & Allende, A. (2009). Quorum sensing inhibitory and antimicrobial activities of honeys and the relationship with individual phenolics. *Food Chemistry, 115*(4), 1337–1344.

Vattem, D. A., Mihalik, K., Crixell, S. H., & McLean, R. J. C. (2007). Dietary phytochemicals as quorum sensing inhibitors. *Fitoterapia, 78*, 302–310.

Vikram, A., Jesudhasan, P. R., Jayaprakasha, G. K., Pillai, B. S., & Patil, B. S. (2010). Grapefruit bioactive limonoids modulate *E. coli* O157:H7 TTSS and biofilm. *International Journal of Food Microbiology, 140*, 109–116.

Wang, C. Y., Wang, S. Y., & Chen, C. (2008). Increasing antioxidant activity and reducing decay of blueberries by essential oils. *Journal of Agricultural and Food Chemistry, 56*, 3587–3592.

Chapter 8
Plant Growth Regulators

Félicie LOPEZ-LAURI

Introduction

Plant growth and development are regulated not only by external factors, as light and temperature, but mainly by internal factors, as its genetic factors and endogenous "phytohormones" also named plant growth regulators (PGRs). The PGRs are defined as organic compounds, naturals, or synthetics, controlling (stimulation or inhibition) plant growth and development functions, and this at very low concentration (nM–pM). In particular, PGRs are involved in the regulation of cell division, cell enlargement, and cell differentiation. Like that, they control organogenesis, senescence, and dormancy from germination stage to fructification. They also play a major role in regulating physiological functions as stomata aperture control, sugar metabolism, and plant stress responses.

The most important natural PGRs belong to one of the five major hormone classes: auxins, gibberellins (GAs), cytokinins (CKs), ethylene (C_2H_4), and abscisic acid (ABA). More recently, other PGRs have been discovered: brassinolide group (BRs), salicylates (SA), jasmonate (JA), and derivatives and polyamines (PAs). Some authors also consider that nitric oxide (NO) is a phytohormone that is also used in postharvest treatment (Lichanporn and Techavuthiporn 2013; Manjunatha et al. 2010).

In plants, PGRs have multiple functions depending on their concentration or their localization and often act in synergy or antagonism with another PGRs. In addition, any given PGR may affect the biosynthesis of another. So, the balance between endogenous hormones has important consequences on physiological responses in fruit maturation and ripening (McAtee et al. 2013). As this equilibrium

F. LOPEZ-LAURI (✉)
Université d'Avignon et des Pays de Vaucluse, UMR95 QualiSud,
301 rue Baruch de Spinoza – Agroparc, BP 21239, 84018 Avignon Cedex 9, France
e-mail: felicie.lauri@univ-avignon.fr

M.W. Siddiqui et al. (eds.), *Postharvest Management Approaches for Maintaining Quality of Fresh Produce*, DOI 10.1007/978-3-319-23582-0_8

could be modified by exogenous applications of PGRs, one can change plant physiology and the developmental program to obtain positive effects on both cultural and postharvest behavior. Nowadays, numerous PGRs are produce synthetically and are used in agriculture to optimize the production and quality of plants, fruits, and vegetables. The application of PGR can provide important economic advantages to fruit farmers in stimulating specific responses such as increase in fruit size and delay or enhance maturity or senescence of orchard and vegetables. Sometimes, preharvest application may have beneficial carryover effects on postharvest quality. Finally, PGR could also be applied after harvest to improve storage, and induce or delay ripening and control quality of fresh produces, of course if its effects are not harmful to the consumer.

The objective of this chapter is to give an overview of properties of PGRs and some recent developments in plant growth regulator efficiencies to improve and maintain fresh product quality.

The Five Major Classes of Plant Growth Regulators

Auxins

Auxin is the first identified plant hormone and whose effects on cell elongation and division have been shown by Frits Went in 1926. In plants, the principal auxin is the indole-3-acetic acid (IAA) synthetized by tryptophan-dependent or tryptophan-independent pathways in meristems and developing organs (Mano and Nemoto 2012; Korasick et al. 2013; Lehmann et al. 2010; Tivendale et al. 2014). Natural auxins, such as phenylacetic acid (PAA), 4-chloro-3-acetic acid (4-Cl-IAA), and indole-3-butyric acid (IBA), also regulate cell division, cell growth, ethylene biosynthesis, root development, leaf formation, stem elongation, apical dominance, and differentiation of vascular tissues and fruit setting (Woodward and Bartel 2005; Simon and Petrášek 2011). However, depending on auxin levels the response to auxin application could be opposed. The structures of most important natural and synthetic auxins are shown in Fig. 11.1.

Synthetic auxins such as α-naphthaleneacetic acid (NAA), and 2,4-dichlorophenoxyacetic acid, better known under the name of 2,4-D, induce similar physiological responses as natural auxins. Due to their properties on organogenesis, NAA and IBA are routinely used to stimulate root initiation and differentiation for the vegetative propagation of plants from stem and leaf cutting. It must not be forgotten that, usually, the synthetic auxins, such as 2,4-D, Picloram®, and Dicamba®, are mainly used as selective herbicide. In fact, used in high concentration, auxin stimulates the production of ethylene that inhibits elongation growth, causes leaf abscission, and finally destroys the plant.

Synthetic auxins were widely used in arboriculture and horticulture for:

- Induction of parthenocarpic fruits in tomato and grapes to obtain seedless fruits appreciated by consumer.

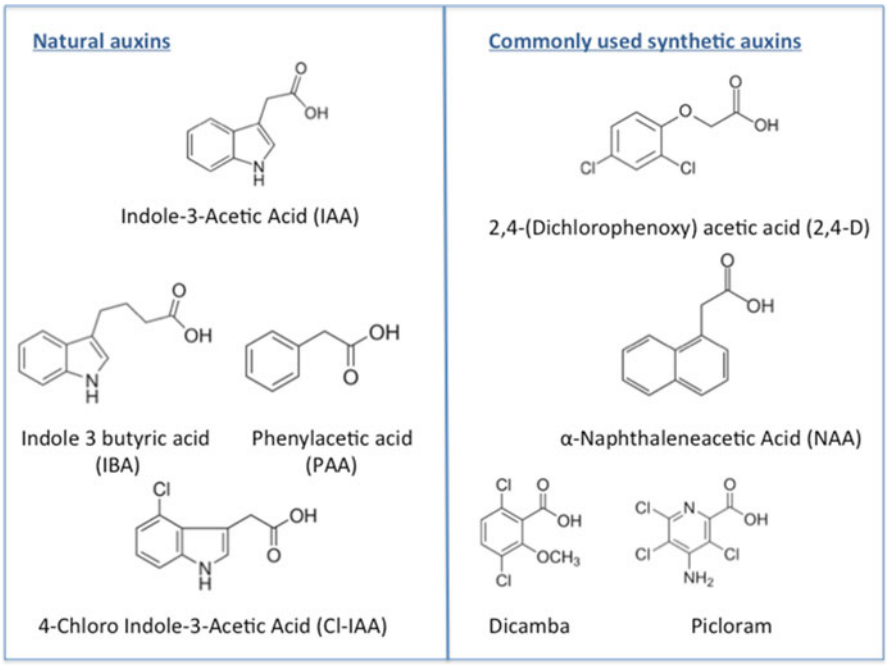


Fig. 11.1 Structures of natural and synthetic auxins

- Inhibition of preharvest fruit drop: application of NAA 10–50 ppm in mango, citrus, and chilies reduces fruit drop by preventing formation of abscission layer.
- Induction of flower and fruit thinning to avoid the biennial bearing in tree fruit and produce higher fruit size (Stern et al. 2007a, b; Schröder et al. 2013).
- Promotion of fruit growth and advance maturity in loquat and Satsuma mandarin fruits (Amorós et al. 2004; Ortolá et al. 1991).

Postharvest treatments with synthetic auxins have been also investigated. Auxin application is well documented in citrus fruits. Postharvest application of 2,4-D has been used to retard calyx abscission, and water loss, which occurs as the consequence of degreening induced by ethylene (Salvador et al. 2010; (Sdiri et al. 2013; Sdiri et al. 2012)). Auxins have also been used to affect biotic stresses of harvested fruits and vegetables. In mango, application of 2,4-D in wax combined with hot water brushing treatment improved quality during storage by limiting side rots caused by *Alternaria alternata* (Kobiler et al. 2001). In addition, Wang et al. (2008) have shown that 2,4-D reduced chilling injury in mango through an increase of ABA and GA levels and an augmentation of antioxidant defenses. In Chilean strawberry, NAA postharvest treatment delays fruit ripening without effect on firmness (Figueroa et al. 2012). The methyl ester of NAA is used to prevent the sprouting of potato tubers and hence increases storage life (Suttle 2003).

In recent years, European Union legislation has restricted the use of 2,4-D even as preharvest application. The level of 2,2-D residue should be below 0.5 ppm on food. Thus, it is absolutely necessary to test safely and environmental friendly new auxins in order to maintain the quality of fresh produce.

Gibberellins

Gibberellins (GAs) are the second most important family of PGR. GAs belong to the group of tetracyclic diterpenoid acids (Fig. 11.2). The ent-gibberelane skeleton (ent-kaurene) of GAs is synthesized from isopentenyl diphosphate (IPP) via the mevalonate or the methyl erythro 4-phosphate (MEP) pathways to give GA12 and GA53. The conversion between the different forms of GAs is provided by gibberellin oxidases. Many advances have been made in understanding the regulation of GA biosynthesis, transport, and signaling (see review of Thomas et al. 2005). Today, over 136 GAs have been identified in plants and fungi. As other hormones, gibberellins play many roles in plant development processes. GAs are implicated in cell elongation and division, stem elongation, breaking dormancy, and the vegetative phase to flowering transition. The first GA identified is the gibberellic acid (GA3), found in *Gibberella fujikuroï* that is responsible of bakanae disease whose symptoms are chlorotic and abnormally elongated leaves. Consequently, GA3 is the most described in literature; however, the bioactive forms of GAs are GA1 and GA4 for stem elongation. The predominant forms of GAs applied are GA3, GA4, GA7, or a mixture of GA4 and GA7.

Synthetic GAs were widely used in arboriculture and horticulture with consequence for postharvest quality (Serrano et al. 2004):

- In table grapes, GA3 increases fruit size and reduce rachis browning and extension of shelf-life (Raban et al. 2013).
- In citrus, pineapple, cherries, and peach, GAs are known to maintain and/or increase color and firmness.
- In cherry, GA3 delays fruit ripening (Zhang and Whiting 2011) and extend crown life of pineapple.
- GA can delay storage disorders such as internal browning (Lurie and Crisosto 2005).
- Preharvest GA treatment can control disease development in persimmon fruit during its storage (Biton et al. 2014). Nevertheless, GA3 application in seedless table grapes could predispose to gray mold caused by *botrytis cinerea* (Zoffoli et al. 2009).
- In leafy vegetable, GA3 treatment during production delays the senescence of product and maintains chlorophyll content during storage (Lers et al. 1998).

Gibberellins are an excellent PGR for postharvest application. Indeed, some GA as GA3 and GA4 are natural hormones and by consequence this forms could be used with any restriction in most part of world. Post harvest applications of GA

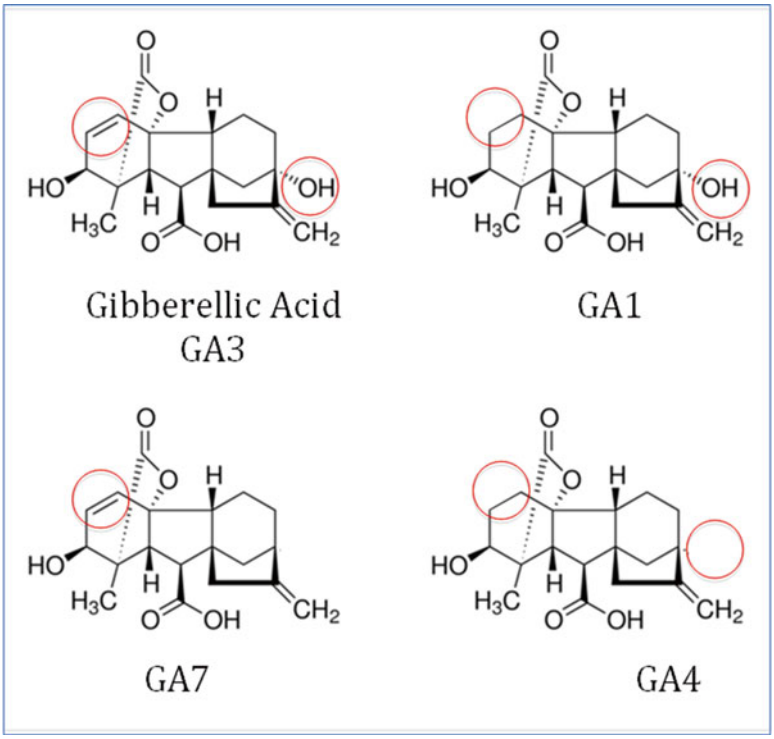


Fig. 11.2 Structures of gibberellin compounds: Gibberellic acid or GA3, gibberellin A1 (GA1), GA4, and GA7. The differences between gibberellin are indicated by circle

allow the maintenance of firmness and the enhancement of storage of fruits and vegetables. In order to extend the green life of bananas after the harvest, some growers apply gibberellic acid directly to the bananas' hands. Subsequently, GA3 treatment also maintains fruit quality longer, ensuring that the bananas will get to their destination in optimum condition (Vargas and Lopez 2011). Postharvest GA3 treatment also induces antioxidant defenses and so reduces chilling injury during storage in tomato (Ding et al. 2015) and delays ripening and maintains green peel color longer in orange (Gambetta et al. 2014) and in papaya (Ramakrishna et al. 2002) and with higher level of ascorbic acid in mango (Khader et al. 1988; Khader 1991).

Cytokinins

Cytokinins (CKs) are PGRs that induce cell division, delay senescence, and regulate pathogen resistance. Cytokinins are N^6-adenine derivates discovered for the first time in coconut (*Cocos nucifera*) milk by Johannes van Overbeek in 1941 (for review,

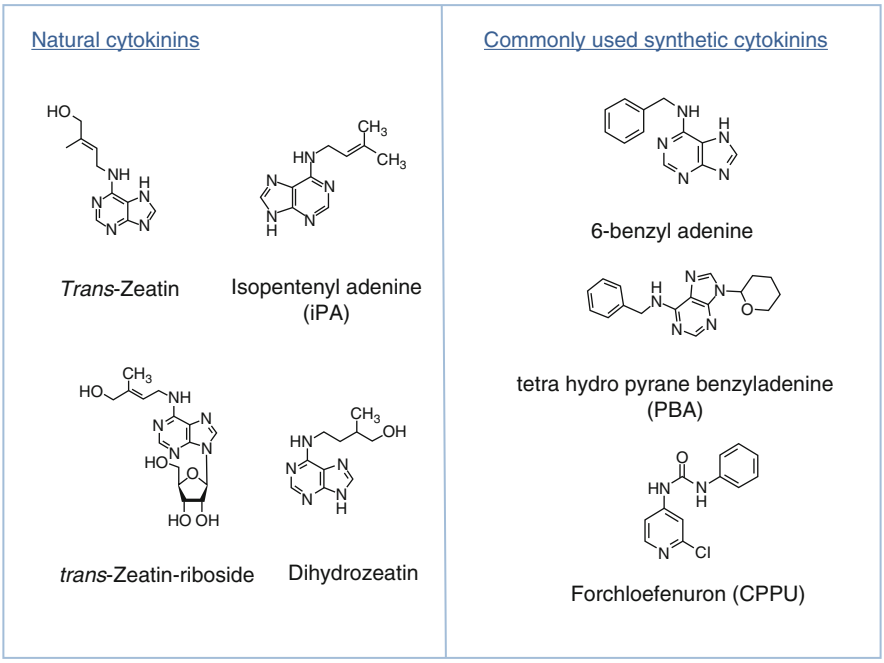


Fig. 11.3 Structures of natural and synthetic cytokinins

Sakakibara 2006). Zeatin (cis and trans isomers) is the most common natural CK. Dihydrozeatin, isopentenyl adenine (iPA), and zeatin riboside naturally occur in plants (Fig. 11.3). CK main application comes from their ability to stimulate the growth and formation of new roots and buds in in vitro cultures and therefore control the regeneration and the growth for the plant micropropagation purpose. For these applications, synthetic cytokinin analogs have been developed from purines (6-benzyladenine (6-BA), tetra hydro pyrane benzyladenine (PBA), and phenylurea (Forchlorfenuron (CPPU)). These analogs could also be used in arboriculture for thinning in apples and pears and so, adjusts crop load, resulting in the best crop yield and guaranteeing return bloom (Flaishman et al. 2001; Hayata et al. 2000). In fruits, synthetic CK acts synergistically with natural auxins to stimulate cell division, resulting in increased fruit size at harvest in cherry (Zhang and Whiting 2011). Moreover, mixture of 6-BA and GA applied during culture improved persimmon quality during storage and enhances tolerance to Alternaria black spot (Biton et al. 2014). In the same way, CPPU applied during fruit development improves cold storage in grapes and kiwifruits (Kim et al. 2006; Marzouk and Kassem 2011). However, no effect of CPPU, applied alone, has been observed by Raban et al. (2013).

Due to its inhibition of senescence, harvest treatment with 6-BA improves appearance of green asparagus spears, rocket, broccoli, and cucumber and positively affect firmness and chlorophyll and ascorbic acid content (An et al. 2006; Koukounaras et al. 2010; Costa et al. 2005; Chen and Yang 2013). Postharvest CK treatment has also effects on fruit texture. So, summer squash sprayed with 6-BA reduces pectin solubilization and later prevents texture deterioration during cold storage. The CPPU is also used combined with GA treatment and the mixture can also delay ripening and increase fungal tolerance in banana and broccoli (Huang and Jiang 2012).

Abscisic Acid

Abscisic acid (ABA) is a 15-carbon sesquiterpene synthetized from carotenoids in chloroplasts and other plastids. The 9-cis-epoxycarotenoid dioxygenase (NCED) is the key enzyme for ABA biosynthesis (Seo and Koshiba 2002). ABA is involved in inhibition of plant growth; it promotes senescence and abscission of leaves and it controls stomatal closure (Garcia-Mata and Lamattina 2007). Dormancy and induction of buds and seeds are also regulated by ABA. This PGR plays a major role in fruit ripening in particular in tomato and mango where ABA controls, via ethylene production, cell wall catabolism leading to a modification of texture and to a diminution of shelf-life (Leng et al. 2014; Zaharah et al. 2013). Therefore, exogenous ABA application enhances the color and maintains postharvest quality of "Crimson Seedless" grapes (Cantín et al. 2007; Ferrara et al. 2013) and induces anthocyanin synthesis in litchi (Singh et al. 2014). In addition, the production of ABA is emphasized by stress, including water loss or freezing temperature during fruit and vegetable storage (Romero et al. 2013; Lafuente and Sala 2002). However, agricultural use of ABA is limited by its susceptibility to light and by the high cost of its production (Gianfagna 1995; Abrams et al. 1997). Therefore, analogs or not and agonist of ABA have been developed that mimic ABA but are more resistant to degradation and less costly to synthesize (Grossmann and Jung 1984; Schubert et al. 1990) (Fig.11.4). So, long-lasting synthetic analogs of ABA, 8' methylene methyl-ester ABA (PBI 365) and 8' acetylene methyl-ester ABA (PBI 429), have been developed and positively checked for maintain quality of cut Baccara roses (Pompodakis and Joyce 2003). Pyrabactin (4-bromo-N-(2-pyridinylmethyl)-1-naphthalenesulfonamide) is the first agonist of ABA that is not an analog. Pyrabactin acts as an activator of abscisic acid receptors and activates ABA pathway in a manner very similar to ABA (Puli and Raghavendra 2012). When it is sprayed onto plants it acts in the same way as ABA, and helps them survive with less water. Sean Cutler of the university of California-Riverside developed another molecule, the quinabactin. It mimics also the functions of ABA that makes plants more tolerant to water deficit (Okamoto et al. 2013). However, the unfairness of these products must be demonstrated before use in pre- and postharvest.

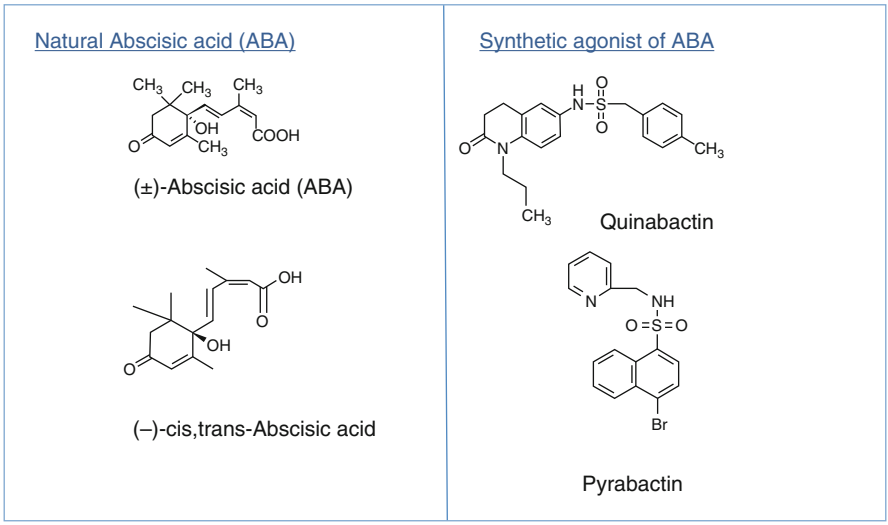


Fig. 11.4 Structures of natural and synthetic abscisic acid

Ethylene

Ethylene (C_2H_4) is the first gaseous hormone identified in plant. Ethylene synthesized from methionine by a well-defined pathway in which 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase catalyze the reactions from S-adenosylmethionine to ACC and ACC to ethylene, respectively (Yang and Hoffman 1984). $C_2H_4$is produced during fruit ripening (see review of Alexander and Grierson 2002; Liu et al. 1999; Mworia et al. 2010), especially in climacteric fruits such as bananas, apples, pears, mangos, and kiwis, and it is involved in senescence and abscission of leaves and flowers. Ethylene synthesis is also induced in response to drought, wounding, chilling injury, and pathogen infection. Climacteric fruits such as in bananas, mangos, tomatoes, and avocados are often harvested at a physiological stage that is considered "commercial maturity" and corresponding to green mature stage just before ripening has initiated. Ripening to obtain "ready-to-eat" fruits can then be conducted under controlled conditions and ethylene fumigation to achieve uniform appearance and quality of ripe fruit. When fruit are exposed to ethylene under these controlled conditions they initiate their respiratory climacteric pattern, induce endogenous ethylene biosynthesis and ripen. In leafy and florets vegetables as broccoli, ethylene application provokes senescence (Costa et al. 2005). Usually, treatments to induce ripening are achieved with ethylene-releasing compounds such as 2-chloroethylphosphonic acid (ethephon) in banana and citrus. However, ethylene promotes calyx abscission in citrus and auxin treatment must be

used to avoid this effect. In addition, fast ripening induced by ethylene treatment can affect fruit quality if the concentration and the timing of application are not adequate.

Other PGRs

Salicylic acid (SA) is a phenolic compound similar to aspirin (acetylsalicylic acid) has been involved in defense responses to several abiotic stresses and to plant pathogens. The most important effect of SA is the induction of systemic resistance acquired (SAR). SA is synthesized from phenylalanine and this synthesis involved the phenylalanine ammonia lyase (PAL) and the benzoic acid 2 hydroxylase (BA2H). SA could be found as free or conjugated compound (Fig. 11.5). It has been shown that exogenous application of salicylic acid led to the accumulation of proteins linked to the resistance and thus significantly reduces the extent of damage caused by pathogens. SA can also inhibit ethylene biosynthesis. SA presents several advantages in postharvest application due their effect on fruit ripening, firmness (Marzouk and Kassem 2011; Ranjbaran et al. 2011), antioxidants (Bal and Celik 2010; Chen et al. 2006; Divya et al. 2014; Huang et al. 2008), and disease resistance (Asghari and Aghdam 2010). Chapter 5 describes the impact of SA postharvest treatments on fresh produce quality.

Jasmonic acid (JA) and its derivates as methyl jasmonate (Me-JA) are volatile fatty compounds, derived from the family of octadecanoic fatty acids, and synthesized from linolenic acid membrane of chloroplast (Fig. 11.5). In the chloroplast, linolenic acid, after the action of lipoxygenase, is converted to a cyclized intermediate 12-keto-phytodienoic acid, which, after transport in the peroxisome, is first reduced and then converted after β -oxidation in jasmonic acid (Fig. 11.6). The exogenous jasmonic acid inhibits plant growth and stimulates various processes related to senescence, ripening, and antioxidant defenses (Kucuker et al. 2014; Flores et al. 2015; Concha et al. 2013). It is also known to induce transcription of genes involved in the synthesis of plant defense proteins in response to biotic stress (Wasternack 2014). So, Me-JA postharvest treatment has been shown to reduce *Alternaria alternata* development in tomato (Chen et al. 2014) and *Penicillium citrinum* in Chinese bayberry (Wang et al. 2014). See also Chap. 6.

Brassinolides or Brassinosteroids (BR) are steroids compounds discovered for the first time in the pollen of oilseed rape (*Brassica napus*) and brassicaceae family (Fig. 11.5). It was subsequently shown that these compounds are ubiquitous among plant species and BRs are present in all parts of the plant but their levels are higher in pollen and seeds. As other PGRs, BRs are involved in many mechanisms of development, photomorphogenesis, leaf senescence, and resistance to stress. BRs have the ability to regulate cell cycle and consequently cell division. BRs have sometimes been used in agriculture to increase production of crops, but up to now, theirs applications to fields is poorly described. BR postharvest treatment induces tomato fruit ripening through stimulation of ethylene biosynthesis (Zhu et al. 2015a, b).

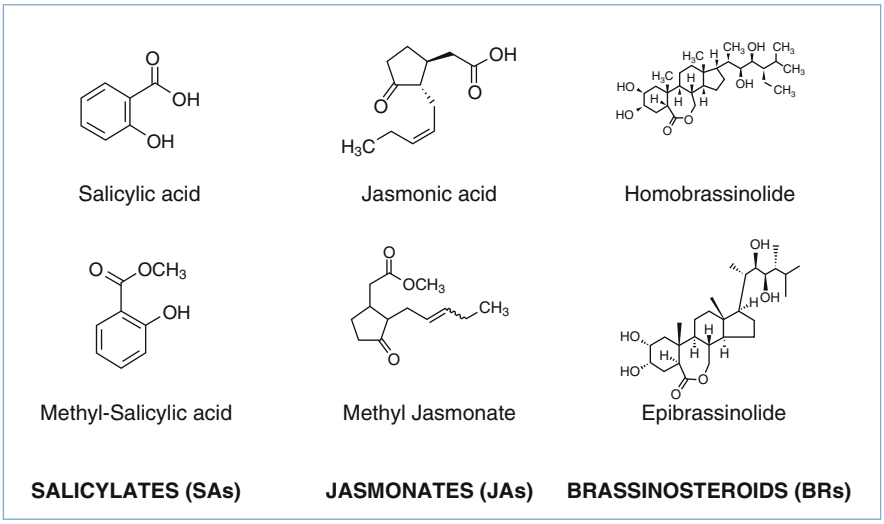


Fig. 11.5 Structures of salicylic acid and methyl-salicylate, Jasmonate, and methyl jasmonate and two brassinolides

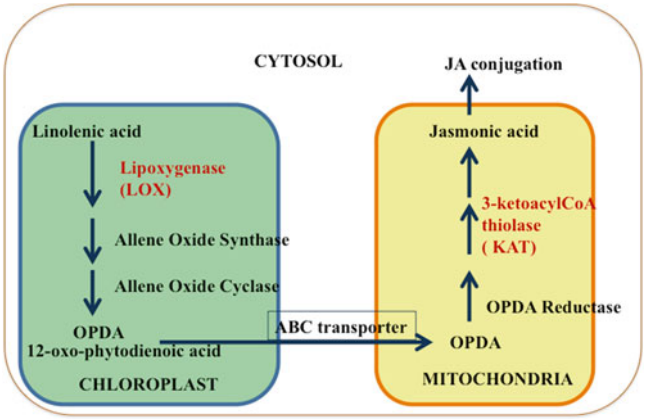


Fig. 11.6 Jasmonic acid biosynthesis in plant cell

Conclusion

In postharvest, PGR may be used to extend shelf-life, to delay senescence, to control ripening, and to limit disease development. These PGRs have also been found to decrease fungal infections, altering or not the quality of the fresh product. The effects of any given PGR depend on many factors as concentration, levels of other endogenous PGR, environmental conditions, signaling factors, or sensitivity of each

plant species or cultivar. Therefore, it is difficult to predict the action of exogenous application of PGR and several researches could be realized before extend the use of the molecules.

The environmental and health effects of PGR used for food production are a problem today. For example, in France, ethylene is the only authorized PGR for postharvest application and only banana and citrus. Thus, it is absolutely necessary to develop environmentally friendly new PGR accepted by the consumer for maintain and/or improve quality of fresh product. Gibberellins and salicylic acid and derivate might be good candidates for postharvest treatments.

Nevertheless, different approaches could be developed to manipulate endogenous hormone balance in the good way for the quality such as environmental factors and in particular using light-emitting diode (LED) irradiation technology.

I should like to recommend the excellent book entitled "Plants hormones: biosynthesis, signal transduction and Action!" by Davies (2010).

References

Abrams, S. R., P. A. Rose, A. J. Cutler, J. J. Balsevich, and M. K. Walker-Simmons (1997) 8 Methylene ABA: An effective and persistent analog of abscisic acid. *Plant Physiology 144*:89–97.

Alexander, L., & Grierson, D. (2002). Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. *Journal of Experimental Botany, 53*(377), 2039–2055. doi:10.1093/jxb/erf072.

Amorós, A., Zapata, P., Pretel, M. T., Botella, M. A., Almansa, M. S., & Serrano, M. (2004). Role of naphthalene acetic acid and phenothiol treatments on increasing fruit size and advancing fruit maturity in loquat. *Scientia Horticulturae, 101*(4), 387–398. doi:10.1016/j.scienta.2003.11.010. doi:http://dx.doi.org/.

An, J., Zhang, M., Lu, Q., & Zhang, Z. (2006). Effect of a prestorage treatment with 6-benzylaminopurine and modified atmosphere packaging storage on the respiration and quality of green asparagus spears. *Journal of Food Engineering, 77*(4), 951–957. doi:10.1016/j.jfoodeng.2005.08.024. http://dx.doi.org/.

Asghari, M., & Aghdam, M. S. (2010). Impact of salicylic acid on post-harvest physiology of horticultural crops. *Trends in Food Science and Technology, 21*(10), 502–509. doi:10.1016/j.tifs.2010.07.009. doi:http://dx.doi.org/.

Bal, E., & Celik, S. (2010). The effects of postharvest treatments of salicylic acid and potassium permanga- nate on the storage of kiwifruit. *Bulgarian Journal of Agricultural Science, 16*, 576–584.

Biton, E., Kobiler, I., Feygenberg, O., Yaari, M., Friedman, H., & Prusky, D. (2014). Control of alternaria black spot in persimmon fruit by a mixture of gibberellin and benzyl adenine, and its mode of action. *Postharvest Biology and Technology, 94*, 82–88. doi:10.1016/j.postharvbio.2014.03.009. http://dx.doi.org/.

Cantín, C. M., Fidelibus, M. W., & Crisosto, C. H. (2007). Application of abscisic acid (ABA) at veraison advanced red color development and maintained postharvest quality of 'Crimson Seedless' grapes. *Postharvest Biology and Technology, 46*(3), 237–241. doi:10.1016/j.postharvbio.2007.05.017. http://dx.doi.org/.

Chen, J.-Y., Wen, P.-F., Kong, W.-F., Pan, Q.-H., Zhan, J.-C., Li, J.-M., et al. (2006). Effect of salicylic acid on phenylpropanoids and phenylalanine ammonia-lyase in harvested grape berries. *Postharvest Biology and Technology, 40*(1), 64–72. doi:10.1016/j.postharvbio.2005.12.017. http://dx.doi.org/.

Chen, B., & Yang, H. (2013). 6-Benzylaminopurine alleviates chilling injury of postharvest cucumber fruit through modulating antioxidant system and energy status. *Journal of the Science of Food and Agriculture, 93*, 1915–1921. doi:10.1002/jsfa.5990.

Chen, J., Zou, X., Liu, Q., Wang, F., Feng, W., & Wan, N. (2014). Combination effect of chitosan and methyl jasmonate on controlling Alternaria alternata and enhancing activity of cherry tomato fruit defense mechanisms. *Crop Protection, 56*, 31–36. doi:10.1016/j.cropro.2013.10.007. http://dx.doi.org/.

Concha, C. M., Figueroa, N. E., Poblete, L. A., Oñate, F. A., Schwab, W., & Figueroa, C. R. (2013). Methyl jasmonate treatment induces changes in fruit ripening by modifying the expression of several ripening genes in Fragaria chiloensis fruit. *Plant Physiology and Biochemistry, 70*, 433–444. doi:10.1016/j.plaphy.2013.06.008. http://dx.doi.org/.

Costa, M. L., Civello, P. M., Chaves, A. R., & Martínez, G. A. (2005). Effect of ethephon and 6-benzylaminopurine on chlorophyll degrading enzymes and a peroxidase-linked chlorophyll bleaching during post-harvest senescence of broccoli (Brassica oleracea L.) at 20 °C. *Postharvest Biology and Technology, 35*(2), 191–199. doi:10.1016/j.postharvbio.2004.07.007. http://dx.doi.org/.

Davies, P. J. (2010) Plant Hormones: Biosynthesis, Signal Transduction, Action!. p. 801pp Springer, Dordrecht, The Netherlands

Ding, Y., Sheng, J., Li, S., Nie, Y., Zhao, J., Zhu, Z., et al. (2015). The role of gibberellins in the mitigation of chilling injury in cherry tomato (Solanum lycopersicum L.) fruit. *Postharvest Biology and Technology, 101*, 88–95. doi:10.1016/j.postharvbio.2014.12.001. http://dx.doi.org/.

Divya, P., Puthusseri, B., & Neelwarne, B. (2014). The effect of plant regulators on the concentration of carotenoids and phenolic compounds in foliage of coriander. *LWT - Food Science and Technology, 56*(1), 101–110. doi:10.1016/j.lwt.2013.11.012. http://dx.doi.org/.

Ferrara, G., Mazzeo, A., Matarrese, A., Pacucci, C., Pacifico, A., Gambacorta, G., et al. (2013). Application of Abscisic Acid (S-ABA) to 'Crimson Seedless' Grape Berries in a Mediterranean Climate: Effects on Color, Chemical Characteristics, Metabolic Profile, and S-ABA Concentration. *Journal of Plant Growth Regulation, 32*(3), 491–505. doi:10.1007/s00344-012-9316-2.

Figueroa, C. R., Opazo, M. C., Vera, P., Arriagada, O., Díaz, M., & Moya-León, M. A. (2012). Effect of postharvest treatment of calcium and auxin on cell wall composition and expression of cell wall-modifying genes in the Chilean strawberry (Fragaria chiloensis) fruit. *Food Chemistry, 132*(4), 2014–2022. doi:10.1016/j.foodchem.2011.12.041.

Flaishman, M. A., Shargal, A., & Stern, R. A. (2001). The synthetic cytokinin CPPU increases fruit size and yield of 'Spadona' and 'Costia' pear (Pyrus communis L.). *Journal of Horticultural Science and Biotechnology, 76*, 145–149.

Flores, G., Blanch, G.P., Ruiz del Castillo, M.L. (2015). Postharvest treatment with (−) and (+)-methyl jasmonate stimulates anthocyanin accumulation in grapes. LWT: Food Science and Technology. (0) doi:http://dx.doi.org/10.1016/j.lwt.2014.12.033.

Gambetta, G., Mesejo, C., Martínez-Fuentes, A., Reig, C., Gravina, A., & Agustí, M. (2014). Gibberellic acid and norflurazon affecting the time-course of flavedo pigment and abscisic acid content in 'Valencia' sweet orange. *Scientia Horticulturae, 180*, 94–101. doi:10.1016/j.scienta.2014.10.021. http://dx.doi.org/.

Garcia-Mata, C., & Lamattina, L. (2007). Abscisic acid (ABA) inhibits light-induced stomatal opening through calcium- and nitric oxide-mediated signaling pathways. *Nitric Oxide, 17*(3–4), 143–151.

Gianfagna, T.J. (1995) Use of natural and synthetic growth regulators, In: P.J. Davis, ed, Plant hormones: Physiology, biochemistry, and molecular biology. *Kluwer Academic Publishers, Boston*, 762–766.

Grossmann, K. and J. Jung. (1984) Influence of terpenoid analogues of abscisic acid on stomatal movement and leaf senescence. *Journal of Agronomy and Crop Science 153*:14–22.

Hayata, Y., Niimi, Y., Inoue, K., & Kondo, S. (2000). CPPU and BA, with and without pollination, affect set, growth, and quality of muskmelon fruit. *HortScience, 35*, 868–870.

Huang, H., & Jiang, Y. (2012). Effect of plant growth regulators on banana fruit and broccoli during storage. *Scientia Horticulturae, 145*, 62–67. doi:10.1016/j.scienta.2012.07.025. http://dx.doi.org/.

Huang, R.-H., Liu, J.-H., Lu, Y.-M., & Xia, R.-X. (2008). Effect of salicylic acid on the antioxidant system in the pulp of 'Cara cara' navel orange (Citrus sinensis L. Osbeck) at different storage temperatures. *Postharvest Biology and Technology, 47*(2), 168–175. doi:10.1016/j.postharvbio.2007.06.018. http://dx.doi.org/.

Khader, S. E. S. A. (1991). Effect of preharvest application of GA3 on postharvest behaviour of mango fruits. *Scientia Horticulturae, 47*(3–4), 317–321. doi:10.1016/0304-4238(91)90014-P. http://dx.doi.org/.

Khader, S. E. S. A., Singh, B. P., & Khan, S. A. (1988). Effect of GA3 as a post-harvest treatment of mango fruit on ripening, amylase and peroxidase activity and quality during storage. *Scientia Horticulturae, 36*(3–4), 261–266. doi:10.1016/0304-4238(88)90060-X. http://dx.doi.org/.

Kim, J. G., Takami, Y., Mizugami, T., Beppu, K., Fukuda, T., & Kataoka, I. (2006). CPPU application on size and quality of hardy kiwifruit. *Scientia Horticulturae, 110*, 219–222.

Kobiler, I., Shalom, Y., Roth, I., Akerman, M., Vinokur, Y., Fuchs, Y., et al. (2001). Effect of 2,4-dichlorophenoxyacetic acid on the incidence of side and stem end rots in mango fruits. *Postharvest Biology and Technology, 23*(1), 23–32. doi:10.1016/S0925-5214(01)00092-8. http://dx.doi.org/.

Korasick, D. A., Enders, T. A., & Strader, L. C. (2013). Auxin biosynthesis and storage forms. *Journal of Experimental Botany, 64*(9), 2541–2555. doi:10.1093/jxb/ert080.

Koukounaras, A., Siomos, A. S., & Sfakiotatis, E. (2010). Effects of 6-BA treatmentson yellowingandquality of storedrocket (Erucasativamill.) leaves. *Journal of Food Quality, 33*, 768–779. doi:10.1111/j.1745-4557.2010.00354.x.

Kucuker, E., Ozturk, B., Celik, S. M., & Aksit, H. (2014). Pre-harvest spray application of methyl jasmonate plays an important role in fruit ripening, fruit quality and bioactive compounds of Japanese plums. *Scientia Horticulturae, 176*, 162–169. doi:10.1016/j.scienta.2014.07.007. http://dx.doi.org/.

Lafuente, M. T., & Sala, J. M. (2002). Abscisic acid levels and the influence of ethylene, humidity and storage temperature on the incidence of postharvest rindstaning of 'Navelina' orange (Citrus sinensis L. Osbeck) fruit. *Postharvest Biology and Technology, 25*(1), 49–57. doi:10.1016/S0925-5214(01)00162-4. http://dx.doi.org/.

Lehmann, T., Hoffmann, M., Hentrich, M., & Pollmann, S. (2010). Indole-3-acetamide-dependent auxin biosynthesis: A widely distributed way of indole-3-acetic acid production? *European Journal of Cell Biology, 89*(12), 895–905. doi:10.1016/j.ejcb.2010.06.021. http://dx.doi.org/.

Leng, P., Yuan, B., & Guo, Y. (2014). The role of abscisic acid in fruit ripening and responses to abiotic stress. *Journal of Experimental Botany, 65*(16), 4577–4588. doi:10.1093/jxb/eru204.

Lers, A., Jiang, W. B., Lomanies, E., & Aharoni, N. (1998). Gibberellic acid and CO2 additive effect in retarding postharvest senescence of parsley, J. *Food Science, 63*, 66–68.

Lichanporn, I., & Techavuthiporn, C. (2013). The effects of nitric oxide and nitrous oxide on enzymatic browning in longkong (Aglaia dookkoo Griff.). *Postharvest Biology and Technology, 86*, 62–65. doi:10.1016/j.postharvbio.2013.06.021. http://dx.doi.org/.

Liu, X., Shiomi, S., Nakatsuka, A., Kubo, Y., Nakamura, R., & Inaba, A. (1999). Characterization of Ethylene Biosynthesis Associated with Ripening in Banana Fruit. *Plant Physiology, 121*(4), 1257–1265. doi:10.1104/pp. 121.4.1257.

Lurie, S., & Crisosto, C. H. (2005). Chilling injury in peach and nectarine. *Postharvest Biology and Technology, 37*(3), 195–208. doi:10.1016/j.postharvbio.2005.04.012. http://dx.doi.org/.

Manjunatha, G., Lokesh, V., & Neelwarne, B. (2010). Nitric oxide in fruit ripening: Trends and opportunities. *Biotechnology Advances, 28*(4), 489–499. doi:10.1016/j.biotechadv.2010.03.001.

Mano, Y., & Nemoto, K. (2012). The pathway of auxin biosynthesis in plants. *Journal of Experimental Botany*. doi:10.1093/jxb/ers091.

Marzouk, H. A., & Kassem, H. A. (2011). Improving yield, quality, and shelf life of Thompson seedless grapevine by preharvest foliar applications. *Scientia Horticulturae, 130*(2), 425–430. doi:10.1016/j.scienta.2011.07.013. http://dx.doi.org/.

McAtee, P., Karim, S., Schaffer, R., & David, K. (2013). A dynamic interplay between phytohormones is required for fruit development, maturation, and ripening. *Frontiers in Plant Science, 4*, 79. doi:10.3389/fpls.2013.00079.

Mworia, E. G., Yoshikawa, T., Yokotani, N., Fukuda, T., Suezawa, K., Ushijima, K., et al. (2010). Characterization of ethylene biosynthesis and its regulation during fruit ripening in kiwifruit, Actinidia chinensis 'Sanuki Gold'. *Postharvest Biology and Technology, 55*(2), 108–113. doi:10.1016/j.postharvbio.2009.08.007. http://dx.doi.org/.

Okamoto, M., Peterson, F. C., Defries, A., Park, S.-Y., Endo, A., Nambara, E., et al. (2013). Activation of dimeric ABA receptors elicits guard cell closure, ABA-regulated gene expression, and drought tolerance. *Proceedings of the National Academy of Sciences, 110*(29), 12132–12137. doi:10.1073/pnas.1305919110.

Ortolá, A. G., Monerri, C., & Guardiola, J. L. (1991). The use of naphthalene acetic acid as a fruit growth enhancer in Satsuma mandarin: a comparison with the fruit thinning effect. *Scientia Horticulturae, 47*(1–2), 15–25. doi:10.1016/0304-4238(91)90023-R. http://dx.doi.org/.

Pompodakis, N. E., & Joyce, D. C. (2003). Abscisic acid analogue effects on the vase life and leaf crisping of cut Baccara roses. *Australian Journal of Experimental Agriculture, 43*(4), 425–428. doi:10.1071/EA02036. http://dx.doi.org/.

Puli, M. R., & Raghavendra, A. S. (2012). Pyrabactin, an ABA agonist, induced stomatal closure and changes in signalling components of guard cells in abaxial epidermis of Pisum sativum. *Journal of Experimental Botany, 63*(3), 1349–1356. doi:10.1093/jxb/err364.

Raban, E., Kaplunov, T., Zutahy, Y., Daus, A., Alchanatis, V., Ostrovsky, V., et al. (2013). Rachis browning in four table grape cultivars as affected by growth regulators or packaging. *Postharvest Biology and Technology, 84*, 88–95. doi:10.1016/j.postharvbio.2013.03.021. http://dx.doi.org/.

Ramakrishna, M., Haribabu, K., & Purushotham, K. (2002). Effect of post-harvest application of growth regulators on storage behavior of papaya (Carica papaya L.). *Journal of Food Science and Technology, 39*(6), 657–659.

Ranjbaran, E., Sarikhani, H., Bakhshi, D., & Pouya, M. (2011). Investigation of Salicylic Acid Application to Reduce Postharvest Losses in Stored 'Bidaneh Ghermez' Table Grapes. *International Journal of Fruit Science, 11*(4), 430–439. doi:10.1080/15538362.2011.630591.

Romero, P., Rodrigo, M. J., & Lafuente, M. T. (2013). Differential expression of the Citrus sinensis ABA perception system genes during postharvest fruit dehydration. *Postharvest Biology and Technology, 76*, 65–73. doi:10.1016/j.postharvbio.2012.09.010. http://dx.doi.org/.

Sakakibara, H. (2006). Cytokinins: activity, biosynthesis, and translocation. *Annual Review of Plant Biology, 57*, 431–449.

Salvador, A., Sdiri, S., Navarro, P., Monterde, A., & Martínez-Jávega, J. M. (2010). The use of auxins to maintain postharvest quality of citrus fruit. *Acta Horticulturae, 877*, 671–677.

Schröder, M., Link, H., & Bangerth, K. F. (2013). Correlative polar auxin transport to explain the thinning mode of action of benzyladenine on apple. *Scientia Horticulturae, 153*, 84–92. doi:10.1016/j.scienta.2013.02.001. http://dx.doi.org/.

Schubert, J., K. Roser, K. Grossmann, H. Sauter, and J. Jung. (1990) Transpiration-inhibiting abscisic acid analogs. *Journal of Plant Growth Regulation 10*:27–32.

Sdiri, S., Navarro, P., Monterde, A., Benabda, J., & Salvador, A. (2012). New degreening treatments to improve the quality of citrus fruit combining different periods with and without ethylene exposure. *Postharvest Biology and Technology, 63*(1), 25–32. doi:10.1016/j.postharvbio.2011.08.005. http://dx.doi.org/.

Sdiri, S., Navarro, P., & Salvador, A. (2013). Postharvest application of a new growth regulator reduces calyx alterations of citrus fruit induced by degreening treatment. *Postharvest Biology and Technology, 75*, 68–74. doi:10.1016/j.postharvbio.2012.08.004. http://dx.doi.org/.

Seo, M., & Koshiba, T. (2002). Complex regulation of ABA biosynthesis in plants. *Trends in Plant Science, 7*(1), 41–48.

Serrano, M., Martínez-Romero, D., Zuzunaga, M., Riquelme, F., & Valero, D. (2004). Calcium, Polyamine and Gibberellin Treatments to Improve Postharvest Fruit Quality. In S. Jain (Ed.), *Dris R* (pp. 55–68). Springer Netherlands: Production Practices and Quality Assessment of Food Crops. doi:10.1007/1-4020-2535-1_3.

Simon, S., & Petrášek, J. (2011). Why plants need more than one type of auxin. *Plant Science, 180*(3), 454–460. doi:10.1016/j.plantsci.2010.12.007. http://dx.doi.org/.

Singh, S. P., Saini, M. K., Singh, J., Pongener, A., & Sidhu, G. S. (2014). Preharvest application of abscisic acid promotes anthocyanins accumulation in pericarp of litchi fruit without adversely affecting postharvest quality. *Postharvest Biology and Technology, 96*, 14–22. doi:10.1016/j.postharvbio.2014.05.005. http://dx.doi.org/.

Stern, R. A., Flaishman, M., Applebaum, S., & Ben-Arie, R. (2007a). Effect of synthetic auxins on fruit development of 'Bing' cherry (Prunus avium L.). *Scientia Horticulturae, 114*(4), 275–280. doi:10.1016/j.scienta.2007.07.010. http://dx.doi.org/.

Stern, R. A., Flaishman, M., & Ben-Arie, R. (2007b). Effect of synthetic auxins on fruit size of five cultivars of Japanese plum (Prunus salicina Lindl.). *Scientia Horticulturae, 112*(3), 304–309. doi:10.1016/j.scienta.2006.12.032. http://dx.doi.org/.

Suttle, J. (2003). Auxin-induced sprout growth inhibition: Role of endogenous ethylene. *American Journal of Potato Research, 80*(5), 303–309. doi:10.1007/BF02854314.

Thomas, S. G., Rieu, I., & Steber, C. M. (2005). Gibberellin metabolism and signaling. In G. Litwack (Ed.), *Vitamins and Hormones* (pp. 289–337). London: Elsevier.

Tivendale, N. D., Ross, J. J., & Cohen, J. D. (2014). The shifting paradigms of auxin biosynthesis. *Trends in Plant Science, 19*(1), 44–51. doi:10.1016/j.tplants.2013.09.012. http://dx.doi.org/.

Vargas, A., & Lopez, J. A. (2011). Effect of dose rate, application method and commercial formulations of GA3 on banana (Musa AAA)fruit greenlife. Global Science Books. *Fresh Produce, 5*(1), 51–55.

Wang, K., Jin, P., Han, L., Shang, H., Tang, S., Rui, H., et al. (2014). Methyl jasmonate induces resistance against Penicillium citrinum in Chinese bayberry by priming of defense responses. *Postharvest Biology and Technology, 98*, 90–97. doi:10.1016/j.postharvbio.2014.07.009. http://dx.doi.org/.

Wang, B., Wang, J., Liang, H., Yi, J., Zhang, J., Lin, L., et al. (2008). Reduced chilling injury in mango fruit by 2,4-dichlorophenoxyacetic acid and the antioxidant response. *Postharvest Biology and Technology, 48*(2), 172–181. doi:10.1016/j.postharvbio.2007.10.005. http://dx.doi.org/.

Wasternack, C. (2014). Action of jasmonates in plant stress responses and development: Applied aspects. *Biotechnology Advances, 32*(1), 31–39. doi:10.1016/j.biotechadv.2013.09.009. http://dx.doi.org/.

Woodward, A. W., & Bartel, B. (2005). Auxin: Regulation, Action, and Interaction. *Annals of Botany, 95*(5), 707–735. doi:10.1093/aob/mci083.

Yang, S. F., & Hoffman, N. E. (1984). Ethylene biosynthesis and its regulation in higher plants. *Annual Review of Plant Physiology, 35*, 155–189.

Zaharah, S. S., Singh, Z., Symons, G. M., & Reid, J. B. (2013). Mode of action of abscisic acid in triggering ethylene biosynthesis and softening during ripening in mango fruit. *Postharvest Biology and Technology, 75*, 37–44. doi:10.1016/j.postharvbio.2012.07.009. http://dx.doi.org/.

Zhang, C., & Whiting, M. D. (2011). Improving 'Bing' sweet cherry fruit quality with plant growth regulators. *Scientia Horticulturae, 127*(3), 341–346. doi:10.1016/j.scienta.2010.11.006. http://dx.doi.org/.

Zhu, T., Tan, W.-R., Deng, X.-G., Zheng, T., Zhang, D.-W., & Lin, H.-H. (2015a). Effects of brassinosteroids on quality attributes and ethylene synthesis in postharvest tomato fruit. *Postharvest Biology and Technology, 100*, 196–204. doi:10.1016/j.postharvbio.2014.09.016. http://dx.doi.org/.

Zhu, F., Yun, Z., Ma, Q., Gong, Q., Zeng, Y., Xu, J., et al. (2015b). Effects of exogenous 24-epibrassinolide treatment on postharvest quality and resistance of Satsuma mandarin (Citrus unshiu). *Postharvest Biology and Technology, 100*, 8–15. doi:10.1016/j.postharvbio.2014.09.014. http://dx.doi.org/.

Zoffoli, J. P., Latorre, B. A., & Naranjo, P. (2009). Preharvest applications of growth regulators and their effect on postharvest quality of table grapes during cold storage. *Postharvest Biology and Technology, 51*(2), 183–192. doi:10.1016/j.postharvbio.2008.06.013. http://dx.doi.org/.

Chapter 9
Active Carbohydrates

Filomena Nazzaro, Florinda Fratianni, Autilia Cozzolino, Tiziana Granese, and Raffaele Coppola

Packaging offers control and protection of foods from chemical, physical, and biological adverse conditions during their distribution and storage. In last decades, different functional and active packaging systems were created, with meliorated characteristics and properties, as answer to consumer requirements (Fig. 9.1).

Global market embraced the increasing consumer demand for more natural foods meeting the criteria of high quality and safety. Thus, lead companies and researchers are involved to explore different ways to improve their productivity in terms of maintaining quality, freshness, and food safety, for example exploring the possibility of using new natural sources as antimicrobial agents (Nazzaro et al. 2009) and/or increasing the use of sustainable materials in food packaging (Mahalik and Nambiar 2010). Thus, several studies are in progress and different materials are experimented to avoid the proliferation of microorganisms, as well as the loss of organoleptic and/or nutritional qualities of food.

Coatings of different composition have been tested and used to prolong storage life of fresh and highly perishable products (for example, fruit, meat and vegetables), retarding their senescence, reducing and/or slowing all metabolic processes, retarding microbial growth, and forming a protective barrier to reduce respiration and transpiration rates (Fisk et al. 2008; Gonzalez-Aguilar et al. 2008; Vargas et al. 2008; Antunes et al. 2012; Dhall 2013; Valencia-Chamorro et al. 2013; Fratianni et al. 2010). Carbohydrates, such as alginate, pectin, chitosan, starch, and hemicelluloses, can represent an interesting area of application, also in the field of food technology. In most cases they are capable to respond, in terms of economy of cost, naturalness,

F. Nazzaro (✉) • F. Fratianni • A. Cozzolino • T. Granese
Institute of Food Science, Via Roma 64, Avellino 83100, Italy
e-mail: mena@isa.cnr.it

R. Coppola
Institute of Food Science, Via Roma 64, Avellino 83100, Italy

DiAAA, University of Molise, Via De Sanctis, Campobasso 86100, Italy
e-mail: mena@isa.cnr.it

M.W. Siddiqui et al. (eds.), *Postharvest Management Approaches for Maintaining Quality of Fresh Produce*, DOI 10.1007/978-3-319-23582-0_9

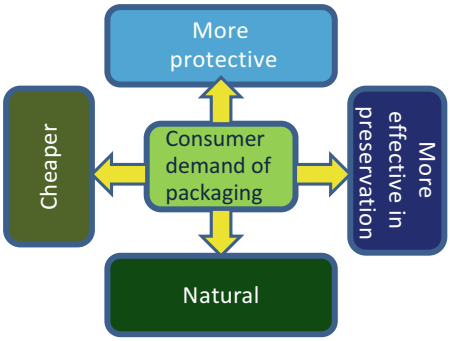


Fig. 9.1 General requirements by consumers about packaging attributes

efficiency, as well as protective materials suitable to be used for packaging (Dutta et al. 2009; Hansen and Plackett 2008; Jiménez et al. 2012). In this chapter, we briefly shall describe some carbohydrates, focusing our attention on their biological activity, in particular their antibacterial and antifungal activity, or regarding their prebiotic effectiveness.

Chitosan

Chitosan is a copolymer giving rise from chitin through a process of deacetylation in an alkaline media (Abdou et al. 2007). Structurally, it consists of β-(1–4)-2-acetamido-d-glucose and β-(1–4)-2-amino-D-glucose units, with this latter exceeding 60 %. Its importance is based mainly on its antimicrobial properties, cationicity, and its film-forming properties. The ability of chitosan to act as a food preservative of natural origin has been reported (Coma et al. 2002, 2003; Durango et al. 2006; Han et al. 2004; Park et al. 2004a, b; Ribeiro et al. 2007; Domard 2001). Chitosan-based films possess a selective permeability to gasses (CO_2 and O_2) and good mechanical properties. Antimicrobial and antifungal properties of such carbohydrate can give rise from its polycationic nature (Kim et al. 2003), mediated by the electrostatic forces forming between the protonated amino group (NH2) present in the chitosan molecule and the negative residues of microbial cell surface (Tsai et al. 2002). Antimicrobial activity of chitosan is also directly improved by the number of its protonated amino groups, the number of protonated amino groups, and the degree of deacetylation (DD) (Liu et al. 2004). In some cases, such features do not affect the antimicrobial activity of chitosan (Qin et al. 2006). In other cases, protonated NH3+ results as not the predominant factor of antimicrobial activity of chitosan; neither the DD value can affect such property (Park et al. 2004a, b). As water-soluble form, chitosan is ineffective in inhibiting the bacterial growth; however, its own state let it to penetrate within bacterial cell, where physiological pH is around neutral; in such case, water-insoluble chitosan molecules can precipitate, and stack on the microbial cell surface, thus forming an resistant layer surrounding the cell which blocks the channels, which are essential for living cells, and can cause a change in the morphology and structure of microbial cells, until to its death.

Listeria monocytogenes is a Gram-positive rod that can cause illness in a variety of food products (Beverlya et al. 2008). Eating foods contaminated with *L. monocytogenes* normally causes the disease listeriosis, more serious for elderly adults and adults with compromised immune systems and can cause meningitis (Levine et al. 2001). In pregnant women, such disease may cause spontaneous abortions or stillborn babies. Roberts and Greenwood (2003) has studied the antimicrobial effect of chitosan (with low and high molecular weight), as an edible film, that was dissolved in lactic acid or acetic acid against *L. monocytogenes* on RTE roast beef. This study showed that the acetic acid chitosan coating was more effective in reducing bacterial counts than the lactic acid chitosan coating. The study indicated also that chitosan coatings could be used to control this microorganism on the surface of roast beef. The molecular weight and amino group of chitosan can affect its antibacterial activity, which is different if related to Gram-positive bacteria and Gram-negative bacteria (Liu et al. 2001). Its activity is also effective against fungi (Sebti et al. 2005; Tsai et al. 2000), acting on the synthesis of some fungal enzymes (Bautista-Banos et al. 2006), inhibiting their growth or inducing morphological changes, structural alterations, and molecular disorganization of the fungal cells. The antibacterial activity of chitosan was successfully tested, for example, against Gram-negative (*Escherichia coli*, *Pseudomonas fluorescens*, *Salmonella typhimurium*, *Vibrio parahaemolyticus*) and Gram-positive bacteria (*L. monocytogenes*, *Bacillus megaterium*, *Bacillus cereus*, *Staphylococcus aureus*, *Lactobacillus plantarum*, *Lactobacillus brevis*, *and Lactobacillus bulgaricus*) (No et al. 2002), showing stronger bactericidal effects for Gram-positive bacteria than Gram-negative bacteria. In other cases (Devlieghere et al. 2004) Gram-negative bacteria were more susceptible; on the other hand, the sensitivity of the Gram-positive bacteria was highly variable: *Brochothrix thermosphacta* and *B. cereus* were very sensitive respect to *L. monocytogenes* and some lactic acid bacteria. Yeasts, such as *Candida lambica* and *Cryptococcus humicolus*, showed an intermediate sensitivity, also depending on pH. The action of chitosan was evaluated with or without the addition of starch in the development of polymers: without gelatinized starch, chitosan was capable to retard the growth of *C. lambica*; such action was practically not existent in the presence of high amount of starch (Devlieghere et al. 2004), but enhanced by the concurrent effect of electron beam (EB) on chitosan (Zhai et al. 2004), or by the incorporation of nisin (N) and garlic oil (GO), against *E. coli*, *S. aureus*, *S. typhimurium*, *L. monocytogenes*, and *B. cereus* (common meat product contaminants) (Pranoto et al. 2005). Chitosan film incorporated with potassium sorbate (KS) exhibited antimicrobial activity against *S. aureus*, *L. monocytogenes*, and *B. cereus*. Li and Xie (2004) studied the mechanical, physical, and antimicrobial properties of Konjac glucomannan–chitosan–nisin ternary blend film. Ziani et al. (2009) ascertained the antifungal properties of films and solutions based on chitosan with different molecular weight and at different concentrations; in particular, the antifungal activity was investigated against *Alternaria alternata*, *Rhizopus oryzae*, and *Aspergillus niger*. Results indicated that the antifungal activity of the different treatment is species dependent and solution dependent. Antimicrobial activity of edible coating solutions based on chitosan and blends of chitosan–starch (obtained from tapioca) with or without potassium sorbate (KS) addition against *Lactobacillus* spp.

and *Zygosaccharomyces bailii* was affected by the presence of KS and/or starch (Vasconez et al. 2009): at molecular level the chitosan antibacterial action could suffer the antagonist effect of the presence of KS and/or tapioca starch, which interactions might inhibit its amino groups bonding to the cell membrane, decreasing the global antimicrobial activity. The concurrent presence of potato starch, KS, and chitosan in the biodegradable film affected its antimicrobial activity against some pathogens, such as *E. coli* and *S. aureus* (Shen et al. 2010). The sweet potato film control did not show any inhibitory effect on *E. coli* or *S. aureus*. Films incorporated with potassium sorbate (KS) ≥15 % or chitosan ≥5 % exhibited anti-*E. coli* effect, whereas the incorporation of chitosan at ≥10 % was effective in suppressing the growth of *S. aureus*. Such results are of particular importance, as *E. coli* and *S. aureus* can be found in several food products, and they are also the human pathogens causing the most economically important food-borne diseases throughout the world (Elizaquıvel and Aznar 2008).

Oligosaccharides

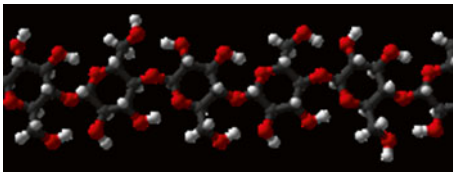
Oligosaccharides are saccharidic compounds, with a variable number of sugar units, generally from 2 to 10, which are considered as low-molecular-weight carbohydrates (Sako et al. 1999; Mussatto and Mancilha 2007). Oligosaccharides are active carbohydrates, considering their functional properties for health purposes. Different oligofructosides or fructans, at higher molecular weight, are produced from many plants and microorganisms through a transfructosylation activity. Depending on the enzyme sources, they have different linkages; fructosyltransferase derived from fungi *Aureobasidium pullulans* and *Aspergillus niger* are able to produce only the lF-type FOS; enzymes giving rise from *Claviceps purpurea* and asparagus produce both lF- and 6G-type oligofructosides (active carbohydrates FOS). Fructooligosaccharides can be distinguished in fructan, glucofructosan, and inulin-type oligosaccharides (active carbohydrates FOS). On the other hand, some scientists use the term fructooligosaccharides only to indicate [lF(1-β-D-fructofuranosyl), sucrose; GF, $n=2-$lo], excluding polyfructans and oligofructosides of different linkages such as neokestose, 6-kestose, and their derivatives. In fact, oligosaccharides have the capability to be selectively fermented in the colon, stimulating the intestinal microbiota and giving rise to important beneficial effects on the body. For this reason, they are also known as prebiotics (Moure et al. 2006; Gullon et al. 2011). Oligosaccharides with prebiotic properties have great appealing for food industry and research, also because they can be used, free or microencapsulated, as functional ingredients in the formulation or manufacturing of functional foods, such as beverages, fruit or vegetal juices, yogurt, dairy products, and ice cream (Barreteau et al. 2006; Mussatto and Mancilha 2007; Nazzaro et al. 2008). The main physiological benefits of these carbohydrates include: the stimulation of the intestinal microbiota; the production of short-chain fatty acids; the growth inhibition of certain pathogens, and the concurrent decrease of some gastrointestinal infections, and finally the lowering of intestinal pH value. They can also act for the

melioration of some dismetabolic status, decreasing insulin response and glucose uptake, and enhancing lipoprotein profile. The presence of prebiotic in the diet can contribute to a reduction of cancer risk too (Agheli et al. 1998; Qiang et al. 2009; Gobinath et al. 2010; Li et al. 2010). Some oligosaccharides demonstrate a certain antioxidant activity (Coelho et al. 2014). The most important oligosaccharides with prebiotic property are certainly fructooligosaccharides (FOS) and galactooligosaccharides (GOS). Recently other oligosaccharides, such as xylooligosaccharides (XOS), pecticoligosaccharides (POS), and agarooligosaccharide (Qiang et al. 2009; Li et al. 2010; Chen et al. 2013; Gullon et al. 2013; Kang et al. 2014), received attention due to prebiotic property. In addition, the concurrent presence of prebiotic and secondary metabolites of vegetal source can enhance the antioxidant properties of different probiotics, such as *Lactobacillus acidophilus* (Nazzaro et al. 2012a, b, c) or *L. plantarum* (Nazzaro et al. 2012a, b, c). The enzyme source of FOS synthesis can be split into two classes; one is plants such as asparagus, sugar beet, onion, and Jerusalem artichoke; the other is formed by FOS of bacterial and fungal origins, such as *Aspergillus*, *Aureobasidium*, *Arthrobacter*, and *Fusarium* sp. FOS can be readily recognized as inulin-type oligosaccharides of o-fructose attached by β-(2 → 1) linkages carrying a o-glucosyl residue at the end of the chain. They constitute a series of homologous oligosaccharides derived from sucrose usually represented by the formula GF. Different interesting properties characterize FOS. Low sweetness intensity is their first feature, so that they are only about one-third as sweet as sucrose. Such property is quite useful in the various kinds of foods, where use of sucrose is restricted, due to its high sweetness. FOS are calorie free; that is, they are not utilized as an energy source in the body; thus they are safe for diabetics. Thirdly, they are noncariogenic. Finally, FOS stimulate the growth of the bifidobacteria and discourage the growth of potentially putrefactive microorganisms with a tendency to cause diarrhea. However, FOS offer, in addition, important physiological properties, such as the effectiveness in decreasing the levels of serum cholesterol, phospholipid, and triglyceride (active carbohydrates FOS). One of the most important FOS is represented by inulin, present in several vegetables.

The importance of inulin is mainly related to its prebiotic activity. A prebiotic is a non digestible food ingredient that beneficially affects the host, stimulating the growth and/or activity of selected bacteria in the colon, and improving the host's health (Gibson and Roberfroid 1995). On the other hand, a prebiotic may repress the growth of pathogens (Roberfroid 2001). Inulin and oligofructose can be digested only through bacterial activity, which may affect the composition of human gut flora through specific fermentation; this gives rise to a community predominated by bifidobacteria (Hidaka et al. 1986; Wang and Gibson 1993). Since 1995 Gibson et al. demonstrated that oligofructose and inulin can modify the in vivo composition of the microbiota, not only by improving the growth of lactic acid bacteria with healthy effect, but also reducing the count of bacteroids, fusobacteria, and clostridia; such effect could be due to several factors, such as the production of bacteriocins by bifidobacteria and lactobacilli, the successful competition for substrates or adhesion sites on the gut epithelium, and the stimulation of the immune system (Gibson and Roberfroid 1995).

Cellulose

Cellulose is one of the most abundant natural biopolymers, occurring primarily in wood, cotton, hemp, and other plant-based materials; its main role is to act as dominant reinforcing phase in plant structure. The following figure describes the structure of cellulose.



Plant fibers are composed of cellulose, hemicellulose, and lignin. Cellulose is ordered in microfibrils enclosed by the other two main components: hemicellulose and lignin (Bledzki and Gassan 1999). Microfibrils of cellulose can be found as intertwined microfibrils in the cell wall (2–20 nm diameter and 100 40,000 nm length depending on source), containing nanofibers with diameters of 5–50 nm and lengths until several millimeters, which are conformed by nanocrystalline domains and amorphous regions (Darder et al. 2007). The structure of cellulose is a linear carbohydrate polymer chain, composed of D-glucopyranose units joined together by β-1,4-glycosidic linkages. In a unit of cellulose, two chains are joined by hydrogen bonding to form a parallel conformation, which is called cellulose. These units are packed, side by side, to tangled microfibrils of cellulose, which also contain disordered or amorphous regions. The arrangement of the microfibrils in the primary wall is casual. Secondary cell walls of plants comprehend cellulose (40–80 %), hemicellulose (10–40 %), and lignin (from 5 to 25 %), where cellulose microfibrils are embedded in lignin. Hemicellulose is a highly branched polymer, when compared to the linearity of cellulose. Its structure contains a variety of sugar units, whereas cellulose contains only 1,4β-D-glucopyranose units and its degree of polymerization is 10–100 times lower than that of cellulose. Lastly, lignin is a complex hydrocarbon polymer, with both aliphatic and aromatic constituents (Soykeabkaew et al. 2008). Its molecules are biosynthesized as nano-sized fibers, called nanocellulose (NC), which are then assembled into fibers, films, walls, and so on. These cellulose nanofibers are with diameters of 5–50 nm and lengths of thousands of nanometers. The network of NC affects the product properties and its functionality. NC fibers are very interesting nanomaterials for production of lightweight and cheap nanocomposites, which on the other hand maintain a certain strength. Generally, NC takes place from the bio-formation of cellulose through bacteria, as well as through the dissolution of plant celluloses using shear forces in refiner techniques. Wood-derived NC can also be made by electrospinning from pulp solutions (Dufresne 1997) or through a planned acid hydrolysis of wood pulp (Beck-Candanedo et al. 2005). Cellulose nanofibers are more effective than their micro-sized counterparts in reinforcement of the polymeric skeleton: this is due to all interactions taking place between the nano-sized elements that form a percolated

network connected by hydrogen bonds, provided there is a good dispersion of the nanofibers in the matrix. NC reinforcements in the polymer matrix might give rise to value-added materials with notable performance and wide applications for the next generation of biodegradable materials, also for food purpose (packaging with antioxidant and antimicrobial features).

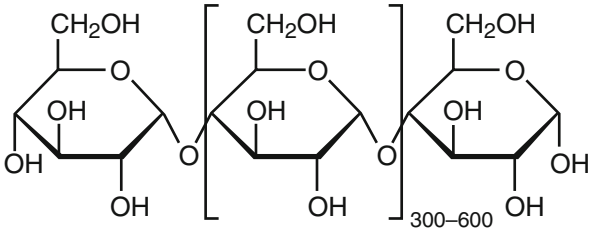
Among the nanostructured celluloses, cellulose-based nanofibers are very interesting, due to relatively easy fabrication via the electrospinning process. Several studies were carried to enhance the short- and long-term antimicrobial activity of nanostructured celluloses, including electrospinning and subsequent UV irradiation, followed by deacetylation, of CA containing AgNO3 against different Gram-negative (such as *E. coli*) and Gram-positive (such as *S. aureus*) pathogenic strains (Jang et al. 2014). Other studies highlighted the utility of enhancing the antimicrobial activity of cellulose fiber by covalently bonding β-cyclodextrin (β-CD) with cellulose fiber and including some antibiotics, such as ciprofloxacin hydrochloride. The structure revealed an excellent antibacterial activity against *E. coli* and *S. aureus*, and the inhibition efficiency against *E. coli* was higher than that against *S. aureus* at the same fiber content generally (Dong et al. 2014).

Recently, Hassan et al. (2015) prepared a new supramolecular metallo-terpyridine carboxymethyl cellulose derivatives with antimicrobial properties, in a one-step procedure, starting from carboxymethyl cellulose (CMC) which reacted with the Cu(BF4)2 complex of 4′-chloro[2,2′:6′,2″]terpyridine to generate the desired CMC–CuII–terpyridine derivative. Such matrix showed antimicrobial activity against Gram-positive bacteria (*S. aureus* and *Streptococcus thermophilus*), Gram-negative bacteria (*E. coli*), and yeast (*(Saccharomyces cerevisiae*). The minimum inhibitory concentration of the prepared metallo-terpyridine CMC derivative against the studied microorganisms ranged from 6 to 8 mg/L to achieve ≥90 % of microbial growth inhibition (Hassan et al. 2015). The incorporation of essential oils in NC can meliorate its antimicrobial activity. This was demonstrated, for example, by Dashipour et al. (2015), which included Zataria multiflora essential oil within carboxymethyl cellulose films, increasing their antioxidant properties and antimicrobial activity against *S. aureus*, *B. cereus*, *E. coli*, *P. aeruginosa*, and *Salmonella typhimurium*, as well as by Atef et al. (2015), which formulated agar-cellulose bionanocomposite films incorporated with savory essential oil, improving their antimicrobial activity against Gram-positive bacteria (*L. monocytogenes*, *S. aureus,* and *B. cereus*) and Gram-negative bacteria (*E. coli*), with superior action against the Gram-positive one.

Starch

Starch is a naturally occurring polysaccharide, the most abundant reserve of polysaccharides present in plants. Principal sources of starch are represented by cereals, legumes, tubers, roots, and seeds; it can be also found in fruits and pollens. Starch granules have different shapes and sizes, from spheres until irregular tubes. Such differences depend on starch source. The extraction of starch from its natural

source is easy and gives rise to a high pure compound, represented by a white, biodegradable, tasteless, and odorless power. Such features makes it as an appealing resource, both in human/animal feeding and for non-industrial purposes, such as the production of paper and pulp, bioethanol, or even adhesives. Chemically, starch is composed of two glucose residues, linked in different forms to build the two main starch polymers, amylose (AM) and amylopectin (AMP), which difference in structure and functionality affects the chemistry, and subsequently the technological, biotechnological, and biological properties of starch.



Basically, amylose is represented by a linear chain of glucose molecules, linked by α-1,4 linkages, with a molecular weight of 105–106 Da, usually located in the periphery of starch granule; in amylopectin, the main component of starch (70–80 %), glucose residues are also joined by α-1,6 linkages, giving rise to a polymer with a molecular weight of 107–109 Da. Amylopectin is composed of three types of glucose chains, A-, B-, and C-chains (Peat et al. 1952): a single C-chain carries one reducing end. B-chains are attached by their reducing end to the C-chain and/or to other B-chains. A-chains are the outermost chains and carry no chain themselves. Amylopectin is generally concentrated in the hilum of starch granule (Kasemsuwan and Jane 1994; Jane and Shen 1993). Lot of amylose molecules are structurally described as in the single helical state (Ring et al. 1985); only some, larger molecules are implicated in double helices with amylopectin (Kasemsuwan and Jane 1994). Amylose shows a high tendency to retrograde and produce tough gels and strong films. On the contrary, amylopectin, when dispersed in water, is more stable and produces soft gels and weak films (Perez and Bertoft 2010). Like other carbohydrates, starch shows antimicrobial activity. Biofilms of quinoa starch containing gold nanoparticles exhibited antimicrobial activity against *E. coli* and *S. aureus* (Pagno et al. 2015). Starch/ε-poly-L-lysine (ε-PL) composite films prepared by combining 4 % (w/v) gelatinized cornstarch and varying the level of ε-PL showed, on the other hand, effective inhibition against *E. coli* and *Bacillus subtilis*: the films containing 2 % (w/w) ε-PL effectively suppressed the growth of the tested microbes; however, they showed a low inhibitory effect on *Aspergillus niger* (Zhang et al. 2015). Kuorwel et al. (2014) investigated the antifungal activity of the antimicrobial (AM) agents linalool, carvacrol, and thymol incorporated in the coatings of starch-based films against *A. niger*, firstly on plate, and then examined on Cheddar cheese. On the solid media, all the AM films demonstrated a significant inhibitory effect against *A. niger* growth. The AM films containing 2.38 % (w/w) linalool, carvacrol, or thymol reduced the population of *A. niger* on the surface of Cheddar cheese by

1.8, 2.0, and 2.2 log CFU/g, respectively, after 35 days of storage at 15 °C. On the other hand, Resa et al. (2014) ascertained that natamycin and nisin supported on starch edible films for controlling mixed culture growth on model systems and Port Salut cheese. This demonstrates the utility of such materials also as packaging and preserving agents for food purposes.

Alginate

Alginate is an anionic polysaccharide giving rise from marine algae and composed of α-L-guluronate and β-D-mannuronate organized as linear homopolymeric and heteropolymeric blocks which can vary in composition and sequence, affecting the physical properties of alginate, such as viscosity of solution, strength, and friability of gel (Pawar and Edgar 2012; Azarakhsh et al. 2012; Gombotz and Wee 1998). Guluronate and mannuronate, two uronates with carboxylate groups at their C5 positions, are different for the configuration of the carboxylate groups. Alginate is an abundant reserve of biomaterials: about 300,000 tons of such material is produced or biosynthesized annually (Pawar and Edgar 2012). Alginate can be considered a food material with good potential to be used as a coating, due to its peculiarity to form strong gels with metal cations, creating tick aqueous solutions (Roopa and Bhattacharya 2008). The enzymatic depolymerization of alginate, catalyzed by alginate lyases, gives rise to the alginate oligosaccharides (AOs) (Kim et al. 2011; Wong et al. 2000). Such oligosaccharides have antioxidant activity, preventing lipid oxidation, and sometimes act as scavengers of hydroxyl and superoxide radicals (Zhao et al. 2012). Like other carbohydrates, alginate exhibits interesting antimicrobial activity, both if used as alone and when other components, such as essential oils, are incorporated into its matrix. Considering the potential benefits of edible coatings and films for storage of food materials, effect of edible calcium alginate film on shelf life of frozen lamb muscles was studied by Koushki et al. (2015). Microbial analyses including total microorganisms count and psychrophilic bacteria count were performed. Considering the role of psychrophilic bacteria in meat spoilage, results of the current research confirmed that calcium alginate films may be to some extent effective in shelf life extension of frozen lamb muscle. The antimicrobial properties of some essential oils (marjoram, clove, cinnamon, coriander, caraway, and cumin) included in alginate/clay nanocomposite films was assayed, The composite films, mainly when contained marjoram, exhibited activity against common pathogenic foodborne bacteria including *E. coli*, *S. aureus*, and *L. monocytogenes* (Alboofetileh et al. 2014). Also polyphenols, when incorporated in nanoparticles of alginate and/or chitosan are capable to exhibit antimicrobial activity. Whang et al. (2015) demonstrated the preservative effectiveness as antimicrobial agents of tea polyphenols/chitosan nanoparticles coated in alginate. Such nanoparticles had preservative effect on tilapia fillets and, in the last step of storage, were capable to retard microbial proliferation, if compared to the untreated ones.

Pectin

Pectin, one of the major components of the plant cell wall, is one of the most complex macromolecules present in nature (Jolie et al. 2010). The main industrial sources for pectin extraction are citrus and apple pomace (Videcoq et al. 2011). Chemically, pectin is poly a1-4-galacturonic acid, which exhibits different degrees of methylation of carboxylic acid residues and/or amidated polygalacturonic acids (Mishra et al. 2012). Methoxylated carboxyl groups give rise by esterification with methanol of carboxyl groups of galacturonic acid; amidated carboxyl group take place when some of the galacturonic acid are transformed with ammonia to carboxylic acid amide. Pectin can be classified as high methoxyl pectin (HMP) or low methoxyl pectin (LMP), according to the degree of esterification (DE) with methanol (Farris et al. 2009). HMP has over 50 % of their carboxyl groups esterified (DE > 50), while LMP have a DE < 50. The DE affects gelling properties of pectins. In this way, LMP forms gel in presence of multivalent ions: gel acts as a bridge between pairs of carboxyl groups of different pectin chains. On the other hand, HMP forms gel in acidic media with the addition of different sugars such as sucrose or glucose (Mishra et al. 2012; Videcoq et al. 2011). Three major pectic polysaccharides are extensively characterized: homogalacturonan (HGA), rhamnogalacturonan, and rhamnogalacturonan II (RG I and RG II) (Willats et al. 2001). Pectin is an ingredient used in food industry without limitation other than current good manufacturing practice; it is considered as generally recognized as safe (GRAS) by the Food and Drugs Administration and it is used in food especially as gelling, stabilizing, or thickening agent in products such as yogurt drinks, fruity milk drinks, jams, and ice cream (Laurent and Boulenguer 2003). One of the most important aspects of edible films is their ability to carry and release a variety of active compounds, including antioxidants, antimicrobial, flavorings, and antibrowning compounds (Falguera et al. 2011). The most active compounds incorporated in pectin edible films are used for their antimicrobial activity. Pectin-based edible films have been incorporated with several antimicrobial substances to obtain antimicrobial active packaging which is capable to prolong extend product shelf life of food, as well as to reduce the risk of pathogen growth on food surfaces. This is a type of packaging that, by controlling the diffusion of the antimicrobial agents from packaging matrix to the product, modifies the conditions surrounding the food; this can negatively affect the growth of pathogen microorganisms (Cagri et al. 2004). Antimicrobial compounds incorporated into pectin edible film give rise from natural sources. They must be generally recognized as safe (GRAS). Antimicrobials with such characteristics include bacteriocins, polyphenols, and essential oils. At present, the only bacteriocin licensed as GRAS food additive is nisin, commercially used as natural preservative (Acuña et al. 2011), and accepted also by the European Community laws (coded as E 234). Nisin was incorporated in pectin edible films and used to preserve (in combination with ionizing radiations) to control *L. monocytogenes* in turkey meat (Jin et al. 2009a, b), allowing, for a microbial reduction by 3.95 and 1.76 log CFU/cm^2, when used with or without the use of 1 kGy ionizing radiation, respectively. Jin et al. (2009a, b) tested pectin-PLA composite films incorporated with nisin against *L. monocytogenes* in three different

media, brain–heart infusion (BHI) broth, liquid egg white, and orange juice, obtaining a microbial reduction by 2.1, 4.5, and 3.7 log CFU/mL, respectively. Pectin edible films were used as carriers of plant essential oils. Edible films based on pectin and apple puree were incorporated with cinnamon, oregano, or lemongrass essential oils (Rojas-Graü et al. 2006), exhibiting antimicrobial activity against *E. coli*. Similarly, Du et al. (2009) tested the antimicrobial activity of allspice, garlic or oregano essential oils (incorporated in edible films based on pectin and tomato puree), against *E. coli*, *Salmonella enterica*, and *L. monocytogenes*. The results indicated that developed edible films presented antimicrobial activity against tested pathogens. Pure main components of essential oils, such as carvacrol, which is the main constituent of oregano and thyme essential oils, or cinnamaldehyde, the principal component of cinnamon essential oil, are effective as antimicrobials in active packaging made from pectin films: for example, edible films based on pectin and tomato puree incorporated with carvacrol exhibited antimicrobial activity against *E. coli* O157:H7 (Du et al. 2008). Cinnamaldehyde or mainly carvacrol, incorporated in edible films based on pectin and apple puree were used as active packaging for the protection of chicken breasts and ham stored at either 23 or 4 °C for 72 h (Ravishankar et al. 2009): they were active against *S. enterica*, *Salmonella enteritidis*, and *E. coli*. Moreover, films with carvacrol were active against *L. monocytogenes*; carvacrol or cinnamaldehyde incorporated in apple pectin edible films showed antimicrobial activity against *Campylobacter jejuni* (Mild et al. 2011), although in different way: Carvacrol disrupts bacterial cell membranes by non-covalent interactions, whereas cinnamaldehyde reacts chemically with the membrane surfaces resulting in cell death (Mild et al. 2011). Ravishankar et al. (2012) formulated edible films based on pectin and apple, carrot or hibiscus incorporated with carvacrol or cinnamaldehyde, and tested their antimicrobial activity against *L. monocytogenes* on contaminated ham and bologna. Results showed that the presence of carvacrol gave better antimicrobial activity against *L. monocytogenes*. Indeed, the use of apple-pectin edible film gave place to a higher inactivation of this pathogen when compared to carrot or hibiscus pectin.

References

Abdou, E. S., Nagy, K. S. A., & Elsabee, M. Z. (2007). Extraction and characterization of chitin and chitosan from local sources. *Bioresource Technology, 99*, 1359–1367.

Acuña, L., Morero, R., & Bellomio, A. (2011). Development of wide-spectrum hybrid bacteriocins for food biopreservation. *Food and Bioprocess Technology, 4*, 1029–1049.

Agheli, N., Kabir, M., Berni-Canani, S., et al. (1998). Plasma lipids and fatty acid synthase activity are regulated by short-chain fructooligosaccharides in sucrose-fed insulin-resistant rats. *Journal of Nutrition, 128*, 1283–1288.

Alboofetileh, M., Rezaei, M., Hosseini, H., & Abdollah, M. (2014). Antimicrobial activity of alginate/clay nanocomposite films enriched with essential oils against three common foodborne pathogens. *Food Control, 36*, 1–7.

Antunes, M., Gago, C., Cavaco, A., & Miguel, M. G. (2012). Edible coatings enriched with essential oils and their compounds for fresh and fresh-cut fruit. *Recent Patents on Food, Nutrition and Agriculture, 4*, 114–122.

Atef, M., Rezaei, M., & Behrooz, R. (2015). Characterization of physical, mechanical, and antibacterial properties of agar-cellulose bionanocomposite films incorporated with savory essential oil. *Food Hydrocolloids, 45*, 150–157.

Azarakhsh, N., Osman, A., Ghazali, H. M., Tan, C. P., & Mohd Adzahan, N. (2012). Optimization of alginate and gellan-based edible coating formulations for fresh-cut pineapples. *International Food Research Journal, 19*, 279–285.

Barreteau, H., Delattre, C., & Michaud, P. (2006). Production of oligosaccharides as promising new food additive generation. *Food Technology and Biotechnology, 44*, 323–333.

Bautista-Banos, S., Hernandez-Lauzardo, A. N., Velazquez-del Valle, M. G., Hernandez-Lopez, M., Ait Barka, E., & Bosquez-Molina, E. (2006). Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. *Crop Protection Review, 25*, 108–118.

Beck-Candanedo, S., Roman, M., & Gray, D. G. (2005). Effect of reaction conditions on the properties and behavior of wood cellulose nanocrystal suspensions. *Biomacromolecules, 6*, 1048–1054.

Beverlya, R. L., Janes, M. E., Prinyawiwatkula, W., & No, H. K. (2008). Edible chitosan films on ready-to-eat roast beef for the control of *Listeria monocytogenes. Food Microbiology, 25*, 534–537.

Bledzki, A. K., & Gassan, J. (1999). Composites reinforced with cellulose based fibres. *Progress in Polymer Science, 24*, 221–274.

Cagri, A., Ustunol, Z., & Ryser, E. T. (2004). Antimicrobial edible films and coatings. *Journal of Food Protection, 67*, 833–848.

Chen, J., Liang, R., Liu, W., et al. (2013). Pectic-oligosaccharides prepared by dynamic high-pressure microfluidization and their in vitro fermentation properties. *Carbohydrate Polymers, 91*, 175–182.

Coelho, E., Rocha, M. A. M., Saraiva, J. A., & Coimbra, M. A. (2014). Microwave superheated water and dilute alkali extraction of brewers' spent grain arabinoxylans and arabinoxylo-oligosaccharides. *Carbohydrate Polymers, 99*, 415–422.

Coma, V., Deschamps, A., & Martial-Gros, A. (2003). Bioactive packaging materials from edible chitosan polymer – antimicrobial activity assessment on dairy-related contaminants. *Journal of Food Science, 68*, 2788–2792.

Coma, V., Martial-Gros, A., Garreau, S., Copinet, A., Salin, F., & Deschamps, A. (2002). Edible antimicrobial films based on chitosan matrix. *Journal of Food Science, 67*, 1162–1169.

Darder, M., Aranda, P., & Ruiz-Hitzky, E. (2007). Bionanocomposites: A new concept of ecological, bioinspired, and functional hybrid materials. *Advanced Materials, 19*, 1309–1319.

Dashipour, A., Razavilar, V., Hosseini, H., Aliabadi, S. S., German, J. B., Ghanati, K., et al. (2015). Antioxidant and antimicrobial carboxymethyl cellulose films containing *Zataria multiflora* essential oil. *International Journal of Biological Macromolecules, 72*, 606–613.

Devlieghere, F., Vermeule, A., & Debevere, J. (2004). Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. *Food Microbiology, 21*, 703–714.

Dhall, R. K. (2013). Advances in edible coatings for fresh fruits and vegetables: A review. *Critical Reviews in Food Science and Nutrition, 53*, 435–450.

Domard, M. (2001). Chitosan: Structure-properties relationship and biomedical applications. In D. Severian (Ed.), *Polymeric biomaterials* (pp. 187–212). New York: Marcel Decker Incorporated.

Dong, C., Qian, L. Y., Zhao, G.-L., He, B.-H., & Xiao, H.-N. (2014). Preparation of antimicrobial cellulose fibers by grafting β-cyclodextrin and inclusion with antibiotics. *Materials Letters, 124*, 181–183.

Du, W.X., Olsen, C.W., Avena-Bustillos, R.J., McHugh, T.H., Levin, C.E., & Friedman, M. (2008). Antibacterial Activity against E. coli O157:H7, Physical Properties, and Storage Stability of Novel Carvacrol-Containing Edible Tomato Films. *Journal of Food Science, 73*, M378-M383.

Dufresne, A. (1997). Mechanical behavior of films prepared from sugar beet cellulose microfibrils. *Journal of Applied Polymer Science, 64*, 1185–1194.

Durango, A. M., Soares, N. F. F., & Andrade, N. J. (2006). Microbiological evaluation of an edible antimicrobial coating on minimally processed carrots. *Food Control, 17*, 336–341.

Dutta, P. K., Tripathi, S., Mehrotra, G. K., & Dutta, J. (2009). Perspectives for chitosan based antimicrobial films in food applications. *Food Chemistry, 114*, 1173–1182.

Elizaquıvel, P., & Aznar, R. (2008). A multiplex RTi-PCR reaction for simultaneous detection of Escherichia coli O157:H7, Salmonella spp. and Staphylococcus aureus on fresh, minimally processed vegetables. *Food Microbiology, 25*, 705–713.

Falguera, V., Quintero, J. P., Jiménez, A., Muñoz, J. A., & Ibarz, A. (2011). Edible films and coatings: Structures, active functions and trends in their use Tr Food. *Science and Technology, 22*, 292–303.

Farris, S., Schaich, K. M., Liu, L., Piergiovanni, L., & Yam, K. L. (2009). Development of polyion-complex hydrogels as an alternative approach for the production of bio-based polymers for food packaging applications: A review. *Trends in Food Science and Technology, 20*, 316–332.

Fisk, C. L., Silver, A. M., Strik, B. C., & Zhao, Y. (2008). Postharvest quality of hardy kiwifruit (*Actinidia arguta* "Ananasnaya") associated with packaging and storage conditions. *Postharvest Biology and Technology, 47*, 338–345.

Fratianni, F., De Martino, L., Melone,A., De Feo, V., Coppola, R., & Nazzaro, F. (2010). Preservation of chicken breast meat treated with thyme and balm essential oils Journal of Food Science 75, M528-M535.

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *Journal of Nutrition, 125*, 1401–1412.

Gobinath, D., Madhu, A. N., Prashant, G., Srinivasan, K., & Prapulla, S. G. (2010). Beneficial effect of xylo-oligosaccharides and fructo-oligosaccharides in streptozotocin-induced diabetic rats. *British Journal of Nutrition, 104*, 40–47.

Gombotz, W. R., & Wee, S. F. (1998). Protein release from alginate matrices. *Advanced Drug Delivery Reviews, 31*, 267–285.

Gonzalez-Aguilar, G. A., Celis, J., Sotelo-Mundo, R. R., De La Rosa, L. A., Rodrigo-Garcia, J., & Alvarez-Parrilla, E. (2008). Physiological and biochemical changes of different fresh-cut mango cultivars stored at 5 °C. *International Journal of Food Science and Technology, 43*, 91–101.

Gullon, B., Gomez, B., Martınez-Sabajanes, M., Yanez, R., Parajo, J. C., & Alonso, J. L. (2013). Pectic oligosaccharides: Manufacture and functional properties. *Trends in Food Science and Technology, 30*, 153–161.

Gullon, B., Gullon, P., Sanz, Y., Alonso, J. L., & Parajo, J. C. (2011). Prebiotic potential of a refined product containing pectic oligosaccharides. *LWT Food Science and Technology, 44*, 1687–1696.

Guo-Jane, M. T., Tsai, J. M., Lee, J., & Zhong, M. J. (2006). Effects of chitosan and a low-molecular-weight chitosan on Bacillus cereus and application in the preservation of cooked rice. *Journal of Food Protection, 69*, 2168–2175.

Han, C., Zhao, Y., Leonard, S. W., & Traber, M. G. (2004). Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (*Fragaria× ananassa*) and raspberries (*Rubus ideaus*). *Postharvest Biology and Technology, 33*, 67–78.

Hansen, N. M. L., & Plackett, D. (2008). Sustainable films and coatings from hemicelluloses: A review. *Biomacromolecules, 9*, 1493–1505.

Hassan, E. A., Hassan, M. L., Moorefield, C. N., & Newkome, G. R. (2015). New supramolecular metallo-terpyridine carboxymethyl cellulose derivatives with antimicrobial properties. *Carbohydrate Polymers, 116*, 2–8.

Hidaka, H., Eida, T., Takiwaza, T., Tokunga, T., & Tashiro, Y. (1986). Effects of fructooligosaccharides on intestinal flora and human health. *Bifidobacteria Microflora, 5*, 37–50.

Jane, J. L., & Shen, J. J. (1993). Internal structure of the potato starch granule revealed by chemical gelatinization. *Carbohydrate Research, 247*, 279–290.

Jang, K. H., Joon, Y. Y., Lee, Y. H., Kang, Y. O., & Park, W. H. (2014). Antimicrobial activity of cellulose-based nanofibers with different Ag phases. *Materials Letters, 116*, 146–149.

Jiménez, A., Fabra, M., Talens, P., & Chiralt, A. (2012). Edible and biodegradable starch films: A review. *Food and Bioprocess Technology, 5*, 2058–2076.

Jin, T., Liu, L., Sommers, C. H., Boyd, G., & Zhang, H. (2009a). Radiation sensitization and post-irradiation proliferation of *Listeria monocytogenes* on ready-to-eat delimeat in the presence of pectin-nisin films. *Journal of Food Protection, 72*, 644–649.

Jin, T., Liu, L., Zhang, H., & Hicks, K. (2009b). Antimicrobial activity of nisin incorporated in pectin and polylactic acid composite films against *Listeria monocytogenes. International Journal of Food Science and Technology, 44*, 322–329.

Jolie, R. P., Duvetter, T., Van Loey, A. M., & Hendrickx, M. E. (2010). Pectin methylesterase and its proteinaceous inhibitor: A review. *Carbohydrate Research, 345*, 2583–2595.

Kang, O. L., Ghani, M., Hassan, O., Rahmati, S., & Ramli, N. (2014). Novel agaro-oligosaccharide production through enzymatic hydrolysis: Physicochemical properties and antioxidant activities. *Food Hydrocolloids*. doi:10.1016/j.foodhyd.2014.04.031.

Kasemsuwan, T., & Jane, J. L. (1994). Location of amylose in normal starch granules. II. Location of phosphodiester cross-linking revealed by phosphorous-31 nuclear magnetic resonance. *Cereal Chemistry, 71*, 282–287.

Kim, H. S., Lee, C. G., & Lee, E. Y. (2011). Alginate lyase: Structure, property, and application. *Biotechnology and Bioprocess Engineering, 16*, 843–851.

Kim, K. W., Thomas, R. L., Lee, C., & Park, H. J. (2003). Antimicrobial activity of native chitosan, degraded chitosan, and O-carboxymethylated chitosan. *Journal of Food Protection, 66*, 1495–1498.

Koushki, M., Azizi, M. H., Koohy-Kamaly, P., Amiri, Z., & Azizkhani, M. (2015). Effect of calcium alginate coating on shelf life of frozen lamb muscle. *Journal of Paramedical Sciences, 6*, 30–35.

Kuorwel, K., Cran, M. J., Sonneveld, K., Miltz, M., & Bigger, S. W. (2014). Evaluation of antifungal activity of antimicrobial agents on Cheddar cheese. *Packaging Technology and Science, 27*, 49–58.

Laurent, M. A., & Boulenguer, P. (2003). Stabilization mechanism of acid dairy drinks (ADD) induced by pectin. *Food Hydrocolloids, 17*, 445–454.

Levine, P., Green, R. S., Ransom, G., & Hill, W. (2001). Pathogen testing of ready-to-eat meat and poultry products collected at federally inspected establishments in the United States, 1990 to 1999. *Journal of Food Protection, 4*, 1188–1193.

Li, T., Li, S., Du, L., et al. (2010). Effects of haw pectic oligosaccharide on lipid metabolism and oxidative stress in experimental hyperlipidemia mice induced by high-fat diet. *Food Chemistry, 121*, 1010–1013.

Li, B., & Xie, B. J. (2004). Synthesis and characterization of konjac glucomannan/poly (vinyl alcohol) interpenetrating polymer networks. *Journal of Applied Polymer Science, 93*, 2775–2780.

Liu, H., Du, Y., Wang, X., & Sun, L. (2004). Chitosan kills bacteria through cell membrane damage. *International Journal of Food Microbiology, 95*, 147–155.

Liu, X. F., Guan, Y. L., Yang, D. Z., Li, Z., & Yao, K. D. (2001). Antibacterial action of chitosan and carboxymethylated chitosan. *Journal of Applied Polymer Science, 79*, 1324–1335.

Mahalik, N. P., & Nambiar, A. N. (2010). Trends in food packaging and manufacturing systems and technology. *Trends in Food Science and Technology, 21*(3), 117–128.

Mild, R. M., Joens, L. A., Friedman, M., Olsen, C. W., McHugh, T. H., Law, B., et al. (2011). Antimicrobial edible apple films inactivate antibiotic resistant and susceptible *Campylobacter jejuni* strains on chicken breast. *Journal of Food Science, 76*, M163–M168.

Mishra, R. K., Banthia, A. K., & Majeed, A. B. A. (2012). Pectin based formulations for biomedical applications: A review. *Journal of Pharmaceutical and Clinical Research, 5*, 1–7.

Moure, A., Gullon, P., Domınguez, H., & Parajo, J. C. (2006). Advances in the manufacture, purification and applications of xylo-oligosaccharides as food additives and nutraceuticals. *Process Biochemistry, 41*, 1913–1923.

Mussatto, S. I., & Mancilha, I. M. (2007). Non-digestible oligosaccharides: A review. *Carbohydrate Polymers, 68*, 587–597.

Nazzaro, F., Fratianni, F., Sada, A., & Orlando, P. (2008). Synbiotic potential of carrot juice supplemented with Lactobacillus spp. and inulin or fructooligosaccharides. *Journal of the Science of Food and Agriculture, 88*, 2271–2276.

Nazzaro, F., Caliendo, G., Arnesi, G., Veronesi, A., Sarzi, P., & Fratianni, F. (2009). Comparative content of some bioactive compounds in two varieties of *Capsicum annuum* L. sweet pepper

and evaluation of their antimicrobial and mutagenic activities. *Journal of Food Biochemistry, 33*, 852–868.

Nazzaro, F., Orlando, P., Fratianni, F., & Coppola, R. (2012a). Microencapsulation in food science and biotechnology. *Current Opinion in Biotechnology, 23*, 182–186.

Nazzaro, F., Fratianni, F., Nicolaus, B., Poli, A., & Orlando, P. (2012b). The prebiotic source influences the growth, biochemical features and survival under simulated gastrointestinal conditions of the probiotic *Lactobacillus acidophilus*. *Anaerobe, 18*, 280–285.

Nazzaro, F., Fratianni, F., Orlando, P., & Coppola, R. (2012c). Biochemical traits, survival and biological properties of the probiotic *Lactobacillus plantarum* grown in the presence of prebiotic inulin and pectin as energy source. *Pharmaceuticals, 5*, 481–492.

No, H. K., Park, N. Y., Lee, S. H., & Meyers, S. P. (2002). Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *International Journal of Food Microbiology, 74*, 65–72.

Pagno, C. H., Costa, T. M. H., de Menezes, E. W., Benvenutti, E. V., Hertz, P. F., Matte, C. R., et al. (2015). Development of active biofilms of quinoa (*Chenopodium quinoa* W.) starch containing gold nanoparticles and evaluation of antimicrobial activity. *Food Chemistry, 173*, 755–762.

Park, S. I., Daeschel, M. A., & Zhao, Y. (2004a). Functional properties of antimicrobial lysozyme-chitosan composite films. *Journal of Food Science, 69*, M215–M221.

Park, P. J., Je, J. Y., Byun, H. G., Moon, S. H., & Kim, S. E. (2004b). Antimicrobial activity of hetero-chitosans and their oligosaccharides with different molecular weights. *Journal of Microbiology and Biotechnology, 14*, 317–323.

Pawar, S. N., & Edgar, K. J. (2012). Alginate derivatization: A review of chemistry, properties and applications. *Biomaterials, 33*, 3279–3305.

Peat, S., Whelan, W. J., & Thomas, G. J. (1952). Evidence of multiple branching in waxy maize starch. *Journal of the Chemical Society*, 4546–4548.

Perez, S., & Bertoft, E. (2010). The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. *Starch-Starke, 62*, 389–420.

Pranoto, Y., Rakshit, S. K., & Salokhe, V. M. (2005). Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. *LWT Food Science and Technology, 38*, 859–865.

Qiang, X., Yonglie, C., & Qianbing, W. (2009). Health benefit application of functional oligosaccharides. *Carbohydrate Polymers, 77*, 435–441.

Qin, C., Li, H., Xiao, Q., Liu, Y., Zhu, J., & Du, Y. (2006). Water-solubility of chitosan and its antimicrobial activity. *Carbohydrate Polymers, 63*, 367–374.

Ravishankar, S., Zhu, L., Olsen, C. W., McHugh, T. H., & Friedman, M. (2009). Edible apple film wraps containing plant antimicrobials inactivate foodborne pathogens on meat and poultry products. *Journal of Food Science, 74*, M440–M445.

Ravishankar, S., Jaroni, D., Zhu, L., Olsen, C., McHugh, T., & Friedman, M. (2012). Inactivation of Listeria monocytogenes on ham and bologna using pectin-based apple, carrot, and hibiscus edible films containing carvacrol and cinnamaldehyde, Journal of Food Science, vol. 77, no. 7, pp. 377–382.

Resa, C. P. O., Gerschenson, L. N., & Rosa, J. (2014). Natamycin and nisin supported on starch edible films for controlling mixed culture growth on model systems and Port Salut cheese. *Food Control, 44*, 146–151.

Ribeiro, C., Vicente, A. A., Teixeira, J. A., & Miranda, C. (2007). Optimization of edible coating composition to retard strawberry fruit senescence. *Postharvest Biology and Technology, 44*, 63–70.

Ring, S. G., L'Anson, K., & Morris, V. J. (1985). Static and dynamic light scattering studies of amylose solutions. *Macromolecules, 18*, 182–188.

Roberfroid, M. B. (2001). Prebiotics: Preferential substrates for specific germs? *American Journal of Clinical Nutrition, 73*, 406–409.

Roberts, M., & Greenwood, M. (2003). *Listeria monocytogenes* (3rd ed., pp. 273–274). Malden, MA: Practical Food Microbiology Blackwell Publishing.

Rojas-Graü, M. A., Avena-Bustillos, R. J., Friedman, M., Henika, P. R., Martín-Belloso, O., & McHugh, T. H. (2006). Mechanical, barrier, and antimicrobial properties of apple puree edible

films containing plant essential oils. *Journal of Agricultural and Food Chemistry, 54*, 9262–9267.

Roopa, B. S., & Bhattacharya, S. (2008). Alginate gels: Characterization of textural attributes. *Journal of Food Engineering, 85*, 123–131.

Sako, T., Matsumoto, K., & Tanaka, R. (1999). Recent progress on research and applications of non-digestible galacto-oligosaccharides. *International Dairy Journal, 9*, 69–80.

Sebti, I., Martial-Gros, A., Carnet-Pantiez, A., Grelier, S., & Coma, V. (2005). Chitosan polymer as bioactive coating and film against Aspergillus niger contamination. *Journal of Food Science, 70*, 100–104.

Shen, X. L., Wu, J. M., Chen, Y., & Zhao, G. (2010). Antimicrobial and physical properties of sweet potato starch films incorporated with potassium sorbate or chitosan. *Food Hydrocolloids, 24*, 285–290.

Soykeabkaew, N., Arimoto, N., Nishino, T., & Peijs, T. (2008). All-cellulose composites by surface selective dissolution of aligned ligno-cellulosic fibres. *Composites Science and Technology, 68*, 2201–2207.

Tsai, G., Su, W., Chen, H., & Pan, C. (2002). Antimicrobial activity of shrimp chitin and chitosan from different treatments and applications of fish preservation. *Fisheries Science, 68*, 70–177.

Tsai, G. J., Wu, Z. Y., & Su, W. H. (2000). Antibacterial activity of a chitooligosaccharide mixture prepared by cellulase digestion of shrimp chitosan and its application to milk preservation. *Journal of Food Protection, 63*, 747–752.

Valencia-Chamorro, S. A., Palou, L., Del Rio, M. A., & Pérez-Gago, M. B. (2013). Antimicrobial edible films and coatings for fresh and minimally processed fruits and vegetables: A review. *Critical Reviews in Food Science and Nutrition, 51*, 872–900.

Vargas, M., Pastor, C., Chiralt, A., McClements, D. J., & González-Martínez, C. (2008). Recent advances in edible coatings for fresh and minimally processed fruit. *Critical Reviews in Food Science and Nutrition, 48*, 496–511.

Vasconez, M. B., Flores, S. K., Campos, C. A., Alvarado, J., & Gerschenson, L. N. (2009). Antimicrobial activity and physical properties of chitosan–tapioca starch based edible films and coatings. *Food Research International, 42*, 762–769.

Videcoq, P., Garnier, C., Robert, P., & Bonnin, E. (2011). Influence of calcium on pectin methylesterase behaviour in the presence of medium methylated pectins. *Carbohydrate Polymers, 86*, 1657–1664.

Wang, X., & Gibson, G. R. (1993). Effects of the in vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine. *Journal of Applied Bacteriology, 75*, 373–380.

Whang, H., Huang, Z. H., Hu, R., & He, J. Y. (2015). Preservative effects of antimicrobial controlled-release coatings containing tea polyphenols nanoparticles on tilapia fillets. In Shang & Wang (Eds.), *Manufacturing and engineering technology* (pp. 433–437). Boca Raton, FL: Taylor and Francis Group.

Willats, W. T., McCartney, L., Mackie, W., & Knox, J. P. (2001). Pectin: Cell biology and prospects for functional analysis. *Plant Molecular Biology, 47*, 9–27.

Wong, T. Y., Preston, L. A., & Schiller, N. L. (2000). Alginate lyase: Review of major sources and enzyme characteristics, structure–function analysis, biological roles, and applications. *Annual Review of Microbiology, 54*, 289–340.

Zhai, M., Zhao, L., Yoshii, F., & Kume, T. (2004). Study on antibacterial starch/chitosan blend film formed under the action of irradiation. *Carbohydrate Polymers, 57*, 83–88.

Zhang, L., Ruich, L., Dong, F., Tian, A., Lia, Z., & Dai, Y. (2015). Physical, mechanical and antimicrobial properties of starch films incorporated with ε-poly-l-lysine. *Food Chemistry, 166*, 107–114.

Zhao, X., Li, B. F., Xue, C. H., & Sun, L. P. (2012). Effect of molecular weight on the antioxidant property of low molecular weight alginate from *Laminaria japonica. Journal of Applied Phycology, 24*, 295–300.

Ziani, K., Fernandez-Pan, I., Royo, M., & Mate, I. J. (2009). Antifungal activity of films and solutions based on chitosan against typical seed fungi. *Food Hydrocolloids, 23*(2009), 2309–2314.

Chapter 10
Active Packaging

M.M. Gutierrez-Pacheco, S.L. Gutierrez-Pacheco, L.A. Ortega-Ramirez, and J.F. Ayala-Zavala

Postharvest Problems in Fresh Produce

Fresh fruits and vegetables have a short postharvest life and are prone to postharvest losses due to mechanical injury, physiological causes, and decay. There has been an enormous increase in the demand for fresh fruit and vegetable products that has required the industry to develop new and improved methods for maintaining food quality and extending shelf life (Aday et al. 2011). The physical damage is a very important aspect of postharvest deterioration and is the primary cause of many losses. Various types of injury can be sustained before, during, or after harvest; for example, bruise may only be show externally (e.g., apples) but there could be internal damage, or it may be evident only after peeling (e.g., potatoes). For instance, studies by Wilson et al. (1999) showed that a single bad bruise on an apple increased the rate of moisture loss by up to 400 %. The presence of bruising and other types of mechanical damage causes significant economic loss of fresh produce due to rejection of the appearance quality by consumers (Prusky 2011).

Besides these normal postharvest changes, the conservation of fresh produce to low temperatures may disrupt the complex sequence of biochemical reactions taking place in plant tissue, and cause irreversible damage known as chilling injury (Snowdon 2010). Low-temperature storage is widely used as a postharvest treatment applied to delay senescence in vegetables, ornamentals and fruits, so upholding their postharvest quality (Aghdam et al. 2015). The application to tropical and subtropical fruits and vegetables is the susceptibility of these to chilling injury at

M.M. Gutierrez-Pacheco • S.L. Gutierrez-Pacheco • L.A. Ortega-Ramirez
J.F. Ayala-Zavala (✉)
Centro de Investigacion en Alimentacion y Desarrollo, A.C. (CIAD, AC),
Carretera a la Victoria Km 0.6, La Victoria, Hermosillo, Sonora 83000, Mexico
e-mail: jayala@ciad.mx

M.W. Siddiqui et al. (eds.), *Postharvest Management Approaches for Maintaining Quality of Fresh Produce*, DOI 10.1007/978-3-319-23582-0_10

temperatures below 12 °C. Chilling injury is a physiological disorder that greatly reduces fruit quality, frequently rendering the product unsellable (Aghdam et al. 2015). In general, tissue which has suffered physiological disruption, is predisposed to microbial decay, one of the main problems in fresh produce.

A main postharvest problem of fresh produce is the presence of microorganisms as fungi and bacteria. This infection may occur during the growing season, at harvest time, during handling, storage, transport, and marketing, or even after purchase by consumer (Ayala-Zavala et al. 2008b). It is well established that pathogenic microorganisms associated with whole or fresh-cut produce can cause disease outbreaks, thereby demonstrating the need to reduce risks associated with these products (Parish et al. 2003). Some microorganisms are associated with outbreaks in some fresh produce (tomato, spinach, lettuce, cabbage, cantaloupe, strawberries, and others) involving a variety of pathogens (Beuchat 1996). The primary concern for fresh produce are *Salmonella spp.,* enterotoxigenic *Escherichia coli, Listeria monocytogenes, Shigella spp., Cryptosporidium spp., Cyclospora spp.* hepatitis A virus and norovirus. However, viruses are emerging as the dominant causative agent associated with fruit and vegetable outbreaks. For example, in Europe during 2009, viruses were identified as the causative agent in 65.1 % of 43 fruit and vegetable outbreaks with verified causes (Foong-Cunningham et al. 2012; Snowdon 2010).

The poor postharvest handling, such as a broken cold chain and unsuitable packaging materials, results in high postharvest losses due to enhanced physiological activities and other metabolic processes that are associated with deterioration of fresh produce. Thus, to meet the market demand for improving microbiological quality and extending shelf life of fresh produce, microbial growth and storage conditions should be controlled during storage (Choi et al. 2015). To extend storage life, postharvest protocols are necessary such as cold storage coupled with heat treatments, temperature preconditioning, intermittent warming, modified and controlled atmosphere storage, ultraviolet light, jasmonates, and other treatments to maintain postharvest quality of fresh produce (Aghdam et al. 2013).

Modified Atmosphere Packaging

Due to the complexities involved with fresh produce many considerations are involved in choosing an acceptable packaging technology (Farber et al. 2003). One of the areas of research that has shown promise, and had success, is that of modified atmosphere packaging (MAP). This is a widely used modern packaging technology used to extend the shelf life of minimally processed fresh produce, as the name suggests, MAP of fresh produce involves packaging produce in polymer films that maintain a commodity specific modified atmosphere such as reduced oxygen and elevated carbon dioxide levels (Kader 2003; Kader and Saltveit 2003). This technique involves either actively or passively controlling or modifying the atmosphere surrounding the product within a package made of various types and/or combinations of films.

Temperature and levels of O_2 and CO_2 in packages are the most important factors that shorten shelf life and trigger deterioration of packaged fresh fruit. Therefore, equilibrium-modified atmosphere packaging and active packaging (AP) have been employed for addressing these problems. Active packaging can be defined as "a mode of packaging in which the package, the product, and the environment interact to prolong shelf life or enhance safety or sensory properties, while maintaining the quality of the product" (Suppakul et al. 2003). AP involves placing absorbers inside the package (Guynot et al. 2003), and includes concepts such as oxygen and carbon dioxide scavenging systems (Suppakul et al. 2003). Oxygen can cause changes in color, flavor, and odor (Suppakul et al. 2003) and encourages growth of aerobic bacteria and molds (Guynot et al. 2003). Hence, oxygen scavengers are used to minimize quality changes and prevent deterioration due to oxidation and growth of microorganisms (Charles et al. 2005). Iron, sodium hydrosulfite, and ascorbic acid are the most used oxygen scavengers in food packaging (Yeh et al. 2008) (Table 10.1).

Moisture-Absorbing Systems

As is well known, fresh produce continues to respire after harvest producing moisture. This humidity leads to accumulation of moisture vapor inside the package, which may saturate the headspace and condense on internal surface of the package and on surface of the produce. This leads to the growth of microorganisms and degradation of texture and flavor, which are the principal causes of fresh produce losses (Akinmusire 2011). Moisture-absorbing systems have been used for a very long time to absorb moisture of foods and inhibit growth of microorganisms. Some of these include desiccants such as silica gel, calcium oxide, calcium chloride, and activated clays and minerals which can be incorporated into sachets, pads, sheets, or blankets (Day 2008). These moisture-absorbing systems that are based on desiccation can maintain a specific relative humidity inside the package by absorbing or releasing the moisture.

Several researchers have studied the use of desiccants such as Mahajan et al. (2008) which elaborated a moisture absorber with a mixture of bentonite (0.55 g/g), sorbitol (0.25 g/g), and $CaCl_2$ (0.2 g/g). These authors reported a moisture-holding capacity of 0.9 g of water by g of mixed desiccant. Additionally, this absorber (5 g), when applied inside the package of fresh mushrooms (250 g), improved the overall appearance. Shirazi and Cameron (1992) observed that 10 g of sorbitol, xylitol, NaCl, KCl, and $CaCl_2$ sealed with one mature green tomato fruit (70–90 g) at 20 °C in simulated packages for 48 days resulted in stable relative humidities of approximately 75, 80, 75, 85, and 35 %, respectively. Villaescusa and Gil (2003) used sorbitol and silica gel in order to control in-package relative humidity for mushrooms. They found that sorbitol promoted tissue leakage and deteriorated texture, whereas silica gel increased weight loss in mushrooms. These disappointing results could be due to excessive amounts of desiccants used, which was 10–15 g/150 g of mushrooms.

Table 10.1 Effect of MAP in fresh produce

Type of atmosphere packaging	Fresh produce	Effect	Reference
Polypropylene trays with O_2 transmission rate of 5200 $cm^3/m/d/atm$	Cantaloupe and honeydew melon	Lower O_2 availability suppressed some of the esters relevant to the aroma of fresh-cut melon and package O_2 levels are more important in determining aroma than other quality attributes and high O_2 levels may be required to reveal desirable aroma compounds	Amaro et al. (2012)
Packages with high and low O_2 (30 and 80 %)	Pear	High O_2 MAP was effective in keeping free radical scavenging capacity as measured by the DPPH assay. After 12 days of storage, phenolics and anthocyanin contents were 2.5 and 12 times, respectively, higher than those in the passive package and 3 and 2 times higher than those in low O_2 package. The sensory evaluation indicated that surface color of cut fruits were stable for at least 12 days. The results suggested that high O_2-modified atmosphere packaging could be used to inhibit browning and prolong the shelf life of pears in spite of more than 50 % loss in vitamin C content	Li et al. (2012)
High CO_2 atmospheric in polyvinyl chloride	Mushrooms (*Volvariella volvacea*)	High CO_2 treatment before or during storage further reduced browning and increased the shelf life of straw mushroom with an optimum storage temperature of 15 °C	Jamjumroon et al. (2012)
MAP1: 2 % O_2+7 % CO_2; MAP_2: 2 % O_2+10 % CO_2; MAP3: 2 % O_2+13 % CO_2	Shiitake Mushrooms (*Lentinula edodes*)	Active MAP extend the shelf life of shiitake mushrooms to 17 days and the concentration of carbon dioxide influence the postharvest quality of shiitake mushrooms. MAP2 treatment inhibited the increase in respiration rate and malondialdehyde contents, delayed the decrease in firmness, soluble sugar and vitamin C, and obviously reduced the activity of polyphenol oxidase and the degree of browning, therefore maintaining better quality	Ye et al. (2012)
3 kPa O_2 + 3 kPa CO_2 inside of packaging film of LDPE (low-density polyethylene)	Broccoli (*Brassica oleracea L.*)	Broccoli packaged with LDPE 30 μm film and storage at 0 °C was most desirable extending shelf-life reducing weight loss, color change, off-odor, and microbial infections	MiAe et al. (2009)
CO_2 (2–5 %) in PE packaging	Green bell peppers (*Capsicum annuum L.*)	Peppers packaged with the PE films did not exhibit significant changes of ascorbic acid during the storage shelf life. Peppers packaged with the two PE films at 5 °C storage, presented significant less chilling injuries compared to the unpackaged peppers additionally, the original green color was preserved	Manolopoulou et al. (2010)
MAP (5.3 % CO_2 + 5.5 % O_2) and UV-C (2 kJ/m^2) irradiation	Cherry tomatoes	The combination of MAP (5.3 % CO_2 + 5.5 % O_2) and UV-C (2 kJ/m^2) irradiation reduced *S.* Typhimurium inoculated in cherry tomatoes. Additionally, MAP reduced changes in firmness and weight, as well as lycopene content	Choi et al. (2015)

Ethylene-Absorbing Systems

Ethylene (C_2H_4) acts as a plant hormone that has different physiological effects on fresh produce. It accelerates respiration, leading to maturity and senescence, and also softening and ripening of many kinds of fruit (Saltveit 1999). Furthermore, ethylene accumulation can cause yellowing of green vegetables and may be responsible for a number of specific postharvest disorders in fresh fruits and vegetables (Vermeiren et al. 1999). To prolong shelf life of fresh produce, accumulation of ethylene should be avoided (Brody et al. 2008). The most commonly used ethylene absorber is potassium permanganate ($KMnO_4$) which is mostly supplied in sachets while other adsorbent or absorbent chemicals may be distributed as sachets or incorporated in the packaging materials (Brody et al. 2008). Due to the presence of double bond, ethylene ($H_2C{=}CH_2$) is very reactive and is rapidly oxidized to acetate and ethanol by $KMnO_4$ (Lopez-Rubio et al. 2004).

Ethylene may also be removed by physical adsorption on active surfaces such as activated carbon or zeolite. Another type of ethylene absorber is based on the absorption of ethylene on activated carbon and subsequent breakdown by metal catalyst. Use of charcoal with palladium chloride prevented the accumulation of ethylene and was effective in reducing the rate of softening in kiwifruits and bananas and chlorophyll loss in spinach leaves (Abe and Watada 1991). Ethylene can also be removed from the package headspace by using polyethylene films into which ceramic powder has been incorporated. The incorporation of ceramic powder increases the permeability of the film and thus helps to reduce the ethylene concentration in the package (Vermeiren et al. 1999).

Flavor Release/Absorbing Systems

The flavor profile is one of the most important sensory attributes of fresh produce and plays a major role in determining consumer acceptability. This is influenced by genetic, preharvest, harvesting, and postharvest factors (Kader 2008). The longer the time between harvest and eating, the greater the losses of characteristic flavor (taste and aroma) and the development of off-flavors in most fruits and vegetables (Forney 2001; Kader 2008). The increased consumer demand for more quality has led the food packaging industry to search for an innovative packaging technology where the package contributes to maintaining quality and safety of the product in addition to simply acting as a protective barrier (Vakakalanka et al. 2012).

Efforts are made to identify methods to embed, entrap, and encapsulate aromas and other compounds within the packaging polymers or plastics or to add them at the headspace of the containers to minimize flavor scalping (Garti 2008). Flavor release might also provide means to mask off-odors coming from the food or the packaging. Further applications of flavor-enriched packaging materials include the possibility to improve the organoleptic quality of the product by emitting desirable

flavors into the food that are released upon opening (Siro 2012). These functionality can be observed in the work of Ayala-Zavala and González-Aguilar (2010) where evaluated the controlled release of garlic oil from β-cyclodextrin capsules into sachets on sensory odor acceptability of fresh-cut tomato (0, 0.25, 0.5, and 1 g of garlic oil capsules/100 g tomato). These authors reported that after 14 storage days the values of odor acceptability were above the limit of acceptability.

On the other hand, in some cases, fresh produce can generate undesirable aromas and other compounds due to diverse physiological factors. Also, packaging materials may also generate undesirable odors, especially plastic materials like polyolefin components. For this reason, aroma absorbers are deigned to eliminate undesirable aromas present in the package headspace. Some of these absorbers include natural clays, zeolites, and active carbon (Siro 2012).

Natural Antimicrobial and Antioxidant Agents Incorporated in Food Packaging

The search of new safe substances for food preservation is being performed around the world (Magnuson et al. 2013). Synthetic additives have been used for many years to maintain the quality of many food products; however the potentially toxic effects attributed to them are leading to the search for more natural alternatives (Ortega-Ramirez et al. 2014). Nowadays, there has been an extensive search for potential natural food additive candidates that retain a broad spectrum of antioxidant and antimicrobial activities while possessing the ability to improve the quality and shelf life of perishable foods (Fratianni et al. 2010).

Natural plant extracts are of increasing interest today due to the great variety of bioactive compounds with antimicrobial and antioxidant activity present in them (Ayala-Zavala et al. 2008c; González-Aguilar et al. 2010). Some of these include polyphenols, terpenoids, alkaloids, lectins, polypeptides, essential oils (EOs), among others. Several essential oils (EOs) such as oils of garlic, cinnamon, thyme, oregano, clove, basil, coriander, citrus peel, laurel, ginger, rosemary, and peppermint, among others, have been demonstrated antimicrobial activity against both bacteria and molds (Burt 2004).

Extensive research has been conducted to the search and study of the antimicrobial and antioxidant activity of natural origin compounds. Tepe et al. (2005) evaluated the antimicrobial activity of *Salvia tomentosa* Miller EO against 14 microorganisms, showing the minimal inhibitory concentrations (MIC) against *Streptococcus pneumonie* (2.25 mg/mL), *Clostridium perfringens* (0.54 mg/mL), and *Mycobacterium smegmatis* (2.25 mg/mL). On the other hand, Singh et al. (2007) reported that cinnamon EO (0.002–0.006 mg/mL) were 100 % antifungal against pathogenic fungi *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Fusarium moniliforme*, *Fusarium graminearum*, *Penicillium citrinum*, and *Penicillium viridicatum*. Also, the growth of *Botrytis cinerea*, *Fusarium sp.*, and *Clavibacter michiganensis* subsp. *michiganensis* was completely inhibited by

oregano, thyme, dictamnus, and marjoram EOs at relatively low concentrations (0.085–0.300 mg/mL) (Daferera et al. 2003).

On the other hand, flavonoids have been identified as an excellent food additive capable of ensure food safety by preventing microbial attack. It has been shown that flavonoids can protect fresh produce against spoilage fungi, such as *Fusarium oxysporum* (banana and grape), *Aspergillus japonicus* (pokhara and apricot), *Aspergillus oryzae* (orange), *A. niger* (apple), and *A. flavus* (mango) (Sharma and Kumar 2009; Al-Hindi et al. 2011). Also, has been reported that flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel shown an inhibitory effect against *E. coli*, *Pseudomonas putida*, and *Salmonella enterica* in the range of 0.200–0.800 mg/mL (Mandalari et al. 2007). This antimicrobial activity has been linked to the ability of the constituents of these extracts to be embedded or interact with the membrane or cell wall of these microorganisms, which causes a destabilization of it and alteration of enzyme systems vital to the survival of the same (Kim et al. 1995; Ayala-Zavala et al. 2011) (Fig. 10.1).

Besides of the antimicrobial activity, these compounds also possess antioxidant properties. Flavonoids from fruits and plants have been shown antioxidant properties in vitro and in vivo studies. Has been reported that methanol extracts of pomegranate peel showed 83 and 81 % antioxidant activity at 0.05 mg/mL using β-carotene-linoleate and DPPH· assays, respectively (Singh et al. 2002). Medicinal plants like *Cymbopogon citratus* have also demonstrated antioxidant activity, which were effective in reducing the DPPH· radical in a 40 and 68 % at 0.033 and 0.05 mg/mL. Also, *Chenopodium* EO showed a 95.66 % of inhibition of $ABTS^{\cdot+}$ radical at 3 mg/mL.

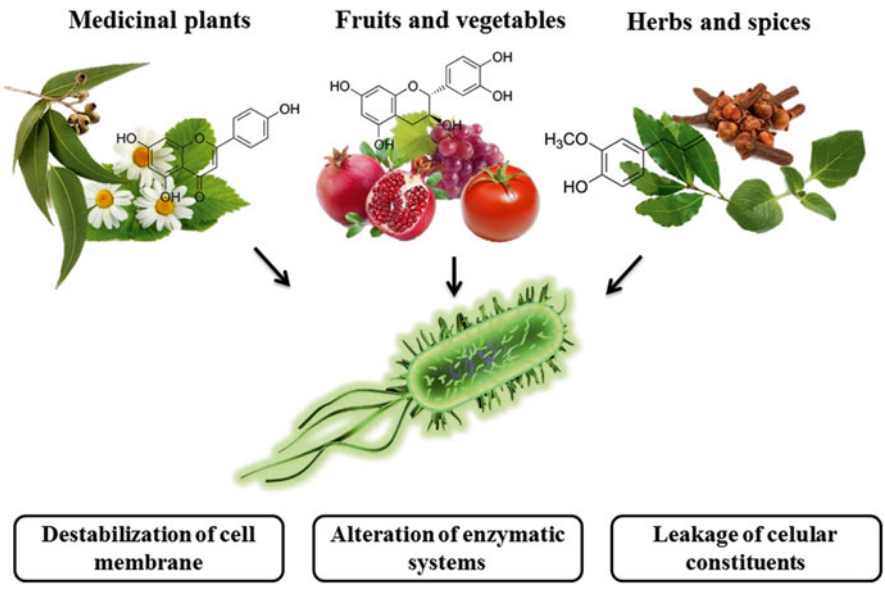


Fig. 10.1 Antibacterial effect of bioactive compounds from natural sources

On the other hand, Vega-Vega et al. (2013) reported that ethanolic extract of mango seed applied to fresh-cut mango presented the highest content of the total phenolics (7.4 times), flavonoids (3.1 times), and antioxidant capacity DPPH·, TEAC, and ORAC (2.9, 2.3, and 2.8 times, respectively) compared to untreated controls. In addition, Silva-Espinoza et al. (2013) applied an aqueous emulsion of cinnamon leaf EO on strawberries stored at 10 °C for 9 days and observed an increment of phenols (78 %) and flavonoids (35 %). Furthermore, increased the antioxidant capacity of the fruits measured with DPPH· (52 %), TEAC (32 %), and ORAC (25 %) assays, compared to control fruits.

Despite the after mentioned properties, the applicability of these compounds directly on the food surface may be sometimes limited because these substances are rapidly neutralized or diffused from the surface into the food product or the environment, thus limiting their antimicrobial and antioxidant properties (Min and Krochta 2005; Ayala-Zavala et al. 2008a). For this reason, their incorporation in a food packaging can protect them and permit a controlled release. The use of packaging with natural bioactive compounds has increased today (Zemljič et al. 2013; Woranuch and Yoksan 2013; Peng et al. 2013). This has allowed the maintenance of foodstuffs for longer time because they maintain effective concentrations of bioactive compounds when is necessary. These compounds could be added in the package allowing their release in the headspace or imbibed into the package material (Ayala-Zavala et al. 2008b).

The effect of antimicrobials in the headspace of the package can be observed in the work of Melgarejo-Flores et al. (2013) which exposed table grapes berries to vapors of cinnamon leaf EO at 0, 0.196, 0.392, and 0.588 mg/mL, and stored in polypropylene trays at 25 °C for 30 min. These authors reported that the exposure of grapes to the oil vapor significantly reduced fungal decay compared to control berries. In addition, the treatments increased phenolic and flavonoid content, related with antioxidant activity. Also, Serrano et al. (2005) observed that the vaporization of eugenol, thymol, menthol, and eucalyptol into polypropylene bags reduced molds and yeasts and total aerobic mesophilic colonies of sweet cherry by 4 and 2 log CFU compared with control, respectively. On the other hand, Ayala-Zavala and González-Aguilar (2010) encapsulated garlic oil in β-cyclodextrin to generate a release system of antimicrobial volatiles and tested on microbial growth and sensory quality of fresh-cut tomato. These authors observed that the highest concentration of garlic oil capsules applied showed the lowest microbial growth and the highest sensory quality.

Edible Films and Coatings as Active Packagings

Edible films and coatings are thin layers of edible materials that play an important role on conservation, distribution, and marketing of fresh produce. Edible coatings may contribute to extending the shelf life of fresh produce by reducing moisture and solute migration, gas exchange, and respiration and oxidative reaction rates, and by reducing or even suppressing physiological disorders (Falguera et al. 2011;

Rojas-Graü et al. 2009). Edible films and coatings can be considered like an "edible packaging" due the characteristics and benefits conferred to the product (Janjarasskul and Krochta 2010). Edible packaging is rapidly advancing by utilizing edible compounds, such as proteins, polysaccharides, lipids and/or resins, and other edible components, derived from diverse renewable sources. Recent innovations have led to the development of edible coatings that can act as carriers of active ingredients. There are a number of compounds that can be incorporated into the coating of fresh produce to delay their senescence, maintain their organoleptic properties, texture, and microbiological safety (antioxidants, antimicrobials, pigments, flavors, spices, salts, nutrient, light absorbers, among others) (Table 10.2).

Depending on individual application, specific controlled-release rates of active solutes are required to fully perform their assigned functions. For example, limiting the migration rate of a preservative can reduce surface microbial growth. In certain cases, modification of an edible film matrix such as cross-linking could be used to reduce bioactive compound mobility (Janjarasskul and Krochta 2010). A controlled rate of release of active solute could be designed based on the chemical affinities between active ingredient, film forming materials, and food product and the conditions (e.g., temperature, pH, aw, time) to which the edible packaging containing the active ingredient would be subjected (Quirós-Sauceda et al. 2014b).

Recently, antimicrobial and antioxidant packaging films have been increasingly investigated (Ayala-Zavala et al. 2008b; Ramos et al. 2014). These properties can be

Table 10.2 Active films and coatings applied to fresh and fresh-cut produce

Active compound	Edible film or coating matrix	Fresh produce	Benefits	Reference
Citral and eugenol	Alginate	Arbutus berries	Preserved sensory and nutritional attributes and reduced microbial spoilage	Guerreiro et al. (2015)
Lemongrass EO	Alginate	Fresh-cut pineapple	Decreased yeast and molds counts	Azarakhsh et al. (2014)
Cinnamic acid	Xanthan gum	Fresh-cut pear	Reduce surface browning and maintain antioxidant capacity	Sharma and Rao (2015)
Lemongrass and oregano EOs	Apple puree-alginate	Fresh-cut apples	Reduce microbial growth	Rojas-Graü et al. (2007)
Carvacrol and methyl cinnamate vapors	Strawberry puree	Strawberries	Maintain firmness and brightness and increased total soluble phenolics	Peretto et al. (2014)
Lemongrass EO	Carnauba-shellac wax	Apple	Maintain hardness and decreased total aerobic bacteria	Jo et al. (2014)
Chitosan	Agar-agar	Garlic cloves	Respiration and moisture loss reduction	Geraldine et al. (2008)
Tea tree EO	Chitosan	Orange	Reduction of microbial growth	Cháfer et al. (2012)

attributed to the incorporation of compounds with these activities or activity of the material itself. These coatings offer advantages over direct application of antimicrobial and antioxidant agents because can be designed to slow down bioactive compounds diffusion (Quirós-Sauceda et al. 2014b). Smaller amounts of these compounds would thus be needed in an edible coating.

Pranoto et al. (2005) reported that alginate-based edible film incorporated with 0.1 % of garlic oil reduced the growth of *Bacillus cereus* and *Staphylococcus aureus* in 5.61 and 4.30 log cycles. Also, Seydim and Sarikus (2006) reported the antimicrobial activity of whey protein-based edible films incorporated with oregano, rosemary, and garlic oils. These authors observed that especially oregano oil (2 %) showed the highest antimicrobial activity showing inhibition zones of 18.99 mm^2. On the other hand, Ayala-Zavala et al. (2013) evaluated the effect of pectin films added with cinnamon leaf oil (36.1 mg/mL) and observed an inhibition zones diameter of 16, 12.3, and 14 mm for *E. coli*, *S. aureus*, and *L. monocytogenes*, respectively. Additionally, these films were applied to fresh-cut peaches and were observed that the same concentration of the oil showed the lowest values of microbial growth (1.86 log UFC/g) and that peaches were positively affected by the coating treatment, increasing its antioxidant status and odor acceptability.

Similarly, Melgarejo-Flores et al. (2013) applied the same coatings to table grapes stored at 10 °C for 15 days and observed a completely inhibition of fungal decay and the highest antioxidant activity compared to control berries. Balaguer et al. (2013) studied the effect of adding cinnamaldehyde (major compound of cinnamon oil) at 1.5, 3, and 5 % in gliadin films against *Penicillium expansum* and *A. niger* in vitro. The tested films were highly effective against fungal growth of *P. expansum* and *A. niger* were completely inhibited after storage in vitro for 10 days in the presence of films incorporated with 3 % cinnamaldehyde.

Other physical factor contributing to postharvest losses of fresh produce is the loss of moisture which causes wilting and/or shrinkage. Rojas-Graü et al. (2006) reported that apple puree films incorporated with oregano, cinnamon, and lemongrass oil decreased water vapor permeability and inhibit the growth of *E. coli*. On the other hand, Siripatrawan and Harte (2010) reported that green tea extract improved water vapor barrier, polyphenol content, and antioxidant activity to chitosan films. Ayranci and Tunc (2004) reported that antioxidants such as citric or ascorbic acids, incorporated into a methylcellulose coating, extensively reduced ascorbic acid losses in whole apricot and peppers. Also, Han et al. (2004) incorporated calcium and vitamin E to chitosan coatings to improve storability and nutritional properties of fresh strawberries and red raspberries.

Another form of active packaging film is the multilayer systems, which is more efficient, even than single films. These can delay or inhibit the diffusion of bioactive compounds to environment (Fig. 10.2) helping to maintain the effective concentrations of bioactive compounds where it is needed and permit a controlled release (Quirós-Sauceda et al. 2014a). A possible mechanism of the multilayered system could include three layers: a matrix layer (e.g., biopolymer based) that contains the bioactive substance; an inner control layer to govern the rate of diffusion of the bioactive substance by allowing its controlled release; and a barrier layer that

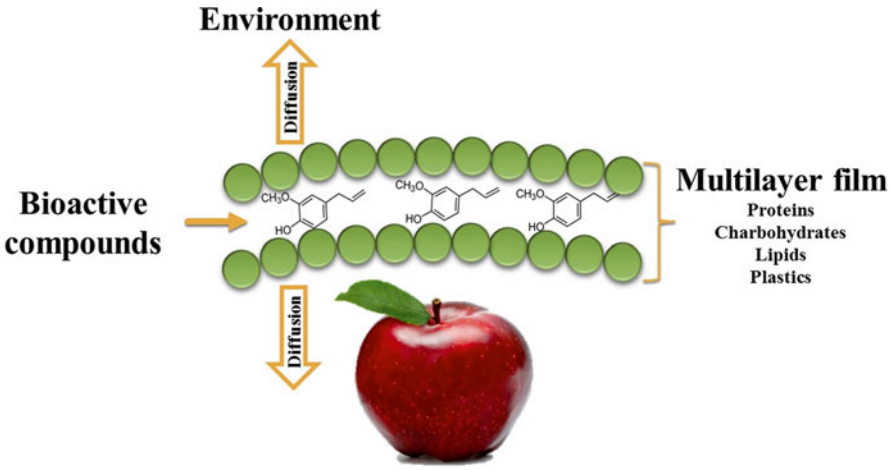


Fig. 10.2 Releasing of bioactive compounds from multilayer film packaging

prevents the migration of the active agent from the covered food as well as controls the permeability to gases.

This particular functionality has been reported by Sipahi et al. (2013) which observed an increment of shelf life of fresh-cut watermelon after to be covered with a alginate-based multilayer (0.5, 1, 2 g/100 g) added with *trans*-cinnamaldehyde microencapsulated (2 g/100 g) in β-cyclodextrins antimicrobial agent. These authors observed that the multilayer coating (2 g/100 g) effectively reduced counts of psychrotrophs by approximately 1.30 logs. Also, Brasil et al. (2012) tested the same antimicrobial agent (2 g/100 g) into chitosan-pectin multilayer coating (2 g/100 g each film forming solution) in enhancing quality of fresh-cut papaya stored at 4 °C for 15 days. The authors observed that multilayer coating maintained firmness, color, and β-carotene content, and showed lower juice leakage. Similarly, Mantilla et al. (2013) studied the effectiveness of a sodium alginate (0.5, 1, and 2 g/100 g) and pectin (2 g/100 g) multilayered edible coating with the same antimicrobial complex (beta-cyclodextrin and *trans*-cinnamaldehyde at 2 g/100 g) in enhancing quality and shelf life of fresh-cut pineapple stored for 15 days at 4 °C. These authors observed that the coatings inhibit the microbial growth and preserve color, texture, and pH of the fruit.

References

Abe, K., & Watada, A. E. (1991). Ethylene absorbent to maintain quality of lightly processed fruits and vegetables. *Journal of Food Science, 56*(6), 1589–1592. doi: 10.1111/j.1365-2621.1991.tb08647.x.

Aday, M. S., Caner, C., & Rahvalı, F. (2011). Effect of oxygen and carbon dioxide absorbers on strawberry quality. *Postharvest Biology and Technology, 62*(2), 179–187. doi:10.1016/j.postharvbio.2011.05.002.

Aghdam, M. S., Sevillano, L., Flores, F. B., & Bodbodak, S. (2013). Heat shock proteins as biochemical markers for postharvest chilling stress in fruits and vegetables. *Scientia Horticulturae, 160*, 54–64. doi:10.1016/j.scienta.2013.05.020.

Aghdam, M., Sevillano, L., Flores, F., & Bodbodak, S. (2015). The contribution of biotechnology to improving post-harvest chilling tolerance in fruits and vegetables using heat-shock proteins. *The Journal of Agricultural Science, 153*(01), 7–24. doi:10.1017/S0021859613000804.

Akinmusire, O. (2011). Fungal species associated with the spoilage of some edible fruits in Maiduguri Northern Eastern Nigeria. *Advances in Environmental Biology, 5*(1), 157–161.

Al-Hindi, R. R., Al-Najada, A. R., & Mohamed, S. A. (2011). Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes. *African Journal of Microbiology Research, 5*, 443–448. doi:org/10.12944/CWE.8.2.20.

Amaro, A. L., Beaulieu, J. C., Grimm, C. C., Stein, R. E., & Almeida, D. P. F. (2012). Effect of oxygen on aroma volatiles and quality of fresh-cut cantaloupe and honeydew melons. *Food Chemistry, 130*(1), 49–57. doi:10.1016/j.foodchem.2011.06.052.

Ayala-Zavala, J. F., del Toro-Sánchez, L., Alvarez-Parrilla, E., Soto-Valdez, H., Martín-Belloso, O., Ruiz-Cruz, S., et al. (2008a). Natural antimicrobial agents incorporated in active packaging to preserve the quality of fresh fruits and vegetables. *Stewart Postharvest Review, 4*(3), 1–9. doi:10.2212/spr.2008.3.9.

Ayala-Zavala, J., Del-Toro-Sánchez, L., Alvarez-Parrilla, E., & González-Aguilar, G. (2008b). High relative humidity in-package of fresh-cut fruits and vegetables: Advantage or disadvantage considering microbiological problems and antimicrobial delivering systems? *Journal of Food Science, 73*(4), R41–R47. doi:10.1111/j.1750-3841.2008.00705.x.

Ayala-Zavala, J. F., & González-Aguilar, G. A. (2010). Optimizing the use of garlic oil as antimicrobial agent on fresh-cut tomato through a controlled release system. *Journal of Food Science, 75*(7), M398–M405. doi:10.1111/j.1750-3841.2010.01723.x.

Ayala-Zavala, J. F., Oms-Oliu, G., Odriozola-Serrano, I., González-Aguilar, G. A., Álvarez-Parrilla, E., & Martín-Belloso, O. (2008c). Bio-preservation of fresh-cut tomatoes using natural antimicrobials. *European Food Research and Technology, 226*(5), 1047–1055. doi:10.1007/s00217-007-0630-z.

Ayala-Zavala, J. F., Silva-Espinoza, B. A., Cruz-Valenzuela, M. R., Leyva, J. M., Ortega-Ramírez, L. A., Carrazco-Lugo, D. K., et al. (2013). Pectin–cinnamon leaf oil coatings add antioxidant and antibacterial properties to fresh-cut peach. *Flavour and Fragrance Journal, 28*(1), 39–45. doi:10.1002/ffj.3125.

Ayala-Zavala, J. F., Vega-Vega, V., Rosas-Dominguez, C., Palafox-Carlos, H., Villa-Rodriguez, J. A., Siddiqui, M. W., et al. (2011). Agro-industrial potential of exotic fruit byproducts as a source of food additives. *Food Research International, 44*(7), 1866–1874. doi:10.1016/j.foodres.2011.02.021.

Ayranci, E., & Tunc, S. (2004). The effect of edible coatings on water and vitamin C loss of apricots (*Armeniaca vulgaris* Lam.) and green peppers (*Capsicum annuum* L.). *Food Chemistry, 87*(3), 339–342. doi:10.1016/j.foodchem.2003.12.003.

Azarakhsh, N., Osman, A., Ghazali, H. M., Tan, C. P., & Mohd Adzahan, N. (2014). Lemongrass essential oil incorporated into alginate-based edible coating for shelf-life extension and quality retention of fresh-cut pineapple. *Postharvest Biology and Technology, 88*, 1–7. doi:10.1016/j.postharvbio.2013.09.004.

Balaguer, M. P., Lopez-Carballo, G., Catala, R., Gavara, R., & Hernandez-Munoz, P. (2013). Antifungal properties of gliadin films incorporating cinnamaldehyde and application in active food packaging of bread and cheese spread foodstuffs. *International Journal of Food Microbiology, 166*(3), 369–377. doi:10.1016/j.ijfoodmicro.2013.08.012.

Beuchat, L. R. (1996). Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection®, 59*(2), 204–216.

Brasil, I. M., Gomes, C., Puerta-Gomez, A., Castell-Perez, M. E., & Moreira, R. G. (2012). Polysaccharide-based multilayered antimicrobial edible coating enhances quality of fresh-cut papaya. *LWT Food Science and Technology, 47*(1), 39–45. doi:10.1016/j.lwt.2012.01.005.

Brody, A. L., Bugusu, B., Han, J. H., Sand, C. K., & McHugh, T. H. (2008). Scientific status summary. *Journal of Food Science, 73*(8), R107–R116. doi:10.1111/j.1750-3841.2008.00933.x.

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods – a review. *International Journal of Food Microbiology, 94*(3), 223–253. doi:10.1016/j.ijfoodmicro.2004.03.022.

Cháfer, M., Sánchez-González, L., González-Martínez, C., & Chiralt, A. (2012). Fungal decay and shelf life of oranges coated with chitosan and bergamot, thyme, and tea tree essential oils. *Journal of Food Science, 77*(8), E182–E187. doi:10.1111/j.1750-3841.2012.02827.x.

Charles, F., Sanchez, J. S., Gontard, N. (2005). Modeling of Active Modified Atmosphere Packaging of Endives Exposed to Several Postharvest Temperatures. Journal of Food Science, 70(8), e443-e449. doi:10.1111/j.1365-2621.2005.tb11512.x.

Choi, D. S., Park, S. H., Choi, S. R., Kim, J. S., & Chun, H. H. (2015). The combined effects of ultraviolet-C irradiation and modified atmosphere packaging for inactivating Salmonella enterica serovar Typhimurium and extending the shelf life of cherry tomatoes during cold storage. *Food Packaging and Shelf Life, 3*, 19–30. doi:org/10.1016/j.fpsl.2014.10.005.

Daferera, D. J., Ziogas, B. N., & Polissiou, M. G. (2003). The effectiveness of plant essential oils on the growth of *Botrytis cinerea,* Fusariumsp. and Clavibacter michiganensis subsp. michiganensis. *Crop Protection, 22*(1), 39–44. doi:10.1016/S0261-2194(02)00095-9.

Day. B. P. (2008). Active packaging of food. In: Kerry J, Butler P (eds) *Smart packaging technologies for fast moving consumer goods*. John Wiley & Sons Ltd, England, 1–18.

Falguera, V., Quintero, J. P., Jiménez, A., Muñoz, J. A., & Ibarz, A. (2011). Edible films and coatings: Structures, active functions and trends in their use. *Trends in Food Science and Technology, 22*(6), 292–303. doi:10.1016/j.tifs.2011.02.004.

Farber, J., Harris, L., Parish, M., Beuchat, L., Suslow, T., Gorney, J., et al. (2003). Microbiological safety of controlled and modified atmosphere packaging of fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety, 2*(s1), 142–160. doi:10.1111/j.1541-4337.2003.tb00032.x.

Foong-Cunningham, S., Verkaar, E. L. C., & Swanson, K. (2012). 1 – Microbial decontamination of fresh produce. In: Demirci A, Ngadi M. O. (eds.) *Microbial decontamination in the food industry*. Cambridge: Woodhead Publishing, 3–29. doi:10.1533/9780857095756.1.3.

Forney, C. F. (2001). Horticultural and other factors affecting aroma volatile composition of small fruit. *HortTechnology, 11*(4), 529–538.

Fratianni, F., De Martino, L., Melone, A., De Feo, V., Coppola, R., & Nazzaro, F. (2010). Preservation of chicken breast meat treated with thyme and balm essential oils. *Journal of Food Science, 75*(8), M528–M535. doi:10.1111/j.1750-3841.2010.01791.x.

Garti, N. (2008). The future of controlled release and delivery technologies. In: Garti N. (ed) *Delivery and controlled release of bioactives in foods and nutraceuticals*. Woodhead Publishing Ltd, Cambridge, 450-464.

Geraldine, R. M., Soares, N. F. F., Botrel, D. A., & de Almeida Gonçalves, L. (2008). Characterization and effect of edible coatings on minimally processed garlic quality. *Carbohydrate Polymers, 72*(3), 403–409. doi:10.1016/j.carbpol.2007.09.012.

González-Aguilar, G. A., Ayala-Zavala, J., Olivas, G., de la Rosa, L., & Álvarez-Parrilla, E. (2010). Preserving quality of fresh-cut products using safe technologies. *Journal für Verbraucherschutz und Lebensmittelsicherheit, 5*(1), 65–72. doi:10.1007/s00003-009-0315-6.

Guerreiro, A. C., Gago, C. M. L., Faleiro, M. L., Miguel, M. G. C., & Antunes, M. D. C. (2015). The effect of alginate-based edible coatings enriched with essential oils constituents on *Arbutus unedo* L. fresh fruit storage. *Postharvest Biology and Technology, 100*, 226–233. doi:10.1016/j.postharvbio.2014.09.002.

Guynot, M., Sanchis, V., Ramos, A., & Marin, S. (2003). Mold-free shelf-life extension of bakery products by active packaging. *Journal of Food Science, 68*(8), 2547–2552. doi:10.1111/j.1365-2621.2003.tb07059.x.

Han, C., Zhao, Y., Leonard, S., & Traber, M. (2004). Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (*Fragaria ananassa*) and raspberries (*Rubus ideaus*). *Postharvest Biology and Technology, 33*(1), 67–78. doi:10.1016/j.postharvbio.2004.01.008.

Jamjumroon, S., Wongs-Aree, C., McGlasson, W. B., Srilaong, V., Chalermklin, P., & Kanlayanarat, S. (2012). Extending the shelf-life of straw mushroom with high carbon dioxide treatment. *Journal of Food, Agriculture and Environment, 10*(1), 78–84.

Janjarasskul, T., & Krochta, J. M. (2010). Edible packaging materials. *Annual Review of Food Science and Technology, 1*, 415–448. doi:10.1146/annurev.food.080708.100836.

Jo, W.-S., Song, H.-Y., Song, N.-B., Lee, J.-H., Min, S. C., & Song, K. B. (2014). Quality and microbial safety of "Fuji" apples coated with carnauba-shellac wax containing lemongrass oil. *LWT Food Science and Technology, 55*(2), 490–497. doi:10.1016/j.lwt.2013.10.034.

Kader, A. A. (2003). A perspective on postharvest horticulture (1978-2003). *HortScience, 38*(5), 1004–1008.

Kader, A. A. (2008). Flavor quality of fruits and vegetables. *Journal of the Science of Food and Agriculture, 88*(11), 1863–1868. doi:10.1002/jsfa.3293.

Kader, A. A., & Saltveit, M. E. (2003). Atmosphere modification. In: Bartz J, Brecht J (eds) *Postharvest physiology and pathology of vegetables*. Marcel Dekker, Inc., New York, 229-246.

Kim, J., Marshall, M. R., & Wei, C.-I. (1995). Antibacterial activity of some essential oil components against five foodborne pathogens. *Journal of Agricultural and Food Chemistry, 43*(11), 2839–2845. doi:10.1021/jf00059a013.

Li, W., Li, X., Fan, X., Tang, Y., & Yun, J. (2012). Response of antioxidant activity and sensory quality in fresh-cut pear as affected by high O2 active packaging in comparison with low O2 packaging. *Food Science and Technology International, 18*(3), 197–205. doi:10.1177/1082013211415147.

Lopez-Rubio, A., Almenar, E., Hernandez-Muñoz, P., Lagarón, J. M., Catalá, R., & Gavara, R. (2004). Overview of active polymer-based packaging technologies for food applications. *Food Reviews International, 20*(4), 357–387. doi:10.1081/FRI-200033462.

Magnuson, B., Munro, I., Abbot, P., Baldwin, N., Lopez-Garcia, R., Ly, K., et al. (2013). Review of the regulation and safety assessment of food substances in various countries and jurisdictions. *Food Additives and Contaminants Part A, 30*(7), 1147–1220. doi:10.1080/19440049.2013.795293.

Mahajan, P. V., Rodrigues, F. A. S., Motel, A., & Leonhard, A. (2008). Development of a moisture absorber for packaging of fresh mushrooms (*Agaricus bisporous*). *Postharvest Biology and Technology, 48*(3), 408–414. doi:10.1016/j.postharvbio.2007.11.007.

Mandalari, G., Bennett, R. N., Bisignano, G., Trombetta, D., Saija, A., Faulds, C. B., et al. (2007). Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel, a byproduct of the essential oil industry. *Journal of Applied Microbiology, 103*(6), 2056–2064. doi:10.1111/j.1365-2672.2007.03456.x.

Manolopoulou, H., Xanthopoulos, G., Douros, N., & Lambrinos, G. (2010). Modified atmosphere packaging storage of green bell peppers: Quality criteria. *Biosystems Engineering, 106*(4), 535–543. doi:10.1016/j.biosystemseng.2010.06.003.

Mantilla, N., Castell-Perez, M. E., Gomes, C., & Moreira, R. G. (2013). Multilayered antimicrobial edible coating and its effect on quality and shelf-life of fresh-cut pineapple (*Ananas comosus*). *LWT Food Science and Technology, 51*(1), 37–43. doi:10.1016/j.lwt.2012.10.010.

Melgarejo-Flores, B. G., Ortega-Ramírez, L. A., Silva-Espinoza, B. A., González-Aguilar, G. A., Miranda, M. R. A., & Ayala-Zavala, J. F. (2013). Antifungal protection and antioxidant enhancement of table grapes treated with emulsions, vapors, and coatings of cinnamon leaf oil. *Postharvest Biology and Technology, 86*, 321–328. doi:10.1016/j.postharvbio.2013.07.027.

Moon-Il C, Yoon-Pyo H, Ji-Weon C, Do-Ham B. (2009). Effect of packaging film and storage temperature on quality maintenance of broccoli. *Korean Journal of Horticultural Science and Technology, 27*(1), 128–139.

Min, S., & Krochta, J. M. (2005). Inhibition of *Penicillium commune* by edible whey protein films incorporating lactoferrin, lacto-ferrin hydrolysate, and lactoperoxidase sistems. *Journal of Food Science, 70*(2), M87–M94. doi:10.1111/j.1365-2621.2005.tb07108.x.

Ortega-Ramirez, L. A., Rodriguez-Garcia, I., Leyva, J. M., Cruz-Valenzuela, M. R., Silva-Espinoza, B. A., Gonzalez-Aguilar, G. A., et al. (2014). Potential of medicinal plants as antimicrobial and

antioxidant agents in food industry: A hypothesis. *Journal of Food Science, 79*(2), R129–R137. doi:10.1111/1750-3841.12341.

Parish, M., Beuchat, L., Suslow, T., Harris, L., Garrett, E., Farber, J., et al. (2003). Methods to reduce/eliminate pathogens from fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety, 2*(s1), 161–173. doi:10.1111/j.1541-4337.2003.tb00033.x.

Peng, Y., Wu, Y., & Li, Y. (2013). Development of tea extracts and chitosan composite films for active packaging materials. *International Journal of Biological Macromolecules, 59*, 282–289. doi:10.1016/j.ijbiomac.2013.04.019.

Peretto, G., Du, W.-X., Avena-Bustillos, R. J., Sarreal, S. B. L., Hua, S. S. T., Sambo, P., et al. (2014). Increasing strawberry shelf-life with carvacrol and methyl cinnamate antimicrobial vapors released from edible films. *Postharvest Biology and Technology, 89*, 11–18. doi:10.1016/j.postharvbio.2013.11.003.

Pranoto, Y., Salokhe, V. M., & Rakshit, S. K. (2005). Physical and antibacte rial properties of alginate-based edible film incorporated with garlic oil. *Food Research International, 38*(3), 267–272. doi:10.1016/j.foodres.2004.04.009.

Prusky, D. (2011). Reduction of the incidence of postharvest quality losses, and future prospects. *Food Security, 3*(4), 463–474. doi:10.1007/s12571-011-0147-y.

Quirós-Sauceda, A., Ayala-Zavala, J., Olivas, G., & González-Aguilar, G. (2014a). Edible coatings as encapsulating matrices for bioactive compounds: A review. *Journal of Food Science and Technology*, 1–12. doi:10.1007/s13197-013-1246-x.

Quirós-Sauceda, A. E., Ayala-Zavala, J. F., Olivas, G. I., & González-Aguilar, G. A. (2014b). Edible coatings as encapsulating matrices for bioactive compounds: A review. *Journal of Food Science and Technology, 51*(9), 1674–1685. doi:10.1007/s13197-013-1246-x.

Ramos, M., Jiménez, A., Peltzer, M., & Garrigós, M. C. (2014). Development of novel nano-biocomposite antioxidant films based on poly (lactic acid) and thymol for active packaging. *Food Chemistry, 162*, 149–155. doi:10.1016/j.foodchem.2014.04.026.

Rojas-Graü, M. A., Avena-Bustillos, R. J., Friedman, M., Henika, P. R., Martín-Belloso, O., & McHugh, T. H. (2006). Mechanical, barrier, and antimicrobial properties of apple puree edible films containing plant essential oils. *Journal of Agricultural and Food Chemistry, 54*(24), 9262–9267. doi:10.1021/jf061717u.

Rojas-Graü, M. A., Raybaudi-Massilia, R. M., Soliva-Fortuny, R. C., Avena-Bustillos, R. J., McHugh, T. H., & Martín-Belloso, O. (2007). Apple puree-alginate edible coating as carrier of antimicrobial agents to prolong shelf-life of fresh-cut apples. *Postharvest Biology and Technology, 45*(2), 254–264. doi:10.1016/j.postharvbio.2007.01.017.

Rojas-Graü, M. A., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). Edible coatings to incorporate active ingredients to fresh-cut fruits: A review. *Trends in Food Science and Technology, 20*(10), 438–447. doi:10.1016/j.tifs.2009.05.002.

Saltveit, M. E. (1999). Effect of ethylene on quality of fresh fruits and vegetables. *Postharvest Biology and Technology, 15*(3), 279–292. doi:10.1016/S0925-5214(98)00091-X.

Serrano, M., Martínez-Romero, D., Castillo, S., Guillén, F., & Valero, D. (2005). The use of natural antifungal compounds improves the beneficial effect of MAP in sweet cherry storage. *Innovative Food Science and Emerging Technologies, 6*(1), 115–123. doi:10.1016/j.ifset.2004.09.001.

Seydim, A. C., & Sarikus, G. (2006). Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. *Food Research International, 39*(5), 639–644. doi:10.1016/j.foodres.2006.01.013.

Sharma, B., & Kumar, P. (2009). *In vitro* antifungal potency of some plant extracts against *Fusarium oxysporum. International Journal of Green Pharmacy, 3*(1), 63–65. doi:10.4103/0973-8258.49377.

Sharma, S., & Rao, T. V. R. (2015). Xanthan gum based edible coating enriched with cinnamic acid prevents browning and extends the shelf-life of fresh-cut pears. *LWT Food Science and Technology, 62*(1, Part 2), 791–800. doi:10.1016/j.lwt.2014.11.050.

Shirazi, A., & Cameron, A. C. (1992). Controlling relative humidity in modified atmosphere packages of tomato fruit. *HortScience, 27*(4), 336–339.

Silva-Espinoza, B. A., Ortega-Ramírez, L. A., González-Aguilar, G. A., Olivas, I., & Ayala-Zavala, J. F. (2013). Protección antifúngica y enriquecimiento antioxidante de fresa con aceite esencial de hoja de canela. *Revista Fitotecnia Mexicana, 36*, 217–224.

Singh, R. P., Chidambara Murthy, K. N., & Jayaprakasha, G. K. (2002). Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using *in vitro* models. *Journal of Agricultural and Food Chemistry, 50*(1), 81–86. doi:10.1021/jf010865b.

Singh, G., Maurya, S., deLampasona, M. P., & Catalan, C. A. N. (2007). A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. *Food and Chemical Toxicology, 45*(9), 1650–1661. doi:10.1016/j.fct.2007.02.031.

Sipahi, R., Castell-Perez, M., Moreira, R., Gomes, C., & Castillo, A. (2013). Improved multilayered antimicrobial alginate-based edible coating extends the shelf life of fresh-cut watermelon (*Citrullus lanatus*). *LWT Food Science and Technology, 51*(1), 9–15. doi:10.1016/j.lwt.2012.11.013.

Siripatrawan, U., & Harte, B. R. (2010). Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. *Food Hydrocolloids, 24*(8), 770–775. doi:10.1016/j.foodhyd.2010.04.003.

Siro, I. (2012). Active and intelligent packaging of food. In: Bhat R, Alias AK, Paliyath G (eds) *Progress in Food Preservation*. Wiley-Blackwell, Oxford, UK . doi:10.1002/9781119962045.ch2.

Snowdon, A. L. (2010). *Post-harvest diseases and disorders of fruits and vegetables: Volume 1: General introduction and fruits* (Vol. 1). Manson Publishing, Ltd., London, UK, 1-68.

Suppakul, P., Miltz, J., Sonneveld, K., & Bigger, S. W. (2003). Active packaging technologies with an emphasis on antimicrobial packaging and its applications. *Journal of Food Science, 68*(2), 408–420. doi:10.1111/j.1365-2621.2003.tb05687.x.

Tepe, B., Daferera, D., Sokmen, A., Sokmen, M., & Polissiou, M. (2005). Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (*Lamiaceae*). *Food Chemistry, 90*(3), 333–340. doi:10.1016/j.foodchem.2003.09.013.

Vakakalanka, M. S., D'Souza, T., Ray, S., & Yam, K. L. (2012). Emerging packaging technologies for fresh produce. In: Yam KL & Sun Lee D (eds) *Emerging food packaging technologies: Principles and practice*. Woodhead Publishing, Ltd., Cambridge, UK, 109-130. doi:10.1533/9780857095664.1.109.

Vega-Vega, V., Silva-Espinoza, B. A., Cruz-Valenzuela, M. R., Bernal-Mercado, A. T., González-Aguilar, G. A., Vargas-Arispuro, I., Corrales-Maldonado, C. G., & Ayala-Zavala, J. F. (2013). Antioxidant enrichment and antimicrobial protection of fresh-cut mango applying bioactive extracts from their seeds by-products. *Food and Nutrition Sciences*, *4*, 197–203. doi:10.1111/j.1750-3841.2010.01792.x.

Vermeiren, L., Devlieghere, F., van Beest, M., de Kruijf, N., & Debevere, J. (1999). Developments in the active packaging of foods. *Trends in Food Science and Technology, 10*(3), 77–86. doi:10.1016/S0924-2244(99)00032-1.

Villaescusa, R., & Gil, M. (2003). Quality improvement of Pleurotus mushrooms by modified atmosphere packaging and moisture absorbers. *Postharvest Biology and Technology, 28*(1), 169–179. doi:10.1016/S0925-5214(02)00140-0.

Wilson, L., Boyette, M., & Estes, E. (1999). Postharvest handling and cooling of fresh fruits, vegetables, and flowers for small farms. Part I: Quality Maintenance. Horticulture Information Leaflet 800, Department of Horticultural Science, North Carolina State University, North Carolina, US. 1–3.

Woranuch, S., & Yoksan, R. (2013). Eugenol-loaded chitosan nanoparticles: II. Application in bio-based plastics for active packaging. *Carbohydrate Polymers, 96*(2), 586–592. doi:10.1016/j.carbpol.2012.09.099.

Ye, J.-J., Li, J.-R., Han, X.-X., Zhang, L., Jiang, T.-J., & Miao, X. (2012). Effects of active modified atmosphere packaging on postharvest quality of shiitake mushrooms (*Lentinula edodes*) stored at cold storage. *Journal of Integrative Agriculture, 11*(3), 474–482. doi:10.1016/S2095-3119(12)60033-1.

Yeh, J. T., Cui, L., Chang, C. J., Jiang, T., Chen, K. N. (2008). Investigation of the oxygen depletion properties of novel oxygen-scavenging plastics. *Journal of Applied Polymer Science*, *110*(3), 1420-1434. doi:10.1002/app.28674.

Zemljič, L. F., Tkavc, T., Vesel, A., & Šauperl, O. (2013). Chitosan coatings onto polyethylene terephthalate for the development of potential active packaging material. *Applied Surface Science, 265*, 697–703. doi:10.1016/j.apsusc.2012.11.086.

Chapter 11
Ozone: A Powerful Tool for the Fresh Produce Preservation

Nikos Tzortzakis

Current Ineffective Sanitation Policies

Freshly harvested fruit and vegetables are prone to spoilage losses sustained during production, transportation, and storage. It is estimated that microbial spoilage losses to be as high as 30 %, with some—Litchi (*Litchi chinensis* Sonn.) fruit for example—subject to postharvest losses up to 50 % between harvest and consumption (Jiang et al. 2001). Economic losses are severe. Based on 30 % loss from harvest-to-consumption, the estimated value of spoilage of fruit and vegetables (including salads) amounts to £ 0.37 and £ 1.19 billion, respectively, in the UK alone (Leatherhead FRA); any reduction in wastage would be of considerable economic benefit to growers, retailers, and consumers alike. The range of microorganisms present on fresh produce can vary widely, depending on the produce nature, agronomic practices, geographical area of production, and weather conditions prior to harvest (Brackett 1999). Most of the natural microflora on fruit and vegetable surfaces are innocuous and usually exert no deleterious effect on sensory qualities. Conversely, some microbes can spoil produce (i.e., plant pathogens and opportunists) and these constitute a serious economic threat for growers, storers, and shippers.

The major pathogens responsible for postharvest losses of fruit and vegetables are fungi including *Alternaria* spp., *Botrytis* spp., *Colletotrichum* spp., *Diplodia* spp., *Monilinia* spp., *Penicillium* spp., *Rhizopus* spp., and *Sclerotinia* spp. plus bacteria such as *Erwinia* spp. and *Pseudomonas* spp.

Spoilage organisms, along with a variety of human pathogens, contaminate fruit and vegetables via several routes at different points in the production chain following harvest. Infection can occur during the growing season, at harvest time,

N. Tzortzakis (✉)
Department of Agricultural Sciences, Biotechnology and Food Science,
Cyprus University of Technology, Limassol 3603, Cyprus
e-mail: nikolaos.tzortzakis@cut.ac.cy

M.W. Siddiqui et al. (eds.), *Postharvest Management Approaches for Maintaining Quality of Fresh Produce*, DOI 10.1007/978-3-319-23582-0_11

during handling, storage, transport, and marketing and/or following purchase by the consumer.

Traditional disinfectants, including chlorinated compounds, fail to adequately control common spoilage microorganisms as well as potential harmful human pathogens (review Graham 1997; Kim et al. 1999; Xu 1999; Crowe et al. 2007). Consequently, microbial contamination remains considerable despite the adoption of current "best practice." Moreover, in recent years, there has been a noticeable rise in the number of documented outbreaks of foodborne diseases associated with the consumption of fresh fruit, vegetables, and unpasteurized fruit juices (De Roever 1998). Recent estimates indicate that contamination of fruit and vegetables is responsible for 2–8 % of all confirmed cases of food-derived illness (Tauxe et al. 1997).

Numerous studies have confirmed the limited effect of chlorinated compounds in controlling pathogenic bacteria (Beuchat et al. 1998; Pirovani et al. 2000), viruses (Sobsey 1989; Tyrrell et al. 1995), and protozoan cysts (Wickramanayake et al. 1984; Korich et al. 1990). Moreover, the use of chlorinated compounds at effective concentrations (20–200 ppm free chlorine, Beuchat et al. 1998) can generate off-tastes and odor defects in treated produce (White 1986) as well as yielding potentially carcinogenic chloro-organic compounds in wash water (Page et al. 1976) and on the surfaces of treated foodstuffs (Graham 1997). Consequently, recent European Union legislation (e.g., EEC 2092/91 and Biocides Directive 98/8/EC) imposed stricter guidelines governing the use of chlorine- and bromine-based sanitizers with the goal of moving toward a complete ban on their use in future.

Safe, effective alternatives to halogenated disinfectants are urgently required to reduce spoilage losses, improve food safety, and gain consumer confidence and approval. Research and commercial applications have established that ozone may provide a viable alternative to traditional disinfectants, with some additional benefits documented (Bott 1991; Graham 1997).

Ozone: Generation, Formation, and Properties

From the early writings of Homer, it is apparent that man has long appreciated the characteristic smell that accompanies lightening. Rideal (1920) noted that scientists had observed the same odor whilst passing air through electrical discharges or during the electrolysis of water (Cruickshank 1801). Yet, it is Schönbein who is generally credited with the discovery of ozone after concluding in a memoir, presented to the Academy of Munich in 1840, that the odors generated during electrical discharge, electrolysis, and after a flash of lightening were as a result of the production of the same substance. He named the substance *ozone*, derived from the Greek word *ozein* meaning "smelly."

The gas constitutes a natural component of both the upper (stratosphere) and lower (troposphere) atmosphere. Stratospheric ozone forms a protective shield against potentially damaging levels of ultraviolet-B radiation (Environmental Protection Agency, EPA), whilst ground-level ozone concentrations are known to be

high enough to endanger human health, animals, vegetation, and building materials (Wellburn 1994). Tropospheric ozone arises through incursions from the stratosphere (Muller and Brausseur 1995), but to a greater extent by in situ photochemical production (Krupa and Manning 1988).

Commercially, ozone is generated at the point of application. It can be produced by submitting oxygen or air to ultraviolet (UV) radiation of 285 nm wavelength (Graham 1997), but many commercial applications employ the corona discharge method of generation. When a high-voltage alternating current is applied across a discharge gap in the presence of air or oxygen, it results in the cleavage of oxygen molecules to form ozone. It is common to supply oxygen, rather than dry air, to maximize the yield of ozone (Hoigné 1998; Xu 1999). Moreover, the use of pure oxygen as feed gas eliminates the production of higher oxides of nitrogen which are known to contaminate ozone generated by air-fed electric discharge generators (Brown and Roberts 1988). Ozone can also be generated by chemical, thermal, chemonuclear, electrolytic (Horvath et al. 1985), and electrochemical methods (Kim et al. 1999).

Ozone does not result in the formation of trihalomethanes unless halogens have been introduced in the wash water, but does form a number of non-halogenated by-products (Adeskaveg 1995). Ozone is generally safer to use (lower Threshold Limit Value—Long Term Exposure Limit, TLV-LTEL) than many alternatives (Pryor 2001; Tzortzakis et al. 2007a, b) and can be cost-effectively generated and controlled onsite.

Biocidal Action of Ozone

Where disinfection by ozone has been compared directly to chlorine, there is a wealth of literature to demonstrate the superiority of ozone (Kessel et al. 1943; Restaino et al. 1995; Sheldon and Brown 1986; Langlais et al. 1991; Kim et al. 1999; Xu 1999). Ozone is a potent oxidizing agent, 1.5 times more powerful in the abstraction of electrons ($O_{3\,(aq)}$ oxidizing potential = +2.07 V) than chlorine ($Cl_{(aq)}$ oxidizing potential = +1.36 V), that decomposes to leave no residues whatsoever (breakdown yields only oxygen [O_2] and water [H_2O]) via a series of reactions involving highly reactive, but short-lived, free radical species (including hydroperoxyl [$\bullet HO_2$], hydroxyl [•OH], and superoxide [$\bullet O_2^-$]) plus several reactive intermediaries (including singlet oxygen [$^1O_2$] and hydrogen peroxide [H_2O_2]) (Grimes et al. 1983). The toxicity of ozone is considered to be due at least in part to the generation of these reactive oxygen species (ROS).

The antimicrobial action of ozone depends on factors such as temperature, pH, and the presence of ozone-consuming compounds that can simultaneously affect its physicochemical properties, i.e., solubility, stability, and reactivity, and hence influence the rate of ozone dissolution. *Temperature*: As with other gases, the solubility of ozone is inversely related to temperature. Moreover, temperature affects the rate of reaction of ozone with microbial constituents (reviewed by Langlais et al. 1991;

Khadre et al. 2001). *pH*: pH changes the rate of ozone dissociation and the nature of the products formed. At low pHs (pH < 7.0), microbial inactivation occurs mainly through reaction with molecular ozone. At higher pHs (pH > 7.0), radical-type chain reactions are promoted and ozone rapidly decomposes in solution via the catalytic activity of the hydroxyl ion (Hoigné 1998). *Ozone-consuming compounds*: the half-life of ozone in aqueous solutions increases with water purity (Hill and Rice 1982). Thus, the presence of potentially competing reaction targets (e.g., organic matter) in wash water can reduce the efficacy of ozone-based disinfection methods (Langlais et al. 1991; Khadre et al. 2001).

Another parameter that should be considered is the initial microbiological load and kind of contaminating microorganisms on the products: the higher the microbial load, the lower the effectiveness of O_3 may be. This is due to the fact that the gas eliminates competitive microflora, but at the same time, ozone is continuously degrading to O_2 and, therefore, it may enhance the aerobic microorganisms' counts.

Phytotoxicity of Ozone

The primary target for ozone is believed to be the plasmalemma, the outer semi-permeable barrier of the cell. Mammalian red blood cell (RBC) membranes are often used as a model to examine the impacts of ozone on cellular membranes (Mudd et al. 1996), because they are well characterized with regard to their composition (Fairbanks et al. 1971).

The reaction of ozone with membrane constituents has been the subject of continuing debate. Lipid peroxidation or classical ozonolysis was first considered to underpin the toxicity of ozone. Hence, the phytotoxicity of ozone was thought to be attributable to its interaction with unsaturated lipids, resulting in the generation of free radicals or toxic intermediates such as hydrogen peroxide (H_2O_2), short-chain aldehydes, and modified phospholipids (Pryor et al. 1991). However, more recent studies show that amino acid components (with cysteine, tryptophan, methionine, and histidine amongst the most reactive of membrane proteins) may constitute the principal site of attack (Dominy and Heath 1985). Ozone reacts significantly with the amine (NH_2) and R- (R = alkyl, alkyl sulfur, or unsaturated) components of amino acids. The reactivity of ozone, and its reactive dissolution products, with amino acids is highly pH dependent and may result in a variety of products depending on the R group (e.g., formaldehyde, sulfoxide, ammonium, and cysteic acid). The importance of the impacts of ozone on amino acids has been identified in the inactivation of several proteins including lysozyme (Dooley and Mudd 1982), cytochrome *c* (Mudd et al. 1997), glutamine synthetase (Berlett and Stadtman 1997), glycophorin (Banerjee and Mudd 1992), acetylcholine esterase (AChE) (Mudd et al. 1996), and glyceraldehyde-3-phosphate dehydrogenase (G-3-PDH) (Knight and Mudd 1984). The reactivity of amino acid residues with ozone varies widely from one protein to another, suggesting that the susceptibility to oxidation is

dependent upon the position of the amino acid in the protein (Mudd et al. 1996); the position of the protein in the membrane and amino acid composition (Mudd et al. 1997). Ozone has been shown to alter membrane permeability by increasing the "leakiness" of the membrane and by inhibiting pumps and/or transporters. The ozone sensitivity of the K^+-stimulated ATPase on the plasma membrane has been demonstrated both in vivo (Dominy and Heath 1985) and in vitro (Heath and Castillo 1988). Castillo and Heath (1990) showed that the plasma membrane Ca^{2+}-transport system was also modified (the pump was inhibited and influx increased) by ozone in vivo. The high influx of free Ca^{2+} in the cytoplasm challenges membrane integrity by initiating irreversible and fatal changes in the cell including protein denaturation, organelle disruption, ATP depletion, and activation or inhibition of key enzymes (Heath and Castillo 1988).

There is also evidence that additional ROIs, including hydroperoxyl ($\bullet HO_2$), hydroxyl ($\bullet OH$), singlet oxygen ($^1O_2$), superoxide ($\bullet O^-_2$), and hydrogen peroxide (H_2O_2), contribute to the oxidative damage sustained by cellular constituents under the influence of ozone (Grimes et al. 1983; Polle 1998). In some instances, these secondary ROIs (e.g., $\bullet OH$) can be more damaging than ozone per se, giving rise to tissue-damaging alkoxy radicals and alkyl radicals (Berlett and Stadtman 1997) that may also be important in triggering the "programmed" cells death.

Mechanism of Microbial Inactivation by Ozone

Ozone has been shown to attack numerous bacterial constituents including proteins, unsaturated lipids and respiratory enzymes in cell membranes, peptidoglycans in cell envelopes, enzymes and nucleic acids in the cytoplasm, as well as proteins and peptidoglycan in spore coats and virus capsids.

Cell Envelopes

Initial studies on *Escherichia coli* (*E. coli*) have suggested that ozone primarily attacks the double bonds of unsaturated membrane lipids, sulfhydryl groups of membrane-bound enzymes, and glycoproteins and glycolipids. This leads to alterations in permeability and, ultimately, cell death (Murray et al. 1965). In aqueous ozone solutions ranging from pH 3 to 7, Perez and colleagues (1995) demonstrated that N-acetyl glucosamine, a substance present in bacterial cell walls and viral capsids, was resistant to the action of ozone. The reaction of glucosamine with ozone was fast but the converse reaction occurred with glucose. This observation may help to explain the greater resistance of gram-positive bacteria (bacteria containing vast amounts of peptidoglycan in their cell wall) to ozone treatments compared with gram-negative bacteria (Khadre et al. 2001).

Enzymes

It has been reported that the residual activities of periplasmic alkaline phosphatase and cytoplasmic β-galactosidase decrease along with the survival rate of *E. coli* in response to ozone exposure (Takamoto et al. 1992). The activity of the latter enzyme decreased to a greater degree than the former. Induction followed by inactivation of catalase and superoxide dismutase activity has also been reported in ozone-treated *E. coli* (Whiteside and Hassan 1987).

Nucleic Material

Ozone has been shown to be mutagenic (L'Herault and Chung 1984) and capable of inducing DNA degradation (i.e., producing single- and double-strand breaks and reducing its transformation capability) in different strains of *E. coli*. Specific modifications of the genetic material of *E. coli*, before destruction of the cell membrane, have been demonstrated, with ozone resistance proposed to be related to DNA repair efficiency (Hamelin 1985).

Virus Inactivation

Owing to the lack of a plasma membrane, alternative mechanisms have been proposed to explain the inactivation of viruses by ozone including damage to the protein coat (Sproul et al. 1982) or nucleic acid component (Roy et al. 1981). Using electron microscopy, Kim et al. (1980) examined the mechanism of ozone inactivation of bacteriophage f2 ribonucleic acid (RNA). They observed that ozonation cleaved the phage coat into its protein subunits, liberating RNA and disrupting adsorption to the host pili. The naked RNA could then be attacked by ozone. In support of these findings, Shinriki and colleagues (1988) showed ozone attacked both protein coat and RNA in tobacco mosaic virus.

Applications

Industrial Applications for Ozone

Since its discovery, ozone has been employed in many commercial applications in both aqueous and gaseous form. In the United States, was issued one of the first patents utilizing ozone to convert a coal–oil mixture into paints and varnish. Other significant ozone patents soon followed, notably for an apparatus to deodorize sewer

gases, and for a full-scale ozone–generating plant for water treatment (reviewed in Hill and Rice 1982). By 1906, ozone was employed for the commercial-scale disinfection of potable water across France, and ozonation is now commonly adopted for the treatment of drinking and wastewater throughout the world (reviewed in Hill and Rice 1982). The number of applications for ozone grows rapidly, and it is now used for the treatment and purification of ground and surface waters, domestic and industrial wastewater plus swimming pools, and cooling tower systems (Langlais et al. 1991; Hoigné 1998; Gottschalk et al. 2002).

Ozone is one of the most potent oxidants in nature, and the mechanism(s) underlying its therapeutic action remain to be understood. Several possible explanations have been proposed, including the activation or generation of reactive oxygen intermediates (ROI) which function as physiological enhancers of various biological processes including the expression of proteins that play key antioxidative roles (see Bocci 1996a, b). Ex vivo studies investigating the effects of ozone treatment on red blood cell metabolism and hemolysis (Zimran et al. 1999) plus neutrophil functions in vitro (Margalit et al. 2001) indicate no adverse effects. Further studies are required to demonstrate the safety of medical application of ozone and these represent a prerequisite for the integration of ozone therapy into conventional medicine.

Aqueous Ozone

Ozone in water is often described as an alternative to hypochlorite as a disinfectant or sanitizer. Ozone can oxidize various soluble metal salts to yield insoluble compounds that can be separated subsequently by precipitation and filtration. Iron, sulfur, and manganese contaminants can be removed from metal sulfates (also from drinking water to control the taste and odor of fresh water) or, for instance, vanadium can be recovered during the treatment of uranium ores, and cobalt separated from nickel salts (Rice 2002). Ozone can oxidize many organic compounds and can thus be used to reduce pesticide residues in process and discharge water (Nickols and Varas 1992). Tests show that 95 % of imazalil, thiabendazole, and sodium orthophenyl phenate (Na-OPP) residues in wash water are destroyed within 30 min (Smilanick 2003). Moreover, ozone is an effective bleaching agent and initiates oxidation reactions with organic compounds. Thus, for example, amine oxides can be produced from tertiary amines, sulphoxides, and sulphones from dialkyl thioethers (Maggiolo and Blair 1959), without the formation of by-products. Ozone is also used for the synthesis of organic materials, e.g., ozone is used to oxidize oleic acid to produce azelaic and pelargonic acids, for various applications in the plastics industry as well as the production of synthetic lubricants (Rubin 2003).

Ozone has been long used for the treatment of waste water–facilitating recycling (Langlais et al. 1991; Williams et al. 1995; Piper 1998; Geering 1999; Rice 1999). It has also been widely adopted for the disinfection of drinking water supplies (e.g., water treatment plants, bottled water/soft drinks plants) as well as for the treatment of poultry and salmon hatcheries and aquaria (Sheldon and Brown 1986; Whistler and Sheldon 1989; Cryer 1992). More recently, dissolved ozone has

been employed for the disinfection and cleansing of wine barrels/casks, eggs, brewing/cider manufacturing facilities, shoes, and various foodstuffs (including supermarket displays of fresh produce in the USA) (Rice 2002). Ozone is also used to treat dairy and swine effluent and water at theme parks as well as public and in-home spas (Suslow 2004). The gas has also been incorporated in ice to assist in odor management during the long distance transport of fresh fish (Rice 2002). In the USA, ozonated water is in use in many fruit packing facilities to disinfect produce, reduce the spread of pathogens, and sanitize dump tanks, flumes, and the surfaces of packing equipment (Hampson and Fiori 1997). An overlooked action of ozone is its potential to induce a suite of defence-related responses in plant tissues (Sandermann et al. 1998).

Gaseous Ozone

Ozone gas is commonly used to eradicate odors (e.g., cigar-cigarette smoke, perspiration, and animal odors; Rice 2002) and is used throughout the world to "freshen" homes and workplaces. The gas is also widely employed on pack lines for medical and pharmaceuticals (reviewed in Bocci et al. 2001), for the pre-treatment of plastic films used for lamination to metals and the pre-treatment of plastics prior to plating as well as the bleaching of paper pulp, kaolin, and textiles (see review Rice 2002). Indeed, the therapeutic effects of ozone exposure and transfusion are commonly utilized in Germany and Eastern Europe for the treatment of a variety of disorders including burns, infections, inflammation, and pressure sores (Bocci 1994).

The gas is also being examined as a weed control agent in crops raised under protected cultivation in the field and as an insecticide as well as a sanitation tool (Kells et al. 2001). Since ozone reacts rapidly with ethylene (effectively scrubbing ethylene from the atmosphere), ozone filtration has been used to eradicate ethylene in stored/shipped fruit (such as bananas and apples), extending "shelf life" and facilitating the controlled ripening of fruit to meet market demands (Rice et al. 1982; Rice 2002).

Applications in the Food Processing for Ozone

There are many potential applications for ozone in the agricultural, horticultural, and food processing sectors. In 1997, an expert panel in conjunction with the US Food and Drug Administration (FDA) awarded ozone "Generally Recognized As Safe (GRAS)" status for use in direct food contact applications (Graham 1997) and in 2000, a Food Additive Petition (FAP, required for approval of a candidate material as a food additive) was filed by the Electric Power Research Institute (EPRI) supported by the FDA to allow the use of ozone as an antimicrobial agent for direct contact with foods (Rice and Graham 2001). This FAP was approved on June 26, 2001

Table 1 Ozone applications in food processing

Ozone usage	Product	References
Storage and transport	Fish	Chen et al. (1997)
	Meats	Kaess and Weidemann (1968); Reagan et al. (1996)
	Poultry	Sheldon and Brown (1986); Whistler and Sheldon (1989)
Wash-water treatment	Fresh produce	Beuchat (1992); Kim et al. (1999)
Shelf life and storage	Cheese	Horvath et al. (1985)
	Grains and spices	Kells et al. (2001)
	Fresh produce	Sarig et al. (1996); Pérez et al. (1999); Skog and Chu (2001); Palou et al. (2002); Tzortzakis et al. (2007a, 2008, 2011)
	Garlic and wheat flour	Galdun et al. (1984); Naitoh et al. (1989)
Process water and effluents	Slaughterhouses, olive oil mills	Joret et al. (1982); Benitez et al. (1997)
Pesticide removal	Fresh produce	Ong et al. (1996); Hwang et al. (2001)
Aflatoxin detoxification	Peanut and cottonseed	Maeba et al. (1988)
Mold control and aging	Wines and beers	Tenney (1973)
Preservation and sterilization	Feeds and tobacco products	Bundschuh and Rilling (1996)

(21 CFR Part 173, Docket No. 00F-1482). This change in legislation has resulted in growing commercial interest in the use of ozone for various applications in the food sector not only in the USA, but all over the world (see Table 1).

Gaseous ozone is also being tested as a replacement for methyl bromide, a widely used fumigant used to control soil-borne fungal pathogens, nematodes, and weeds. Methyl bromide was scheduled for worldwide withdrawal by 2005 in developed countries, and by 2015 in developing countries, under the United Nations Directive on ozone-depleting substances, which constitutes part of the Montreal Protocol (Bell 2000). Gaseous ozone is also being tested as a weed control agent in crops forced early with the aid of various forms of protection (e.g., clear plastic film) (see Pryor 2001).

Impacts of Ozone on Fresh Produce

The highest microbial reductions are often observed at the highest doses of ozone (Kim and Yousef 2000; Bialka and Demirci 2007; Alexandre et al. 2011); however, as the sensitivity to ozone varies among different commodities, it is necessary to establish an optimal treatment (dose) for each product (Karaca 2010) to avoid

tissue damage, which among other things leads to an increased susceptibility to microbial infection. A number of studies (Kim and Yousef 2000; Khadre et al. 2001; Ketteringham et al. 2006) focused on the antimicrobial efficacy of ozone treatment and not on the effects of ozone on nutritional and sensory quality of the product. Only those treatments that reduce microbial contamination of the product without having an adverse effect on product's visual, textural, and nutritional quality (Allende et al. 2008) can be recommended and subsequently incorporated into the supply chain.

In other studies, ozone enrichment of stored foodstuffs has been shown to reduce microbial spoilage, provide odor control, and result in extended storage life by destroying the ripening/senescence-promoting hormone ethylene (Skog and Chu 2001; Palou et al. 2001). Ozone can be a good alternative sanitizer for fresh fruits and vegetables and it destroys microorganisms by progressive oxidation of vital cellular components (Das et al. 2006). Recently, high research interest is taking place, examining the possible molecular and biochemical aspects of ozone impacts on fruit and/or pathogen with possible induced resistance at the fresh produce level (Minas et al. 2010; Tzortzakis et al. 2007a, b, 2008, 2011, 2013).

Aqueous Ozone

In the aqueous phase, ozone has been employed in the sanitation of wash water (Ogawa et al. 1990; Spotts and Cervantes 1992) and processing equipment (Gorman et al. 1995). Aqueous ozone treatments have also proved effective in degrading pesticide residues on fresh produce (Ong et al. 1996; Hwang et al. 2001), thereby reducing concerns over food and environmental safety. Recently, various applications have utilized ozonated ice and the low-pressure administration of ozonated water in food chillers and display cabinets to retain the freshness and quality of supermarket produce (reviewed in Rice 2002).

Previous experimentation has identified ozonated water to be an effective means of decontaminating lettuce (Kim et al. 1999; Olmez and Akbas 2009), apples (Achen and Yousef 2001), table grapes (Sarig et al. 1996), carrots (Williams et al. 1995; Chauhan et al. 2011), artichoke (Restuccia et al. 2014), Chinese cabbage (Kondo et al. 1989), celery (Zhang et al. 2005), spinach (Rahman et al. 2010; Karaca and Velioglu 2014), and strawberries (Alexandre et al. 2011; Aday et al. 2014). Studies conducted on pears (Spotts and Cervantes 1992) and tomatoes (Ogawa et al. 1990) reveal that exposure to 3.8 g of ozone ml^{-1} water for 10 min results in equivalent depressions in fungal pathogen counts as traditional chlorine-based disinfection treatments. Spore germination on the surface of fruit was much reduced, but spores placed in wounds were unaffected by ozone treatment—resulting in a situation similar to that for chlorine-based disinfection methods without concerns over halogenated residues (Eckert and Eaks 1989). A similar ozone exposure (3.8 g of ozone ml^{-1} water for 10 min) regime was shown to kill sporangia of *Bremia lactucae* in water within a minute of contact, while those on lettuce leaves

were protected (25 min contact at a similar level of ozone exposure did not reduce viability of sporangia) Scherm et al. (1993). Ozone exposure (1–5 ppm for different time of exposure ranging into 0.5–5 min) in lettuce (including fresh-cut lettuce) reduced microbial loan (*E. coli* O157:H7; *Listeria monocytogenes*) with positive effects on lettuce quality regarding vitamin C, β-carotene, and sensory quality (Rico et al. 2006; Olmez and Akbas 2009). The suitability of chlorine dioxide (60 mg L^{-1}/10 min), peracetic acid (100 mg L^{-1}/15 min), and ozonated water (1.2 mg L^{-1}/1 min) as alternative sanitizers to sodium hypochlorite (150 mg L^{-1} free chlorine/15 min) was evaluated for minimally processed lettuce, resulting in a 2.5, 1.1, and 0.7 reduction of log cycle on count of microbial load (total coliforms, *E. coli*, *Salmonella* spp., psychrotrophic and mesophilic bacteria, yeasts, and molds) as reported by Bachelli et al. (2013).

Ozone-treated (50,000 ppm for 1 up to 64 min) raspberries and strawberries achieved reduction in microbial loan of *E. coli* O157:H7 and *Salmonella enteric* (Bialka et al. 2008). The efficiency of three aqueous ozone concentrations (0.075 ppm, 0.15 ppm, 0.25 ppm) and two exposure times (2 and 5 min) were investigated for maintaining strawberry quality. The results have shown that low (0.075 ppm) and middle (0.15 ppm) ozone concentrations can be applied to extend the shelf life of strawberries by at least 3 weeks under refrigerated conditions, as delayed the changes in pH, total soluble solids, firmness and electrical conductivity as well as prevented mold growth during storage (Aday et al. 2014). Restaino et al. (1995) also investigated the antimicrobial effects of ozonated water against food related microorganisms and determined that ozone effectively killed such gram-positive bacteria as *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis*, and such Gram-negative bacteria as *Pseudomonas aeruginosa*, and *Yersinia enterocolitica*. Restaino et al. (1995) also determined that ozone destroyed the yeasts *Candida albicans* and *Zygosaccharomyces bacilli* and spores of *Aspergillus niger*.

The efficacy of ozonated water (for 1, 5, or 10 min) at different temperatures (room temperature, mild heated [50 °C] and refrigerated [4 °C] temperature) and/or pH washing for inactivation of *Salmonella enterica* Typhimurium on green onions, grape tomatoes, and green leaf lettuces revealed that different surface structures of fresh produce significantly impact the antimicrobial efficacy of ozonated water washing operated under various parameters (time, temperature, and pH) (Xu and Wu 2014).

Examining the efficacy of ozone (12 mg L^{-1}) toward chlorine (100 mg L^{-1}) water against *E. coli* and *Listeria innocua* on lettuce, spinach, and parsley, it was found that chlorine and ozone washes resulted in average log reductions (±standard error) of 2.9 ± 0.1 and 2.0 ± 0.3 for *E. coli* in the vegetables tested, respectively, while the efficiency of ozone (2.2 ± 0.1 log) was very close to that of chlorine (2.3 ± 0.1 log) on *L. innocua*. Moreover, ascorbic acid and total phenolic contents and antioxidant activity in ozone-treated samples were 40.1 %, 14.4 %, and 41.0 %, respectively, less than the control samples (Karaca and Velioglu 2014).

Gaseous Ozone

Conceivable benefits of adding ozone to air in packinghouses and storage rooms include control of postharvest diseases on fruit, retarding the production of spores from decaying fruit, sanitation of surfaces, and ethylene removal (see Table 2). One use under investigation is the ozone enrichment of storage rooms and refrigerated containers, but there were contrasting reports in the past as to the efficacy of the gas deployed for such applications (Rice et al. 1982).

Treatment of fresh produce with ozone has reduced fungal development, measured as lesion size, on a number of products, e.g., in apples (Sharpe et al. 2009) exposed to gaseous ozone at 0.45 ppm for 2 days, in broccoli (Forney et al. 2003) continuously exposed to ozone at 0.2 and 0.7 ppm for 12 days, in carrots exposed to gaseous ozone at 0.45 ppm for 2 days (Sharpe et al. 2009), at 1 ppm for 4 days (Forney et al. 2007), intermittently (8 h per day) exposed to ozone at 15 ppm for 4 weeks (Liew and Prange 1994), or continuously exposed to low level of ozone at 0.05 ppm over a 6-month storage period (Hildebrand et al. 2008).

Previous work by Spalding (1968), and more recently by Palou et al. (2001, 2002), reveals that atmospheres enriched by 0.3–0.5 ppm ozone significantly retard the growth of *Monilinia fructicola* (brown rot), *Rhizopus stolonifer* (rhizopus rot), and *Botrytis cinerea* (grey mold) on artificially inoculated peaches. On the other hand, Sarig and colleagues (1996, 1997) reported ozone exposure to be an effective means of controlling *R. stolonifer* and *B. cinerea* proliferation in table grapes, but these effects were not reproduced during similar experiments performed by Spalding (1968) and Shimizu et al. (1982). Liew and Prange (1994) reported a 50 % reduction in the daily growth rate of *B. cinerea* and *Sclerotinia sclerotiorum* in carrots exposed to 60 ppm ozone—with the action of ozone clearly fungistatic and not fungicidal. Lower levels of ozone (1.0 ppm O_3 at 8 °C) administered during storage has been shown to significantly reduce the development of *B. cinerea* and *S. sclerotiorum* on carrots, with ozonation capable of reducing the proliferation of fungicide resistant strains (Forney et al. 2001). Barth and colleagues (1995) also report that ozone enrichment (0.3 ppm O_3 at 2 °C) suppresses pathogen development, in this case during storage of thornless blackberries. Indeed, studies contacted by Ewell (1940) and Kuprianoff (1953) indicate that the "shelf life" of strawberries, raspberries, apples, currants, and grapes can be doubled if produce is maintained in 2.0–3.0 ppm gaseous ozone for a few hours per day. Bulbs and tubers may also benefit from ozone enrichment. For example, Baranovskaya and coworkers (1979) and Tallentire (2007) highlight the advantages of introducing ozone into refrigerated potato stores for a skin finish perspective without effects on tuber quality.

Several studies report reduced sporulation in fungi maintained in an ozone-enriched atmosphere. For example, Krause and Weidensaul (1978) report that ozone treatment (0.3 ppm for two 6 h exposure periods) significantly inhibits sporulation and germination during in vitro studies on *B. cinerea*, whilst Palou et al. (2001) show that spore production by lesions on citrus fruit infected with *Penicillium digitatum* (green mold) and *Penicillium italicum* (blue mold) is much reduced by exposure to ozone. Moreover, Margosan and Smilanick (2000) and Smilanick et al. (2002)

Table 2 Effect of gaseous ozone treatment on postharvest fungal and bacteria pathogens of fruit and vegetables

	Commodity	Pathogen(s)	Ozone (ppm; mg L^{-1};µl L^{-1}; µmol mol^{-1})	Effects		Reference
				Antimicrobial activity	Produce quality	
FUNGI						
Berry fruits	Blackberry (*Rubus* sp.)	*Botrytis cinerea*; *Rhizopus* sp.	100; 300 ppm	++	+	Barth et al. (1995)
	Blueberry (*Vaccinium angustifolium*)	–	0.5 ppm 0.7 ppm	0 +	Na +	Spalding (1968); Song et al. (2003b)
	Cranberries (*Vaccinium vitis-idaea*)	Rooting	0.27–0.60 ppm	–	–	Norton et al. (1968)
	Strawberry (*Fragaria ananassa*)	*Botrytis cinerea*	1.5 µl L^{-1}	+	+,±	Nadas et al. (2003)
	Strawberry (*Fragaria ananassa*)	*Botrytis cinerea*; *Rhizopus stolonifer*	0.5 ppm	0	–	Spalding (1968)
	Strawberry (*Fragaria ananassa*)	*Botrytis cinerea*	350 ppm	±	+	Pérez et al. (1999)
	Strawberry (*Fragaria ananassa*)	*Botrytis cinerea*	0.05–5.0 ppm	+	+	Tzortzakis et al. (2005)
	Strawberry (*Fragaria ananassa*)	Yeast and mold counts	5000 mg L^{-1}	0	–	Allende et al. (2007)
	Strawberry (*Fragaria ananassa*)	*Botrytis cinerea*	0.075 mg L^{-1}	+	+	Aday and Caner (2014)
	Grape (*Vitis vinifera*)	*Botrytis cinerea*	5000 ppm (1 h)	+	Na	Gabler et al. (2010)
	Grape (*Vitis vinifera*)	*Botrytis cinerea*	0.3–1.0 ppm	–	0	Palou et al. (2002)
	Grape (*Vitis vinifera*)	*Botrytis cinerea*	0.5 ppm	0	Na	Spalding (1968)
	Grape (*Vitis vinifera*)	*Botrytis cinerea*	0.1 ppm	+	Na	Tzortzakis et al. (2007a)
	Grape (*Vitis vinifera*)	*Botrytis cinerea*	0.05–1.0 ppm	+	Na	Pintado et al. (2004)
	Grape (*Vitis vinifera*)	*Rhizopus stolonifer*	8 mg/min (500 ml min^{-1})	+	+	Sarig et al. (1996)

(continued)

Table 2 (continued)

	Commodity	Pathogen(s)	Ozone (ppm; mg L^{-1};µl L^{-1}; µmol mol^{-1})	Effects		Reference
				Antimicrobial activity	Produce quality	
	Grape (*Vitis vinifera*)	*Botrytis cinerea*; *Penicillium digitatum*; *Penicillium italicum*	200–350 µl L^{-1}	+	Na	Ozkan et al. (2011)
	Grape (*Vitis vinifera*)	*Botrytis cinerea*; *Penicillium digitatum*; *Penicillium italicum*	2 ppm	+	±, −	Cayuela et al. (2009)
	Grape (*Vitis vinifera*)	*Botrytis cinerea*; *Penicillium* spp.; *Alternaria* spp.	0.075–0.500 µl L^{-1}	+ −	+ +	Feliziani et al. (2014)
	Grape (*Vitis vinifera*)	*Botrytis cinerea*	0.1 ppm continually 8 ppm (30 min every 2.5 h)	0	−	Artes-Hernandez et al. (2004, 2007)
Pome fruits	Apple (*Malus domestica*)	–	0.006 ppm (4 h/day)	+	+	Bazarova (1982)
	Apple (*Malus domestica*)	*Botrytis cinerea*; *Penicillium expansum*; *Phialophora malorum*	3.25 ppm	+	++	Schomer and McColloch (1948)
	Apple (*Malus domestica*)	–	0.04 µl L^{-1}	Na	+	Skog and Chu (2001)
	Apple (*Malus domestica*)	–	2–10 cm^3/m^3	Na	+	Kuprianoff (1953)
	Pear (*Pyrus communis*)	–	0.04 µl L^{-1}	Na	+	Skog and Chu (2001)
	Pear (*Pyrus communis*)	–	2.14–6.24–21.4 mg m^{-3} (1 h/day)	Na	+	Zao et al. (2013)
Stone fruits	Peach (*Prunus persica*)	*Botrytis cinerea*; *Mucor fructicola*; *M. piriformis*; *Penicillium expansum*	0.3–1.0 ppm	+	0	Palou et al. (2002)
	Peach (*Prunus persica*)	*Monilinia fructicola*; *Rhizopus stolonifer*	0.5 ppm	0	+	Spalding (1968)

	Plum (*Prunus domestica*)	*Botrytis cinerea*	0.1 ppm	+	Na	Tzortzakis et al. (2007a)
Citrus fruits	Citrus spp.	*Penicillum* spp.	0.05–5.0 ppm	+	Na	Pintado et al. (2004)
	Clementine (*Citrus* × *clementina*)	*Botrytis cinerea*	0.1 ppm	+	Na	Tzortzakis et al. (2007a)
	Lemons (*Citrus limon*)	*Penicillium italicum*	0.3 ppm	+	0	Palou et al. (2001)
	Orange (*Citrus sinensis*)	–	Up to 40 ppm	Na	+	Kuprianoff (1953)
	Orange (*Citrus sinensis*)	*Penicillium digitatum*; *P. italicum*	0.3–0.72 ppm	+	0	Palou et al. (2001, 2003)
	Tangerines (Citrus tangerina)	*Penicillium digitatum*	200 mg L^{-1} (2 h/day)	+	0	Whangchai et al. (2010)
Tropical fruits	Banana (*Musa* spp.)	–	1.5–90 cm^3 m^{-3} air	Na	±	Gane (1937)
	Kiwi (*Actinidia deliciosa*)	*Botrytis cinerea*	4 mg h^{-1}	+	+	Barboni et al. (2010)
	Kiwi (*Actinidia deliciosa*)	*Botrytis cinerea*	0.3 µl L^{-1} (2–144 h)	+	+	Minas et al. (2010)
	Longan (*Dimocarpus longan*)	*Lasiodiplodia* sp.; *Cladosporium* sp.	0.02 ml L^{-1} (0–15–30–60–120 min)		+	Whangchai et al. (2006)
	Papaya (*Carica papaya*)	*Colletotrichum gloeosporioides*	1.5–2.5–3.5–5.0 µL L^{-1}; 96 h	+	+	Ong and Ali (2015); Ong et al. (2014)
	Persimmon (*Diospyros lotus*)	–	0.15 ppm	Na	+	Salvador et al. (2006)
Vegetables and others	Bean sprouts (*Phaseolus aureus*; *Glycine max*)	–	20–200 ppm	+	+	Naitoh and Shiga (1989)
	Broccoli (*Brassica oleracea*)	–	0.04 µl L^{-1}	Na	+	Skog and Chu (2001)
	Cantaloupe (*Cucumis melo*)	*Penicillium* sp.; *Alternaria tenuis*	0.5 ppm	+	Na	Spalding (1968)
	Carrot (*Daucus carota*)	*Botrytis cinerea*; *Sclerotinia sclerotiorum*	7.5–60 µg L^{-1}	+	–	Liew and Prange (1994)

(continued)

Table 2 (continued)

Commodity	Pathogen(s)	Ozone (ppm; mg L^{-1};µl L^{-1}; µmol mol^{-1})	Effects		Reference
			Antimicrobial activity	Produce quality	
Carrot (*Daucus carota*)	*Botrytis cinerea*; *Sclerotinia sclerotiorum*	0.115–1.0 µl L^{-1} 0.05 µl L^{-1}	+, ± +	+ ±	Forney et al. (2007); Hildebrand et al. (2001); Hildebrand et al. (2008)
Carrot (*Daucus carota*)	*Botrytis cinerea*; *Sclerotinia sclerotiorum*	0.3–1.0 µl L^{-1}	+	+	Song et al. (2003a)
Corn (*Maize* sp.)	*Aspergillus parasiticus*	20,000–50,000 ppm	+	Na	Kells et al. (2001)
Cucumber (*Cucumis sativus*)	–	0.04 µl L^{-1}	+	+	Skog and Chu (2001)
Cucumbers (*Cucumis sativus*)	Rotting	0.1 µmol mol^{-1}	+	+	Glowacz et al. (2015)
Green bean (*Phaseolus* sp.)	*Alternaria tenuis*	0.5 ppm	0	–	Spalding (1968)
Mushroom (*Agaricus bisporus*)	–	0.04 µl L^{-1}	Na	–	Skog and Chu (2001)
Onion (*Allium cepa*)	*Penicillium* sp.; *Aspergillus* sp.	50–250 ppm	+	+	Song et al. (2000)
Onion (*Allium cepa*)		0.05–0.25 ppm	+	+	Fan et al. (2001)
Onion (*Allium cepa*)		0.2 µg L^{-1} (8 h/d, 5 days/week)	+	+	Faitel'berg-Blank et al. (1979)
Pepper (*Capsicum annuum*)	Rotting	0.1–0.3 µmol mol^{-1}	+	+	Glowacz et al. (2015)
Potato (*Solanum tuberosum*)	*Helminthosporium solani*	0.05–5.0 ppm	+	Na	Tallentire (2007)
Potato (*Solanum tuberosum*)		0.2 µg L^{-1} (8 h/day, 5 days/week)	+	+	Faitel'berg-Blank et al. (1979)
Tomato (*Solanum lycopersicum*)	*Botrytis cinerea*; *Alternaria alternata*; *Colletotrichum coccodes*; *Cladosporium herbarum*	0.05–5.0 ppm	+	+	Tzortzakis et al. (2007a, 2007b, 2008) Roberts (2005)

	Tomato (*Solanum lycopersicum*)	–	10 µL L^{-1}; 10 min	Na	+	Rodoni et al. (2010)
	Zucchini (*Cucurbita pepo*)	Rotting	0.1 µmol mol^{-1}	+	+	Glowacz et al. (2015)
BACTERIA or OTHER						
Berry fruits	Blueberry (*Vaccinium angustifolium*)	*Enterobacter agglomerans*; *Pseudomonas fluorescens*	1.0 ppm	+	Na	Crowe et al. (2007)
	Blueberry (*Vaccinium angustifolium*)	*Escherichia coli* O157:H7; *Salmonella enterica*	30,000 ppm (2–64 min)	+	Na	Bialka and Demirci (2007)
	Raspberries (*Rubus idaeus*)	*Escherichia coli* O157:H7; *Salmonella enterica*	50,000 ppm (2–64 min)	+	Na	Bialka and Demirci (2007)
	Strawberry (*Fragaria ananassa*)	*Escherichia coli* O157:H7; *Salmonella enterica*	50,000 ppm (2–64 min)	+	Na	Bialka and Demirci (2007)
Tropical fruits	Papaya (*Carica papaya*)	Coliforms, mesophilic bacteria	9.2 µL L^{-1} (10–20–30 min)	+	+	Yeoh et al. (2014)
Vegetables and others	Cantaloupe (*Cucumis melo*)	*Salmonella enterica*	10,000 ppm (10 min)	+	Na	Selma et al. (2008a)
	Carrots (*Daucus carota*)	*Escherichia coli* O157:H7	1000–3500 ppm (5–15 min)	+	Na	Singh et al. (2002)
	Melons (*Cucumis melo*)	*Escherichia coli* O157:H7; mesophilic and psychrotrophic bacteria, molds and coliforms	10,000 ppm, 30 min	+	+	Selma et al. (2008b)
	Pepper (*Capsicum annuum*)	*Salmonella enteritidis*; *Escherichia coli*	2–8 mg L^{-1} (10–40 min)	+	Na	Han et al. (2002)
	Pepper (*Capsicum annuum*)	*Salmonella enteritidis*; *Escherichia coli*; *Staphylococcus aureus*	6–7 mg L^{-1} (10–60 min)	+	Na	Zhao and Cranston (1995)

(continued)

Table 2 (continued)

	Commodity	Pathogen(s)	Ozone (ppm; mg L^{-1};µl L^{-1}; µmol mol^{-1})	Effects		Reference
				Antimicrobial activity	Produce quality	
	Pepper (*Capsicum annuum*)	*Escherichia coli* O157; *Listeria monocytogenes* and *Salmonella enterica sv. Typhimurium*	0–1–3–5–7–9 ppm (for 0.5, 3, 6, and 24 h)	+	Na	Alwi and Ali (2014)
	Pepper (*Capsicum annuum*)	Mesophilic and psychrotrophic bacteria, molds	0.7 ppm (1–5 min)	+	Na	Horvitz and Cantalejo (2012)
	Pepper (*Capsicum annuum*)	*Escherichia coli* O157:H7; *B. cereus*	0.1–9.0 ppm (up to 360 min)	+	±, −	Akbas and Ozdemir (2006)
	Pepper (*Capsicum annuum*)	*Escherichia coli* O157:H7; *B. cereus*	16.33–66 mg L^{-1} (7.5–15–30–60 min)	+	+	Inan et al. (2007)
	Spinach (*Spinacia oleracea*)	*Escherichia coli* O157:H7	750–2000 ppm (5 min) 935 ppm (5–30 min) 5–10 ppm (3 days)	+	Na	Klockow and Keener (2009); Vurma et al. (2009)
	Tomato cherry (*Solanum lycopersicum*)	*Salmonella enteritidis*	5–30 mg L^{-1} (0–20 min)	+	Na	Das et al. (2006)
	Tomato cherry (*Solanum lycopersicum*)	Mesophilic and psychrotrophic bacteria	4 ppm (every 30 min/3 h)	+	+	Aguayo et al. (2006)
	Tomato cherry (*Solanum lycopersicum*)	*Escherichia coli* O157:H7	5 ppm (5–15 min)	+	Na	Bermudez-Aguirre and Barbosa-Canovas (2013)
	Tomato (*Solanum lycopersicum*)	*Escherichia coli* ATCC 25922	0.025–0.045 ppm	+	+	Venta et al. (2010)

Table key:
Pathogen(*s*): (not recorded)
Effects: + (controlled microbial growth/produce quality enhanced); ++ (complete elimination of surface microflora); − (no effect on or stimulation in microbial growth/adverse effect on produce quality); ± (variable dependent on storage conditions); Na (not assessed); 0 (no effect on microbial growth or produce quality)

reported that germination of spores of *B. cinerea*, *M. fructicola*, *P. digitatum*, and *R. stolonifer* is inhibited by ozone enrichment. Similarly, exposure of 'Valencia' oranges and 'Eureka' lemons to ozone (1.0 ± 0.05 ppm at 10 °C) delays attack by *P. digitatum* and *P. italicum* and reduces the rate of proliferation of both fungal diseases. In other studies, continuous exposure of fresh commodities to ozone during storage was reported to reduce decay. Hildebrand et al. (2001) observed a reduction of decay when carrots inoculated with *S. sclerotiorum* and *B. cinerea* were held in 0.115–0.530 μL L^{-1} ozone at 10 °C for 20 days.

Sanitation of equipment and fruit surfaces with ozone gas has been reported, but doses of ozone that kill postharvest pathogenic fungi in a few hours or days are very high and to use them requires very ozone-tolerant products, corrosion-resistant facilities that contain the gas, and presumably other measures to scrub ozone from vented air and other safety measures (Smilanick 2003). To kill spores of the pathogens that cause green mold, blue mold, and sour rot (*Penicillium digitatum*, *P. italicum*, and *Geotrichum citri-aurantii*, respectively) in humid air (about 95 % RH) at 5 °C within 1 h, about 200 ppm ozone was required. If the air was dry (35 % RH), a dose five to ten times higher was required. The feasibility of using ozone gas to rapidly kill fungi on fresh fruit is limited because in a survey of more than 60 fresh products it was found that many were injured by 200 ppm applied for 1 h. Onions, citrus fruit, russet potatoes, cantaloupes, waxed apples, and kiwi fruit were unharmed when examined 1 week after treatment, while stone fruit, mushrooms, bananas, leafy vegetables of many kinds, snow peas, mangos, broccoli, brussels sprouts, and un-waxed apples and pears were severely harmed (Smilanick 2003).

When strawberries exposed to ozone (up to 8 ppm), the results indicated that the treatment of 4 ppm ozone could inhibit the decrease of ascorbic acid, peroxidise (POD) activity, and catalase (CAT) activity, and reduce weight loss rate and malondialdehyde (MDA) content. The treatment delayed the senescence of strawberry, with a significantly lower respiration rate. Thus, the best concentration of ozone was 4 ppm, and ozone treatment could be a good candidate for maintaining postharvest quality of strawberry and provide a longer storage life (Zhang et al. 2011). The effectiveness of ultrasound (30 W) and the chemical sanitizers ozone (0.075 mg L^{-1}) and chlorine dioxide (6 mg L^{-1}) alone and in combination were examined on strawberry storage life. All treatments inhibited mold growth during storage. However, individual ozone treatment causes bleaching of the fruit. Ultrasound treatments with ozone and chlorine dioxide were more beneficial for quality factors such as pH, total soluble solids, electrical conductivity, and texture compared with the individual treatment or untreated fruit. Thus, it was suggested that combinations of ultrasound plus ozone and chlorine dioxide could be used for prolonging shelf life of strawberries (Aday and Caner 2014).

Carrots and tomatoes exposed to ozone enhanced several fruit quality parameters such as sugars, antioxidative status, as well as aroma and stress volatiles (ethanol, hexanal) and maintained fruit firmness (Forney et al. 2007; Tzortzakis et al. 2007a, b). Additionally, ozone-enriched atmosphere (0.05, 0.2, 1.0, and 5.0 ppm) reduced disease development on tomatoes by *B. cinerea*, *Alternaria alternata*, *Colletotrichum coccodes*, and *Cladosporium herbarum*, with great effects on spore germination and spore production suppression as well as the induced resistance of

pre-exposed tomatoes to ozone against fungal attack (Tzortzakis et al. 2005, 2007a, b, 2008, 2011, 2013). Han et al. (2002) reported more than 5 log reductions of *E. coli* O157:H7 on green peppers after the treatments with 7 mg L^{-1} ozone for 20 and 40 min, under 85 % relative humidity and at 22 °C. Similarly, Zhao and Cranston (1995) reported that 3–6 log reduction of *E. coli*, *Salmonella* spp., and *Staphylococcus aureus* on black pepper could be achieved by passing ozonized air (6–7 mg L^{-1}; 6 L min^{-1}) through black pepper for 60 min.

Impacts of Ozone on Human Health

Worldwide, the incidence of notified foodborne disease has increased significantly during the past decade, giving rise to serious public health concerns. The transmission of respiratory infections in indoor environments represents a major public concern, whereas inside building might exist as epidemiology, especially by ventilation systems (Kowalski et al. 1998).

In any food processing environment, surfaces that appear clean visually can still be contaminated with large number of viable microorganisms that could contaminate food (Adams and Moss 1997). After removal of food residues, therefore, additional measures may be needed to reduce the number of microorganisms present. Such measures, known as terminal disinfection or microbiological cleaning, are especially important in food handling environments, where food contact surfaces must have only minimum levels of microbial contamination, for example, in the production of ready-to-eat foods (Moore et al. 2000).

E. coli O157:H7, which is known to cause hemorrhagic colitis and hemolytic uremic syndrome, has emerged as a foodborne pathogen of major public health concern. A wide variety of foods including meat, milk, fruit juices, and vegetables are possible vehicles of *E. coli* O157:H7. The *E. coli* O157:H7 outbreaks occurring from the consumption of fresh-cut vegetables (FCVs) have led investigators to search for novel methods of controlling *E. coli* O157:H7 contamination of FCVs (Singh et al. 2002).

Ozone in the gaseous or aqueous state is a proven biocide and inactivates a broader spectrum of microorganisms than traditional disinfectants (Kim et al. 1999; Xu 1999; Khadre et al. 2001). Microbiological safety is an extremely important aspect of food quality. Consumers, as well as regulatory agencies, insist that foods are safe to eat, regardless of other quality indices. Currently, much emphasis is being placed on the eradication and control of a number of foodborne microbes capable of causing human disease (Tauxe et al. 1997).

There are recognized ozone thresholds for the protection of vegetation, building materials, and human health (Palou et al. 2002). Human exposure to high ozone concentrations can cause dryness in the mouth and throat, coughing, headache, and chest restriction.

Table 3 illustrates the efficacy of aqueous ozone for the eradication of targeted human pathogens. Several studies highlight the ozone efficiency. Thus, Moore et al. (2000) reported that ozone exposure (2 ppm for 4 h) for *E. coli* ATCC 25922,

Table 3 Results of comparative studies on the inactivation of human pathogenic microorganisms by aqueous ozone treatments

Species	Ozone (mg L^{-1})	Treatment time (min)	Inactivation (log_{10})	Treatment conditions		Medium	References
				°C	pH		
Bacteria							
Bacillus cereus	0.12	5	>2.0	28	–	O_3 demand-free water[a]	Broadwater et al. (1973)
Bacillus spp.	0.011	1	6.1	22	–	Deionized water	Khadre and Yousef (2001)
Enterococcus faecalis	0.188	<1	>3.0	19–21	6.1	Deionized water	Restaino et al. (1995)
Escherichia coli	0.23–0.26	1.67	4.0	24	7.0	O_3 demand-free water	Farooq and Akhlaque (1983)
E. coli	0.188	<1	>5.0	19–21	6.1	Deionized water	Restaino et al. (1995)
E. coli O157:H7	25	3	>2.6	22–24		Deionized water	Achen and Yousef (2001)
Legionella pneumophila	0.32	20	>4.5	24	7.0	Distilled water	Edelstein et al. (1982)
Listeria monocytogenes	0.188	<1	>4.0	19–21	6.1	Deionized water	Restaino et al. (1995)
Mycobacterium fortuitum	0.23–0.26	1.67	1.0	24	7.0	O_3 demand-free water	Farooq and Akhlaque (1983)
Salmonella typhimurium	0.23–0.26	1.67	4.3	24	7.0	O_3 demand-free water	Farooq and Akhlaque (1983)
S. typhimurium	0.188	<1	>5.0	19–21	6.1	Deionized water	Restaino et al. (1995)
Fungi and yeasts							
Aspergillus parasiticus spores	1.74	2.08	>1.0	25	7.0	Phosphate buffer	Beuchat et al. (1999)
Candida albicans	0.188	<1	>4.5	19–21	6.1	Deionized water	Restaino et al. (1995)
Candida tropicalis	0.02–1.0	0.30–0.08	2.0	20	7.2	O_3 demand-free water	Kawamura et al. (1986)
Zygosaccharomyces bailii	0.188	<1	>4.5	19–21	6.1	Deionized water	Restaino et al. (1995)

(continued)

Table 3 (continued)

Species	Ozone (mg L^{-1})	Treatment time (min)	Inactivation (log_{10})	Treatment conditions		Medium	References
				°C	pH		
Virus							
Bacteriophage f2	0.1	10	0.7	20	7.2	Activated sludge effluent	Harakeh and Butler (1985)
Coxsackie virus B5	0.4	2.5	4.0	20	7.2	Sludge effluent	Harakeh and Butler (1985)
Enteric	0.02–4.1	29	>1.7	18	7.0	Raw wastewater	Joret et al. (1982)
Hepatitis *A*	0.25	0.02	2.7	20	7.2	Phosphate buffer	Herbold et al. (1989)
Human rotavirus	0.31	10	0.7	20	7.2	Sludge effluent	Harekeh and Butler (1985)
Poliovirus type 1 (Mahoney)	0.51	0.53	1.0	20	7.2	Water	Roy et al. (1981)
Protozoans							
Cryptosporidium parvum	1	5	>1.0	25	7.0	O_3 demand-free water	Korich et al. (1990)
Giardia lamblia	0.7	1.1	2.0	5	5.0	Water	Wickramanayake et al. (1984)
G. murus	0.5	2.8	2.0	5	5.0	Water	Wickramanayake et al. (1984)
Naegleria gruberi	2.0	2.1	2.0	5	5.0	Water	Wickramanayake et al. (1984)

[a]O_3 demand-free water is autoclaved (at 121 °C for 15 min) ozonated deionized water

Staphylococcus aureus, *Serratia liquefaciens*, *Listeria innocua*, and *Rhodotorula rubra* resulted in greater ($P < 0.05$) loss of viability than that observed in the absence of ozone. Indeed, gram-negative bacteria were more sensitive to ozone than gram-positive organisms; bacteria were more sensitive than yeast strain tested (Moore et al. 2000). Singh et al. (2002) reported that bactericidal effect in population of *E. coli* O157:H7 increased with concentration and length of gaseous ozone exposure on lettuce and carrots, becoming significant after 15 min of exposure. Kim et al. (1999) treated shredded lettuce with ozone and reported that bubbling ozone gas (49 mg L^{-1}, 0.5 L min^{-1}) in a lettuce–water mixture decreased the natural microbial load by 1.5–1.9 log in 5 min. Kowalski et al. (1998) reported that the disinfection action of ozone (300–1500 ppm for 10–480 s) in air parallels the action of ozone in water in experiments with *E. coli* and *Staphylococcus aureus*.

Conclusion

Widely used sanitizers, such as chlorine, have some disadvantages because of their limited effects in reducing microorganisms and concerns about their probable effects on health. Methyl bromide, a fumigant commonly used in farming and industry, has detrimental effects on ozone layer. Because of these reasons, the usage of these chemicals has been restricted, and they are thought to be phased out in near future. Therefore, the food industry is in search of applications that will substitute for these agents.

Ozone seems to be an effective sanitizer with great potential applications in the food industry. It decomposes into simple oxygen with no safety concerns about consumption of residual by-product. Due to its high oxidation capacity and microbial inactivation potential, ozone has prevented various kinds of microbial spoilages usually encountered in fruits and vegetables. Decontamination of products by ozone depends on number and kind of contaminating microorganisms, physiology of the product, ozone application system, temperature, pH, and other factors. If improperly used, ozone can cause some deleterious effects on physiology and quality of products such as losses in sensory quality.

For effective and safe use in food processing, optimum ozone concentration, contact time, and other treatment conditions should be defined for all products. Pilot trials must be conducted before starting commercial application, because every ozone application is unique.

Ozone application has also given promising results for important problems of food industry, such as mycotoxin and pesticide residues. Degradation products, formed after ozonation of these residues, have not exactly been determined, and this seems to be the most crucial obstacle on this subject. In vivo and in vitro toxicological tests are needed to be conducted to screen the effects of degradation products on human and animal health. Through emerging new techniques, as well as improvements and innovations in ozone generating and application systems, the subject will be evaluated more effectively in future.

References

Achen, M., & Yousef, A. E. (2001). Efficacy of ozone against *Escherichia coli* O157:H7 on apples. *Journal of Food Science, 66*, 1380–1384.

Adams, M.R., & Moss, M.O. (1997). Controlling the microbiological quality of foods. In *Food microbiology* (pp. 323–358). Cambridge: The Royal Society of Chemistry.

Aday, M. S., Büyükcan, M. B., Temizkan, R., & Caner, C. (2014). Role of ozone concentrations and exposure times in extending shelf life of strawberry. *Ozone: Science & Engineering, 36*, 43–56.

Aday, M. S., & Caner, C. (2014). Individual and combined effects of ultrasound, ozone and chlorine dioxide on strawberry storage life. *LWT—Food Science and Technology, 57*, 344–351.

Adeskaveg, J.E. (1995, May). Postharvest sanitation to reduce decay of perishable commodities. *Perishables Handling Newsletter*, p. 82.

Aguayo, E., Escalona, V. H., & Artes, F. (2006). Effect of cyclic exposure to ozone gas on physicochemical, sensorial and microbial quality of whole and sliced tomatoes. *Postharvest Biology and Technology, 39*, 169–177.

Akbas, M. Y., & Ozdemir, M. (2006). Effect of different ozone treatments on aflatoxin degradation and physicochemical properties of pistachios. *Journal of the Science of Food and Agriculture, 86*, 2099–2104.

Alexandre, E. M. C., Santos-Pedro, D. M., Brandao, T. R. S., & Silva, C. L. M. (2011). Influence of aqueous ozone, blanching and combined treatments on microbial load of red bell peppers, strawberries and watercress. *Journal of Food Engineering, 105*, 277–282.

Allende, A., Marın, A., Buendıa, B., Tomas-Barberan, F., & Gil, M. I. (2007). Impact of combined postharvest treatments (UV-C light, gaseous O_3, superatmospheric O_2 and high CO_2) on health promoting compounds and shelf-life of strawberries. *Postharvest Biology and Technology, 46*, 201–211.

Allende, A., Selma, M. V., Lopez-Galvez, F., Villaescusa, R., & Gil, M. I. (2008). Role of commercial sanitizers and washing systems on epiphytic microorganisms and sensory quality of fresh-cut escarole and lettuce. *Postharvest Biology and Technology, 49*, 155–163.

Alwi, N. A., & Ali, A. (2014). Reduction of *Escherichia coli* O157, *Listeria monocytogenes* and *Salmonella enterica* sv. Typhimurium populations on fresh-cut bell pepper using gaseous ozone. *Food Control, 46*, 304–311.

Artes-Hernandez, F., Aguayo, E., Artes, F., & Tomas-Barberan, F. A. (2007). Enriched ozone atmosphere enhances bioactive phenolics in seedless table grapes after prolonged shelf life. *Journal of the Science of Food and Agriculture, 87*, 824–831.

Artes-Hernandez, F., Aguayo, E., & Artes, F. (2004). Alternative atmosphere treatments for keeping quality of "Autumn seedless" table grapes during long term cold storage. *Postharvest Biology and Technology, 31*, 59–67.

Bachelli, M. L. B., Amaral, R. D. A., & Benedetti, B. C. (2013). Alternative sanitization methods for minimally processed lettuce in comparison to sodium hypochlorite. *Brazilian Journal of Microbiology, 44*, 673–678.

Banerjee, S. K., & Mudd, J. B. (1992). Reaction of ozone with glycophorin in solution and in lipid vesicles. *Archives of Biochemistry and Biophysics, 295*, 84–89.

Baranovskaya VA, Zapolskii OB, Ovrutskaya IV, Obodovskaya NN, Oshenichnaya EE, Yushkevich OI (1979). Use of ozone gas sterilization during storage of potatoes and vegetables. *Koshervnaya I Ovoshchesushhil naya Prom. 4*,10–12.

Barboni, T., Cannac, M., & Chiaramonti, N. (2010). Effect of cold storage and ozone treatment on physicochemical parameters, soluble sugars and organic acids in *Actinidia deliciosa. Food Chemistry, 121*, 946–951.

Barth, M. M., Zhou, C., Mercier, J., & Payne, F. A. (1995). Ozone storage effects on anthocyanin content and fungal growth in blackberries. *Journal of Food Science, 60*, 1286–1288.

Bazarova, V. (1982). Use of ozone in storage of apples. *Food Science, 14*, 11.

Bell, C. N. (2000). Fumigation in the 21st century. *Crop Protection, 19*, 563–569.

Benitez, F., Beltran-Heredia, J., Torregrosa, J., Acero, J., & Cercas, V. (1997). Chemical pretreatment by ozone of wastewaters from olive oil mills. *Toxicological and Environmental Chemistry, 60*, 97–109.

Berlett, B. S., & Stadtman, E. R. (1997). Protein oxidation in aging, disease, and oxidative stress. *Journal of Biological Chemistry, 272*, 20313–20316.

Bermudez-Aguirre, D., & Barbosa-Canovas, G. V. (2013). Disinfection of selected vegetables under non-thermal treatments: Chlorine, acid citric, ultraviolet light and ozone. *Food Control, 29*, 82–90.

Beuchat, L. R. (1992). Surface disinfection of raw produce. *Dairy, Food and Environmental Sanitation, 12*, 6–9.

Beuchat, L. R., Chmielewski, R., Keswani, J., Law, S. E., & Frank, J. F. (1999). Inactivation of aflatoxigenic *Aspergilli* by treatment with ozone. *Letters in Applied Microbiology, 29*, 202–205.

Beuchat, L. R., Nail, B. V., Adler, B. B., & Clavero, M. R. S. (1998). Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. *Journal of Food Protection, 61*, 1305–1311.

Bialka, K. L., & Demirci, A. (2007). Decontamination of *Escherichia coli* O157:H7 and *Salmonella enterica* on blueberries using ozone and pulsed UV-light. *Journal of Food Science, 72*, 391–396.

Bialka, K. L., Demirci, A., & Puri, V. M. (2008). Modeling the inactivation of *Escherichia coli* O157:H7 and *Salmonella enterica* on raspberries and strawberries resulting from exposure to ozone or pulsed UV-light. *Journal of Food Engineering, 85*, 444–449.

Bocci, V. (1994). Autohemotherapy after treatment of blood with ozone. A reappraisal. *Journal of International Medical Research, 22*, 131–144.

Bocci, V. (1996a). Does ozone therapy normalize the cellular redox balance? Implications for therapy of human immunodeficiency virus infection and several other diseases. *Medical Hypotheses, 46*, 150–154.

Bocci, V. (1996b). Ozone as a bioregulator. Pharmacology and toxicology of ozone therapy today. *Journal of Biological Regulators and Homeostatic Agents, 10*, 31–54.

Bocci, V., Aldinucci, C., Borrelli, E., Corradeschi, F., Diadori, A., Fanetti, G., & Valacchi, G. (2001). Ozone in medicine. *Ozone Science and Engineering, 23*, 207–217.

Bott, T.R. (1991). Ozone as a disinfectant in process plant. *Food Control*, 1–3.

Brackett, R. E. (1999). Incidence, contributing factors, and control of bacterial pathogens in produce. *Postharvest Biology and Technology, 15*, 305–311.

Broadwater, W., Hoehn, R., & King, P. (1973). Sensitivity of three selected bacterial species to ozone. *Applied Microbiology, 26*, 391–393.

Brown, K. A., & Roberts, T. M. (1988). Effects of O_3 on foliar leaching in Norway spruce: Confounding factors due to NO_x production during O_3 generation. *Environmental Pollution, 55*, 55–73.

Bundschuh, G., & Rilling, S. (1996). Ozone fumigation process. *Trends in Food Science and Technology, 7*, 307–308.

Castillo, F. J., & Heath, R. L. (1990). Ca^{2+}transport in membrane vesicles from pinto bean leaves and alteration after ozone exposure. *Plant Physiology, 94*, 788–795.

Cayuela, J. A., Vazquez, A., Perez, A. G., & Garcıa, J. M. (2009). Control of table grapes postharvest decay by ozone treatment and resveratrol induction. *Food Science and Technology International, 15*, 495–502.

Chauhan, O. P., Raju, P. S., Ravi, N., Singh, A., & Bawa, A. S. (2011). Effectiveness of ozone in combination with controlled atmosphere on quality characteristics including lignification of carrot sticks. *Journal of Food Engineering, 102*, 43–48.

Chen, H. H., Chiu, E. M., & Huang, J. R. (1997). Color and gel-forming properties of horse mackerel (*Trachurus japonicus*) as related to washing conditions. *Journal of Food Science, 62*, 985–991.

Crowe, K. M., Bushway, A. A., Bushway, R. J., Davis-Dentici, K., & Hazen, R. A. (2007). A comparison of single oxidants versus advanced oxidation processes as chlorine-alternatives

for wild blueberry processing (*Vaccinium angustifolium*). *International Journal of Food Microbiology, 116*, 25–31.

Cryer, E. (1992). Recent application of ozone in freshwater fish hatchery systems. In W. J. Blogoslawski (Ed.), *Proceedings of the 3rd International Symposium on the Use of Ozone in Aquatic Systems* (pp. 134–154). Norwalk, CT: International Ozone Association Pan American Committee.

Das, E., Gurakan, G. C., & Bayındırlı, A. (2006). Effect of controlled atmosphere storage, modified atmosphere packaging and gaseous ozone treatment on the survival of *Salmonella enteritidis* on cherry tomatoes. *Food Microbiology, 23*, 430–438.

De Roever, C. (1998). Microbiological safety evaluations and recommendations on fresh produce. *Food Control, 9*, 321–347.

Dominy, P. J., & Heath, R. L. (1985). Inhibition of the K^+-stimulated ATPase of the plasmalemma of pinto bean leaves by ozone. *Plant Physiology, 77*, 43–45.

Dooley, M. M., & Mudd, J. B. (1982). Reaction of ozone with Lysozyme under different exposure conditions. *Archives of Biochemistry and Biophysics, 218*, 459–471.

Eckert, J. W., & Eaks, I. L. (1989). Postharvest disorders and diseases of citrus fruits. In: Smilanick JL, Margosan DM and Mlikota Gabler F, 2002. Impact of ozonated water on the quality and self-life of fresh citrus fruit, stone fruit, and table grapes. *Ozone Science and Engineering, 24*, 343–356.

Edelstein, P., Whittaker, R., Kreiling, R., & Howell, C. (1982). Efficacy of ozone in eradication of *Legionella pneumophila* from hospital plumbing fixtures. *Applied and Environmental Microbiology, 44*, 1330–1334.

Ewell, A. W. (1940). Ozone and its applications in food preservation. "Refrigeration Application Data Book", 2nd ed., Sect. II. "Cold Storage Practice", pp. 199–203. Am. Soc. Refrig. Eng., Menasha, Wis. In: Liangji X, 1999. Use of ozone to improve the safety of fresh fruits and vegetables. *Food Technology, 53*, 58–62.

Fairbanks, G., Steck, T. C., & Wallach, D. F. H. (1971). Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. *Biochemistry, 10*, 2606–2624.

Faitel'berg-Blank, V. R., Bykove, E. V., Orlova, A. V., Ostapenko, L. G., & Stepanenko, V. A. (1979). Improvement of keeping quality of potatoes and onions by means of ionized air. *Vestn S'kh Nauki, 4*, 110–112.

Fan, L. H., Song, J., Hildebrand, P. D., & Forney, C. F. (2001). Corona discharge reduces mold on commercially stored onions. *Acta Horticulturae, 553*, 427–428.

Farooq, S., & Akhlaque, S. (1983). Comparative response of mixed cultures of bacteria and virus to ozone. *Water Research, 17*, 809–812.

Feliziani, E., Romanazzi, G., & Smilanick, J. L. (2014). Application of low concentrations of ozone during the cold storage of table grapes. *Postharvest Biology and Technology, 93*, 38–48.

Forney, C. F., Fan, L., Hildebrand, P. D., & Song, J. (2001). Do negative air ions reduce decay of fresh fruits and vegetables. Proc. 4th Int. Conf. On Postharvest. *Acta Horticulturae, 553*, 421–424.

Forney, C. F., Song, J., Fan, L., Hildebrand, P. D., & Jordan, M. A. (2003). Ozone and 1-methylcyclopropene alter the postharvest quality of broccoli. *Journal of the American Society for Horticultural Science, 128*, 403–408.

Forney, C. F., Song, J., Hildebrand, P. D., Fan, L., & McRae, K. B. (2007). Interactive effects of ozone and 1-methylcyclopropene on decay resistance and quality of stored carrots. *Postharvest Biology and Technology, 45*, 341–348.

Gabler, F. M., Mercier, J., Jimınez, J. I., & Smilanick, J. L. (2010). Integration of continuous biofumigation with *Muscodor albus* with pre-cooling fumigation with ozone or sulfur dioxide to control postharvest gray mold of table grapes. *Postharvest Biology and Technology, 55*, 78–84.

Galdun, T., Postol, A., & Lukovnikova, G. (1984). Changes in the carbohydrate complexes of garlic during long-term storage cold storage with ozonation of air. *Tovarovedenie, 17*, 43–47.

Gane, R. (1937). The respiration of bananas in the presence of ethylene. *New Phytologist, 36*, 170–178.

Geering, F. (1999). Ozone applications—The state of the air in Switzerland. *Ozone Science and Engineering, 21*, 99–118.

Glowacz, M., Colgan, R., & Rees, D. (2015). Influence of continuous exposure to gaseous ozone on the quality of red bell peppers, cucumbers and zucchini. *Postharvest Biology and Technology, 99*, 1–8.

Gorman, B. M., Sofos, J. N., Morgan, J. B., Schmidt, G. R., & Smith, G. C. (1995). Evaluation of hand-trimming, various sanitizing agents, and hot water spray-washing as decontamination interventions for beef brisket adipose tissue. *Journal of Food Protection, 58*, 899–907.

Gottschalk, C., Libra, J. A., & Saupe, A. (2002). Ozonation of water and wastewater. In C. Gottschalk, J. A. Libra, & A. Saupe (Eds.), *A practical guide to understanding ozone and its applications*. Germany: Wiley-VCH.

Graham, D. M. (1997). Use of ozone for food processing. *Food Technology, 51*, 72–75.

Grimes, H. D., Perkins, K. K., & Boss, W. F. (1983). Ozone degrades into hydroxyl radical under physiological conditions. *Plant Physiology, 72*, 1016–1020.

Hamelin, C. (1985). Production of single-and double-stranded breaks in plasmid DNA by ozone. *International Journal of Radiation Oncology, Biology, Physics, 11*, 253–257.

Hampson, B., & Fiori, S. (1997). Application of ozone in food processing operations. In *Proceedings of 1997 IOA PAG conference* (pp. 261–267). Nevada: Lake Tahoe.

Han, Y., Floros, J. D., Linton, R. H., Nielsen, S. S., & Nelson, P. E. (2002). Response surface modeling for the inactivation of *E. coli* O157:H7 on green peppers (*Capsicum annuum*) by ozone gas treatment. *Journal of Food Science, 67*, 1188–1193.

Harakeh, M., & Butler, M. (1985). Factors influencing the ozone inactivation of enteric viruses in effluent. *Ozone Science and Engineering, 6*, 235–243.

Heath, R. L., & Castillo, F. J. (1988). Membrane disturbances in response to air pollutants. In S. Schulte-Hostede, N. M. Darrall, L. W. Blank, & A. R. Wellburn (Eds.), *Air pollution and plant metabolism* (pp. 53–75). London: Elsevier.

Herbold, K., Flehmig, B., & Botzenhart, K. (1989). Comparison of ozone inactivation, in flowing water, of hepatitis A virus, poliovirus 1, and indicator organisms. *Applied and Environmental Microbiology, 55*, 2949–2953.

Hildebrand, P. D., Forney, C. F., Song, J., Fan, L., & McRae, K. B. (2008). Effect of a continuous low ozone exposure (50 nl l^{-1}) on decay and quality of stored carrots. *Postharvest Biology and Technology, 49*, 397–402.

Hildebrand, P. D., Song, J., Forney, C. F., Renderos, W. E., & Ryan, D. A. J. (2001). Effects of corona discharge on decay of fruits and vegetables. *Acta Horticulturae, 553*, 425–426.

Hill, A. G., & Rice, R. G. (1982). Historical, background, properties and applications. In R. G. Rice & A. Netzer (Eds.), *Handbook of ozone technology and applications* (pp. 1–37). Ann Arbor, MI: Ann Arbor Science.

Hoigné, J. (1998). Chemistry of aqueous ozone and transformation of pollutants by ozonation and advanced oxidation processes. In J. Hrubec (Ed.), *The handbook of environmental chemistry: Quality and treatment of drinking water II* (pp. 83–141). Berlin Heidelberg Germany: Springer-Verlag.

Horvath, M., Bilitzky, L., & Huttner, J. (1985). Fields of utilization of ozone. In R. J. K. Clark (Ed.), *Ozone* (pp. 257–316). Inc, New York: Elsevier Science publishing Co.

Horvitz, S., & Cantalejo, M. J. (2012). Effects of ozone and chlorine postharvest treatments on quality of fresh-cut red bell peppers. *International Journal of Food Science and Technology, 47*, 1935–1943.

Hwang, E., Cash, J., & Zabik, M. (2001). Postharvest treatments for the reduction of mancozeb in fresh apples. *Journal of Agricultural and Food Chemistry, 49*, 3127–3132.

Inan, F., Pala, M., & Doymaz, I. (2007). Use of ozone in detoxification of aflatoxin B_1 in red pepper. *Journal of Stored Products Research, 43*, 425–429.

Jiang, J. M., Zhu, X. R., & Li, Y. B. (2001). Postharvest control of Litchi fruit rot by *Bacillus subtilis*. *Lebensmittel-Wissenschaft und Technologie, 34*, 430–436.

Joret, J. C., Block, J. C., Hartemann, P., & Richard, Y. (1982). Waste-water disinfection: elimination of fecal bacteria and enteric viruses by ozone. *Ozone Science and Engineering, 4*, 91–99.

Kaess, G., & Weidemann, J. F. (1968). Ozone treatment of chilled beef. I. Effect of low concentrations of ozone on microbial spoilage and surface color of beef. *International Journal of Food Science and Technology, 3*, 325–334.

Karaca, H. (2010). Use of ozone in the citrus industry. *Ozone Science and Engineering, 32*, 122–129.

Karaca, H., & Velioglu, Y. S. (2014). Effects of ozone treatments on microbial quality and some chemical properties of lettuce, spinach, and parsley. *Postharvest Biology and Technology, 88*, 46–53.

Kawamura, K., Kaneko, M., Hirata, T., & Taguchi, K. (1986). Microbial indicators for the efficiency of disinfection processes. *Water Science and Technology, 18*, 175–184.

Kells, S. A., Mason, L. J., Maier, D. E., & Woloshuk, C. P. (2001). Efficacy and fumigation characteristics of ozone in stored maize. *Journal of Stored Products Research, 37*, 371–382.

Kessel, J., Allison, D., Moore, F., & Kairne, M. (1943). Comparison of chlorine and ozone as virucidal agents of poliomyelitis virus. *Experimental Biology and Medicine, 53*, 71–73.

Ketteringham, L., Gausseres, R., James, S. J., & James, C. (2006). Application of aqueous ozone for treating pre-cut green peppers (*Capsicum annuum* L.). *Journal of Food Engineering, 76*, 104–111.

Khadre, M. A., Yousef, A. E., & Kim, J. G. (2001). Microbiological aspects of ozone applications in food: A review. *Journal of Food Science, 66*, 1242–1252.

Kim, C., Gentile, D., & Sproul, O. (1980). Mechanism of ozone inactivation of bacteriophage *f2*. *Applied and Environmental Microbiology, 39*, 210–218.

Kim, J. G., & Yousef, A. E. (2000). Inactivation kinetics of foodborne spoilage and pathogenic bacteria by ozone. *Journal of Food Science, 65*, 521–528.

Kim, J. G., Yousef, A. E., & Chism, G. W. (1999). Use of ozone to inactivate microorganism on lettuce. *Journal of Food Safety, 19*, 17–34.

Klockow, P. A., & Keener, K. M. (2009). Safety and quality assessment of packaged spinach treated with a novel ozone-generation system. *LWT—Food Science and Technology, 42*, 1047–1053.

Knight, K. L., & Mudd, J. B. (1984). The reaction of ozone with glyceraldehyde-3-phosphate dehydrogenase. *Archives of Biochemistry and Biophysics, 229*, 259–269.

Kondo, F., Utoh, K., & Rostamibashman, M. (1989). Bactericidal effects of ozone water and ozone ice on various microorganisms. *Bull. Faculty of Agriculture Miyazaki University, 36*, 93–98.

Korich, D. G., Mead, J. R., Madore, M. S., Sinclair, N. A., & Sterling, C. R. (1990). Effects of ozone, chlorine dioxide, chlorine, and monochloramine on *Cryptosporidium parvum* oocyst viability. *Applied and Environmental Microbiology, 56*, 1423–1428.

Kowalski, W. J., Bahnfleth, W. P., & Whittant, T. S. (1998). Bactericidal effects of high airborne ozone concentrations on *Escherichia coli* and *Staphylococcus aureus. Ozone Science and Engineering, 20*, 205–221.

Krause, C. R., & Weidensaul, T. C. (1978). Effects of ozone on the sporulation, germination and pathogenicity of *Botrytis cinerea. Phytopathology, 68*, 195–198.

Krupa, S. V., & Manning, W. J. (1988). Atmospheric ozone: Formation and effects on vegetation. *Environmental Pollution, 50*, 101–137.

Kuprianoff, J. (1953). The use of ozone in cold storage of fruits. In: Xu L, 1999. Use of ozone to improve the safety of fresh fruits and vegetables. *Food Technology, 53*, 58–62.

L'Herault, P., & Chung, Y. S. (1984). Mutagenicity of ozone in different repair-deficient strains of *Escherichia coli. Molecular and General Genetics, 197*, 472–477.

Langlais, B., Reckhow, D. A., & Brink, D. R. (1991). Practical application of ozone: Principle and case study. In B. Langlais, D. A. Reckhow, & R. Brink (Eds.), *Ozone in water treatment: Application and engineering cooperative research report* (pp. 133–316). Chelsea, Michigan: Lewis Publishers.

Liew, C. L., & Prange, R. K. (1994). Effect of ozone and storage temperature on postharvest diseases and physiology of carrots (*Daucus carota* L.). *Journal of the American Society for Horticultural Science, 119*, 563–567.

Maeba, H., Takamoto, Y., Kamimura, M., & Miura, T. (1988). Destruction and detoxification of aflatoxins with ozone. *Journal of Food Science, 53*, 657–668.

Maggiolo A, Blair EA (1959). Ozone Oxidation of Sulfides and Sulfoxides. In Ozone Chemistry and Toxicology. Advances in Chemistry Series, Washington, DC: American Chemical Society, 21:200–201.

Margalit, M., Attias, E., Attias, D., Elstein, D., Zimran, A., & Matzner, Y. (2001). Effect of ozone on neutrophil function in vitro. *Clinical and Laboratory Haematology, 23*, 243–247.

Margosan, D.A., & Smilanick, J.L. (2000). Mortality of spores of *Botrytis cinerea*, *Monilinea*, *Penicillium digitatum* and *Rhizopus stolonifer* after exposure to ozone gas, and a survey of the tolerance of produce to sporicidal ozone doses. In Proceedings of postharvest integrated pest management meeting, University of California, Davis, CA, August 2000.

Minas, I. S., Karaoglanidis, G. S., Manganaris, G. A., & Vasilakakis, M. (2010). Effect of ozone application during cold storage of kiwifruit on the development of stem-end rot caused by *Botrytis cinerea. Postharvest Biology and Technology, 58*, 203–210.

Moore, G., Griffith, C., & Peters, A. (2000). Bactericidal properties of ozone and its potential applications as a terminal disinfectant. *Journal of Food Protection, 63*, 1100–1106.

Mudd, J., Dawson, P., Adams, J., Wingo, J., & Santrock, J. (1996). Reaction of ozone with enzymes of erythrocyte membranes. *Archives of Biochemistry and Biophysics, 335*, 145–151.

Mudd, J. B., Dawson, P. J., Tseng, S., & Liu, F. L. (1997). Reaction of ozone with protein tryptophans: Band III, serum albumin, and cytochrome C. *Archives of Biochemistry and Biophysics, 338*, 143–149.

Muller, J. F., & Brausseur, G. (1995). Images: A three-dimensional chemical transport model of the global troposphere. *Journal of Geophysical Research, 100*, 16445–16490.

Murray, R., Pamela, S., & Elson, H. (1965). Location of mucopeptide of selection of the cell wall of *E. coli* and other gram-negative bacteria. *Canadian Journal of Microbiology, 11*, 547–560.

Nadas, A., Olmo, M., & Garcia, J. M. (2003). Growth of *Botrytis cinerea* and strawberry quality in ozone-enriched atmospheres. *Journal of Food Science, 68*, 1798–1802.

Naitoh, S., Sawada, Y., & Yamaguchi, N. (1989). Studies on utilization of ozone in food preservation: effect of ozone treatment on storage of packaged Namamen Japanese raw noodle. *Journal of Antibacterial and Antifungal Agents, 17*, 517–526.

Naitoh, S., & Shiga, I. (1989). Studies on utilizing of ozone in food preservation. IX. Effect of ozone treatment on elongation of hypocotyls and microbial counts of bean sprouts. *Journal of the Japanese Society for Food Science and Technology, 36*, 181–188.

Nickols, D., & Varas, A. J. (1992). Ozonation. In E. A. Bryat, G. P. Fulton, & G. C. Budd (Eds.), *Disinfection alternatives for safe drinking water* (pp. 197–258). New York: Van Nostrand Reinhold.

Norton, J. S., Charig, A. J., & Demoranville, I. E. (1968). The effect of ozone on storage of cranberries. *Proceedings of the American Society for Horticultural Science, 93*, 792–796.

Ogawa, J. M., Feliciano, A. J., & Manji, B. T. (1990). Evaluation of ozone as a disinfectant in postharvest dumptank treatments for tomato. *Phytopathology, 80*, 1020.

Olmez, H., & Akbas, M. Y. (2009). Optimization of ozone treatment of fresh-cut green leaf lettuce. *Journal of Food Engineering, 90*, 487–494.

Ong, M. K., & Ali, A. (2015). Antifungal action of ozone against *Colletotrichum gloeosporioides* and control of papaya anthracnose. *Postharvest Biology and Technology, 100*, 113–119.

Ong, M. K., Ali, A., Alderson, P. G., & Forney, C. F. (2014). Effect of different concentrations of ozone on physiological changes associated to gas exchange, fruit ripening, fruit surface quality and defence-related enzymes levels in papaya fruit during ambient storage. *Scientia Horticulturae, 179*, 163–169.

Ong, K. C., Cash, J. N., Zabik, M. J., Siddiq, M., & Jones, A. L. (1996). Chlorine and ozone washes for pesticide removal from apples and processed applesauce. *Food Chemistry, 55*, 153–160.

Ozkan, R., Smilanick, J. L., & Karabulut, O. A. (2011). Toxicity of ozone gas to conidia of *Penicillium digitatum*, *Penicillium italicum*, and *Botrytis cinerea* and control of gray mold on table grapes. *Postharvest Biology and Technology, 60*, 47–51.

Page, T., Harris, R., & Epstein, S. (1976). Drinking water and cancer mortality in Louisiana. *Science, 193*, 55–57.

Palou, L., Crisosto, C., Smilanick, J., Adaskaveg, J., & Zoffoli, J. (2002). Effects of continuous 0.3 ppm ozone exposure on decay development and physiological responses of peaches and table grapes in cold storage. *Postharvest Biology and Technology, 24*, 39–48.

Palou, L., Smilanick, J. L., Crisosto, C. H., Mansour, M., & Plaza, P. (2003). Ozone gas penetration and control of sporulation of *Penicillium digitatum* and *Penicillium italicum* within commercial packages of oranges during cold storage. *Crop Protection, 22*, 1131–1134.

Palou, L., Smilanick, J. L., Crisosto, C. H., & Mansour, M. (2001). Effect of gaseous ozone exposure on the development of green and blue molds on cold stored citrus fruit. *Plant Disease, 85*, 632–638.

Perez, R. R., Nunez, S. A., Baluja, C., & Otero, M. L. (1995). Ozonation kinetics of glucosamine and N-acetyl glucosamine in aqueous medium. *Ozone Science and Engineering, 17*, 463–467.

Pérez, A. G., Sanz, C., Ríos, J. J., & Olías, J. M. (1999). Effects of ozone treatment on postharvest strawberry quality. *Journal of Agricultural and Food Chemistry, 47*, 1652–1656.

Pintado, C., Tzortzakis, N.G., Roberts, R., Singleton, I., & Barnes, J. (2004). Preservation of fresh produce by ozone. International Centenary Conference of Association of Applied Biologists. Advances in applied biology: providing new opportunities for consumers and producers in the 21st century, Dec 15–17, 2004, Oxford, UK.

Piper, J. (1998). Tomato washing using ozonated water. Presented at ERPI ozone workshop, Memphis, May. In: Xu L, 1999. Use of ozone to improve the safety of fresh fruits and vegetables. *Food Technology, 53*, 58–62.

Pirovani, M., Güemes, D., Di Pentima, J., & Tessi, M. (2000). Survival of *Salmonella hadar* after washing disinfection of minimally processed spinach. *Letters in Applied Microbiology, 31*, 143–148.

Polle, A. (1998). Photochemical oxidants: uptake and detoxification. In L. de Kok & I. Stuhlen (Eds.), *Responses of plant metabolism to air pollution and global change* (pp. 95–116). Leiden: Backhuys Publishing.

Pryor, A. (2001). *Ozone as a gaseous soil treatment agent as an alternative to methyl bromide for pre-plant fumigation*. Presented at the International Ozone Association PAG Conference, Newport Beach, CA.

Pryor, W. A., Das, B., & Church, D. F. (1991). The ozonation of unsaturated fatty acids: Aldehydes and hydrogen peroxide as products and possible mediators of ozone toxicity. *Chemical Research in Toxicology, 4*, 341–348.

Rahman, S. M. E., Ding, T., & Oh, D. H. (2010). Inactivation effect of newly developed low concentration electrolyzed water and other sanitizers against microorganisms on spinach. *Food Control, 21*, 1383–1387.

Reagan, J. O., Acuff, G. F., Buege, D. R., Buyck, M. J., Dickson, J. S., Kastner, C. L., Marsden, J. L., Morgan, J. B., Nickelson, R., II, Smith, G. C., & Sofos, J. N. (1996). Trimming and washing of beef carcasses as a method of improving the microbiological quality of meat. *Journal of Food Protection, 59*, 751–756.

Restaino, L., Frampton, E. W., Hemphill, J. B., & Palnikar, P. (1995). Efficacy of ozonated water against various food-related microorganisms. *Applied and Environmental Microbiology, 61*, 3471–3475.

Restuccia, C., Lombardo, S., Pandino, G., Licciardello, F., Muratore, G., & Mauromicale, G. (2014). An innovative combined water ozonisation/O_3-atmosphere storage for preserving the overall quality of two globe artichoke cultivars. *Innovative Food Science and Emerging Technologies, 21*, 82–89.

Rice, R. G. (1999). Ozone in the United States. State of the art. *Ozone Science and Engineering, 21*, 99–118.

Rice, R. G. (2002). Century 21-pregnant with ozone. *Ozone Science and Engineering, 24*, 1–15.

Rice, R. G., Farquhar, J. W., & Bollyky, L. J. (1982). Review of the applications of ozone for increasing storage times of perishable foods. *Ozone Science and Engineering, 4*, 147–163.

Rice, R. G., & Graham, D. M. (2001). Milestone event-ozone for food processing. In B. Loeb (Ed.), *Ozone News* (pp. 15–17). San Francisco, CA: International Ozone Association.

Rico, D., Martin-Diana, A. B., Frias, J. M., Henehan, G. T. M., & Barry-Ryan, C. (2006). Effects of ozone and calcium lactate treatments on browning and texture properties of fresh-cut lettuce. *Journal of the Science of Food and Agriculture, 86*, 2179–2188.

Rideal, E. K. (1920). *Ozone*. London: Constable & Co., Ltd.

Roberts, R. (2005). *Harnessing the potential of gaseous ozone for the disinfection of fresh produce in storage and transit*. Ph.D. Thesis, Newcastle University, UK.

Rodoni, L., Casadei, N., Concello, A., Chaves Alicia, A. R., & Vicente, A. R. (2010). Effect of short-term ozone treatments on Tomato (*Solanum lycopersicum* L.) fruit quality and cell wall degradation. *Journal of Agricultural and Food Chemistry, 58*, 594–599.

Roy, D., Chian, E. S. K., & Engelbreeht, R. S. (1981). Kinetics of enteroviral inactivation by ozone. *Journal of the Environmental Engineering Division, 107*, 887–901.

Rubin, M. B. (2003). The history of ozone. CD Harries and the introduction of ozone into organic chemistry. *Helvetica Chimica Acta, 86*, 930–940.

Salvador, A., Abad, I., Arnal, L., & Martınez-Javega, J. M. (2006). Effect of ozone on postharvest quality of persimmon. *Journal of Food Science, 71*, S443–S446.

Sandermann, H., Jr., Ernst, D., Heller, W., & Langebartels, C. (1998). Ozone: An abiotic elicitor of plant defense reactions. *Trends in Plant Science, 3*, 47–50.

Sarig, P., Zahavi, T., Zutkhi, Y., Yannai, S., Lisker, N., & Ben-Arie, R. (1996). Ozone for the control of post-harvest decay of table grapes caused by *Rhizopus stolonifer. Physiological and Molecular Plant Pathology, 48*, 403–415.

Sarig, P., Zutkhi, Y., Monjauze, A., Lisker, N., & Ben-Arie, R. (1997). Phytoalexin elicitation in grape berries and their susceptibility to *Rhizopus stolonifer. Physiological and Molecular Plant Pathology, 50*, 337–347.

Scherm H, Pryor BM, van Bruggen AHC (1993). Effects of ozonated water on sporangial germination of Bremia lactucae in vitro and in vivo. *Ann. Appl. Biol., 122*, 40–41.

Schomer, H.A., & McColloch, L.P. (1948). Ozone in relation to storage of apples. Circular No. 765, United States Department of Agriculture, pp 1–24.

Selma, M. V., Ibanez, A. M., Allende, A., Cantwell, M., & Suslow, T. (2008a). Effect of gaseous ozone and hot water on microbial and sensory quality of cantaloupe and potential transference of *Escherichia coli* O157:H7 during cutting. *Food Microbiology, 25*, 162–168.

Selma, M. V., Ibanez, A. M., Cantwell, M., & Suslow, T. (2008b). Reduction by gaseous ozone of *Salmonella* and microbial flora associated with fresh-cut cantaloupe. *Food Microbiology, 25*, 558–565.

Sharpe, D., Fan, L., McRae, K., Walker, B., MacKay, R., & Doucette, C. (2009). Effects of ozone treatment on *Botrytis cinerea* and *Sclerotinia sclerotiorum* in relation to horticultural product quality. *Journal of Food Science, 74*, 250–257.

Sheldon, B. W., & Brown, A. L. (1986). Efficacy of ozone as a disinfectant for poultry carcasses and chill water. *Journal of Food Science, 51*, 305–309.

Shimizu, Y., Makinott, J., Sato, J., & Iwamoto, S. (1982). Preventing rot of Kyoho grapes in cold storage with ozone. In: Palou L, Crisosto CH, Smilanick JL, Adaskaveg JE, Zoffoli JP 2002. Effects of continuous 0.3 ppm ozone exposure on decay development and physiological responses of peaches and table grapes in cold storage. *Postharvest Biology and Technology, 24*, 39–48.

Shinriki, N., Ishizaki, K., Yoshizaki, T., Miura, K., & Ueda, T. (1988). Mechanism of inactivation of tobacco mosaic virus with ozone. *Water Research, 22*, 933.

Singh, N., Singh, R. K., Bhunia, A. K., & Stroshine, R. L. (2002). Efficacy of chlorine dioxide, ozone, and thyme essential oil or a sequential washing in killing *Escherichia coli* O157:H7 on lettuce and baby carrots. *LWT—Food Science and Technology, 35*, 720–729.

Skog, L. J., & Chu, C. L. (2001). Effect of ozone on qualities of fruits and vegetables in cold storage. *Canadian Journal of Plant Science, 81*, 773–778.

Smilanick, J.L. (2003, August). Postharvest use of ozone on citrus fruit. In M. Ritenour (Ed.), *Packinghouse Newsletter*, p. 199.

Smilanick, J. L., Adaskaveg, J. E., & Zoffoli, J. P. (2002). Effects of continuous 0.3 ppm ozone exposure on decay development and physiological responses of peaches and table grapes in cold storage. *Postharvest Biology and Technology, 24*, 39–48.

Sobsey, M. D. (1989). Inactivation of health-related microorganisms in water by disinfection processes. *Water Science and Technology, 21*, 179–195.

Song, J., Fan, L., Forney, C. F., Hildebrand, P. D., Jordan, M. A., Renderos, W., & Mc Rae, K. B. (2003a). Ozone and 1-MCP treatments affect the quality and storage life of fresh carrots. *Acta Horticulturae, 628*, 295–301.

Song, J., Fan, L., Forney, C. F., Jordan, M. A., Hildebrand, P. D., Kalt, W., & Ryan, D. A. J. (2003b). Effect of ozone treatment and controlled atmosphere storage on quality and phytochemicals in highbush blueberries. *Acta Horticulturae, 600*, 417–423.

Song, J., Fan, L., Hildebrand, P., & Forney, C. (2000). Biological effects of corona discharge on onions in a commercial storage facility. *Horticulture Technology, 10*, 608–612.

Spalding, D.H. (1968). *Effects of ozone atmospheres on spoilage of fruits and vegetables after harvest*. Marketing Research Report No.801, pp. 1–9.

Spotts, R. A., & Cervantes, L. A. (1992). Effect of ozonated water on postharvest pathogens of pear in laboratory and packinghouse tests. *Plant Disease, 76*, 256–259.

Sproul, O., Pfister, R., & Kim, C. (1982). The mechanism of ozone inactivation of waterborne viruses. *Water Science and Technology, 14*, 303–314.

Suslow, T. V. (2004). *Ozone applications for postharvest disinfection of edible horticultural crops*. Davis, CA: Agriculture and Natural Resources. Publication 8133.

Takamoto, Y., Maeba, H., & Kamimura, M. (1992). Changes in survival rate and enzyme activities in *Escherichia coli* with ozone. *Applied Microbiology and Biotechnology, 37*, 393–395.

Tallentire, E. (2007). *Novel methods to decontaminate fresh food produce*. Ph.D. Thesis, Newcastle University Upon Tyne, UK.

Tauxe, R., Kruse, H., Hedeerg, C., Potter, M., Madden, J., & Wachsmuth, K. (1997). Microbial hazards and emerging issues associated with produce-a preliminary report to the National Advisory Committee on microbiological criteria for food. *Journal of Food Protection, 60*, 1400–1408.

Tenney, R. I. (1973). Ozone generation and use in the brewery. *Brewers Digest, 48*, 64–66.

Tyrrell, S., Rippey, S., & Watkins, W. (1995). Inactivation of bacterial and viral indicators in secondary sewage effluents, using chlorine and ozone. *Water Research, 29*, 2483–2490.

Tzortzakis, N. G., Borland, A., Singleton, I., & Barnes, J. (2007a). Impact of atmospheric ozone-enrichment on quality-related attributes of tomato fruit. *Postharvest Biology and Technology, 45*, 317–326.

Tzortzakis, N.G., Pintado, C., Tallentire, E., Singleton, I., & Barnes, J. (2005). *Impact of atmospheric ozone-enrichment on microbial spoilage of fresh produce*. OZONE IV: Agricultural & Food Processing Applications of Ozone as an Antimicrobial Agent, March 2–4, 2005, Fresno, CA, USA.

Tzortzakis, N. G., Singleton, I., & Barnes, J. (2007b). Deployment of low-level ozone-enrichment for the preservation of chilled fresh produce. *Postharvest Biology and Technology, 43*, 261–270.

Tzortzakis, N. G., Singleton, I., & Barnes, J. (2008). Impacts of low-level atmospheric ozone-enrichment on black spot and anthracnose rot of tomato fruit. *Postharvest Biology and Technology, 47*, 1–9.

Tzortzakis, N. G., Taybi, T., Antony, E., Singleton, I., Borland, A., & Barnes, J. (2013). Profiling shifts in protein complement in tomato fruit induced by atmospheric ozone-enrichment and/or wound-inoculation with *Botrytis cinerea. Postharvest Biology and Technology, 78*, 67–75.

Tzortzakis, N. G., Taybi, T., Roberts, R., Singleton, I., Borland, A., & Barnes, J. (2011). Low-level atmospheric ozone exposure induces protection against *Botrytis cinerea* with down regulation of ethylene-, jasmonate- and pathogenesis-related genes in tomato fruit. *Postharvest Biology and Technology, 61*, 152–159.

Venta, M. B., Broche, S. S. C., Torres, I. F., Pérez, M. G., Lorenzo, E. V., Rodriguez, Y. R., & Cepero, S. M. (2010). Ozone application for postharvest disinfection of tomatoes. *Ozone Science and Engineering, 32*, 361–371.

Vurma, M., Pandit, R. B., Sastry, S. K., & Yousef, A. E. (2009). Inactivation of *Escherichia coli* O157:H7 and natural microbiota on spinach leaves using gaseous ozone during vacuum cooling and simulated transportation. *Journal of Food Protection, 72*, 1538–1546.

Wellburn, A. (1994). *Air pollution and climate change: The biological impact* (pp. 123–144). Essex: Longman.

Whangchai, K., Saengnil, K., Singkamanee, C., & Uthaibutra, J. (2010). Effect of electrolyzed oxidizing water and continuous ozone exposure on the control of *Penicillium digitatum* on tangerine cv. 'Sai Nam Pung' during storage. *Crop Protection, 29*, 386–389.

Whangchai, K., Saengnil, K., & Uthaibutra, J. (2006). Effect of ozone in combination with some organic acids on the control of postharvest decay and pericarp browning of longan fruit. *Crop Protection, 25*, 821–825.

Whistler, P. E., & Sheldon, B. W. (1989). Bactericidal activity, eggshell conductance, and hatchability effects of ozone versus formaldehyde disinfection. *Poultry Science, 68*, 1074–1077.

White, G. C. (1986). *Chlorination of potable water. In Handbook of chlorination* (pp. 256–393). New York: Van Nostrand Reinhold Company Ltd.

Whiteside, C., & Hassan, H. M. (1987). Induction and inactivation of catalase and superoxide dismutase of *Escherichia coli* by ozone. *Archives of Biochemistry and Biophysics, 257*, 464–471.

Wickramanayake, G., Rubin, A., & Sproul, O. (1984). Inactivation of *Naegleria* and *Giardia* cysts in water by ozonation. *Journal of the Water Pollution Control Federation, 56*, 983–988.

Williams D, Montecalvo JrJ, Mueller E, Earls D, Swanson K, Peterson L (1995). Ozonation as an alternative disinfectant for carrot washwater. International Food Technologists Annual Meeting, Book of abstracts. p. 81.

Xu, L. (1999). Use of ozone to improve the safety of fresh fruits and vegetables. *Food Technology, 53*, 58–63.

Xu, W., & Wu, C. (2014). Different efficiency of ozonated water washing to inactivate *Salmonella enterica* Typhimurium on green onions, grape tomatoes, and green leaf lettuces. *Journal of Food Science, 79*, 378–383.

Yeoh, W. K., Ali, A., & Forney, C. F. (2014). Effects of ozone on major antioxidants and microbial populations of fresh-cut papaya. *Postharvest Biology and Technology, 89*, 56–58.

Zao, Z., Xu, G., Han, Z., Li, Q., Chen, Y., & Li, D. (2013). Effect of ozone on the antioxidant capacity of 'Qiushui' pear (*Pyrus pyrifolia* Nakai cv. Qiushui) during postharvest storage. *Journal of Food Quality, 36*, 190–197.

Zhang, L., Lu, Z., Yu, Z., & Gao, X. (2005). Preservation of fresh-cut celery by treatment of ozonated water. *Food Control, 16*, 279–283.

Zhang, X., Zhang, Z., Wang, L., Zhang, Z., Li, J., & Zhao, C. (2011). Impact of ozone on quality of strawberry during cold storage. *Frontiers of Agriculture in China, 5*, 356–360.

Zhao, J., & Cranston, P. (1995). Microbial decontamination of black pepper by ozone and the effect of the treatment on volatile oil constituents of the spice. *Journal of the Science of Food and Agriculture, 68*, 11–18.

Zimran, A., Wasser, G., Forman, L., Gelbart, T., & Beutler, E. (1999). Effect of ozone on red blood cell enzymes and intermediates. *Acta Haematologica, 102*, 148–151.

Chapter 12
Chlorine Dioxide (ClO_2)

Vivian Chi-Hua Wu

Introduction

Chlorine dioxide (ClO_2) has been used to control microbiological contamination. Since the 1920s, ClO_2 has been acknowledged for its disinfection properties. After first being recognized as a chemical sterilizing agent in 1984, it was recognized by the US Environmental Protection Agency (EPA) in 1988 as a sterilant effective against bacteria, viruses, and protozoa, including oocysts of *Cryptosporidium* and *Giardia* cysts.

Chlorine dioxide is also recognized by the US Food and Drug Administration (FDA) for disinfecting drinking water and trihalomethane and haloacetic acid control in drinking water, bacteria control in cooling water and in paper processing, sanitizing uncut fruits and vegetables, controlling bacteria in produce flume water, controlling bacteria in poultry processing, controlling bacteria in beverage and brewing equipment, as well as sanitizing food facilities and equipment (International Dioxcide, Inc. 2000). The US EPA has approved the use of ClO_2 as a disinfectant for potable water treatment with a monitoring requirement of 1-ppm limit to chlorite ion in the treated water (Anonymous 2000a). The US FDA has also approved the use of ClO_2 as a bactericidal agent in poultry processing water at a level of up to 3 ppm residual ClO_2 (Anonymous 1995). Aqueous ClO_2 was approved by the US FDA for washing fruits and vegetables at concentrations not exceeding 3 ppm residual ClO_2 (Anonymous 1998). The use of ClO_2 in an isolator (sealed chamber) has increased in popularity within the industries of the USA and Europe. Pharmaceutical and medical device industries have adopted ClO_2 in gaseous form as it maintains the properties of a true gas at ambient temperatures, is unaffected by temperature, and

V.C.-H. Wu (✉)
The Pathogenic Microbiology Laboratory, School of Food and Agriculture, The University of Maine, 5735 Hitchner Hall, Orono, ME 04469, USA
e-mail: vivian.wu@umit.maine.edu

M.W. Siddiqui et al. (eds.), *Postharvest Management Approaches for Maintaining Quality of Fresh Produce*, DOI 10.1007/978-3-319-23582-0_12

is biocidal over a wide range of pH. Gaseous ClO_2 is also known for its process consistency since it can be accurately and precisely monitored and controlled (Czarneski and Lorcheim 2005).

Definition

Chlorine dioxide (ClO_2) is a synthetic, green-yellowish gas with a chlorine-like, irritating odor. It is different from elementary chlorine in its chemical structure and behavior. In water, ClO_2 exists as free radicals. At high concentrations, it reacts strongly with reducing agents. Chlorine dioxide is an unstable gas that dissociates into chlorine gas (Cl_2) and oxygen and producing heat. When ClO_2 is photo-oxidized by sunlight, it breaks apart. The end products of ClO_2 reactions are chloride (Cl^-), chlorite (ClO^-), and chlorate (ClO_3^-). One of the most important qualities of ClO_2 is its high water solubility, especially in cold water. Chlorine dioxide does not hydrolyze when it enters water where it remains a dissolved gas in solution. Chlorine dioxide is approximately 10 times more soluble in water than chlorine. Chlorine dioxide can be removed by aeration or carbon dioxide (Lenntech 2007; Herdt and Feng 2009).

Chlorine dioxide is a strong oxidation agent and one of the few compounds that exist almost entirely as monomeric free radicals (Anonymous 2000b). Researchers have focused on ClO_2 as an alternative sanitizer to chlorine since it has an oxidation capacity approximately 2.5 times higher than that of chlorine and is less reactive to organic compounds and less affected by pH (Benarde et al. 1967; Richardson et al. 1998; Beuchat et al. 2004; Han et al. 2004; Lee et al. 2004; Sy et al. 2005a). The interest in using ClO_2 as decontaminant for vegetables is largely due to its efficacy, which is less affected by low pH and organic matter, and its inertness towards ammonia to form chloramines (Beuchat 1998).

Aqueous and Gaseous Chlorine Dioxide

Studies have shown that both of the aqueous and gaseous ClO_2 are effective sanitizing agents (Benarde et al. 1967; Richardson et al. 1998; Beuchat et al. 2004; Han et al. 2004; Lee et al. 2004; Sy et al. 2005a; Wu and Kim 2007; Wu and Rioux 2010). Both forms inactivate a broad spectrum of microorganisms such as bacteria, spores, viruses, protozoa, and algae. At equal concentrations, gaseous ClO_2 is more effective than aqueous ClO_2 as it offers greater penetration into small spaces where the aqueous form cannot reach (Czarneski and Lorcheim 2005; Wu and Kim 2007). The efficacy of treatment depends on the gas concentration, exposure time, relative humidity, temperature, and ratio of volume of product to the amount of ClO_2 applied (Han et al. 2001). In treatments using aqueous ClO_2, surface integrity plays a role in treatment efficacy. Most investigators working on ClO_2 sanitization use gaseous ClO_2 for its greater penetration capability rather than aqueous ClO_2; hence

it is considered more effective in reducing microorganisms on fruits and vegetables (Han et al. 2001; Lee et al. 2004). In addition, aqueous ClO_2 treatments may result in residual water that promotes mold growth on fresh produce if the residual water is not removed or dried off.

For applications in the food industry, the use of gaseous ClO_2 may be limited because the treatment must be conducted in a firmly and safely sealed chamber (Lee et al. 2004) since high concentrations of the gas are potentially explosive, and numerous mechanical devices or steps are necessary to handle ClO_2 gas as well as to provide precise concentrations for sanitization (Han et al. 2004; Lee et al. 2006). Aqueous ClO_2 offers several advantages for food sanitization, especially for processing of vegetables and fruits. A special chamber is not required for the sanitizing process, handling is easier than with gaseous ClO_2, and the liquid can be easily applied to the existing process during washing without modifying subsequent processing steps. In contrast to the hydrolysis of chlorine gas in water, ClO_2 in water does not degrade appreciably and remains in solution as a dissolved gas. Compared to chlorine, ClO_2 produces fewer potentially carcinogenic by-products such as trihalomethanes in the presence of organic material (Richardson et al. 1998; Wu and Kim 2007).

The use of chlorine dioxide has advantage over other chlorine-based compounds. Gaseous ClO_2 is able to break down odor-causing phenolic compounds and will not react with substances such as ammonia (Sy et al. 2005b). Upon chlorination with chlorine, hypochlorite, a very unstable compound, degrades to a mixture of chloride and chlorate, especially when not kept cold (Lenntech 2007). Chlorine is also not effective against many bacterial and fungal spores; therefore higher concentrations of chlorine and a longer treatment time are needed. Chlorine dioxide's major chemical reaction is oxidation; therefore prevent the formation of trihalomethane and haloacetic acid. This allows for low phytotoxicity, making it safe to humans and the environment. Chlorine dioxide, unlike chlorine, will not react with and not be consumed by other impurities. The efficacy of ClO_2 is less affected by pH and organic matter, and it does not react with ammonia to form chloramines, as do liquid chlorine and hypochlorites. As the consumption of fruits and vegetables has increased in the USA, outbreaks of foodborne illness have also risen. Consequently, an effective way to eliminate foodborne contamination is critical to reduce foodborne illnesses. The use of gaseous ClO_2 to reduce microbial contamination on fresh produce is an advantageous alternative to the use of chlorine.

Antimicrobial Mechanisms

Cell membrane was reported as the primary target of ClO_2. The mode of action of ClO_2 involves the disruption of cell protein synthesis and membrane permeability control (Wu and Kim 2007). Damage to the genetic materials is also a possibility. Upon treatment, ClO_2 reacts with the amino acids and RNA within the cell, prevents the production of proteins, affects the cell membrane by switching membrane

proteins and fats, and prevents inhalation (Lenntech 2007). The effect of ClO_2 was related to nonspecific oxidative damage of the outer membrane leading to the destruction of the trans-membrane ionic gradient. Young and Setlow (2003) reported that ClO_2 contributed to *Bacillus subtilis* spores death through the initial steps of spore germination.

Generation Methods for Chlorine Dioxide

Chlorine dioxide cannot be compressed and stored under pressure because it is explosive; therefore the shipping of gaseous ClO_2 is impossible, and it has to be generated on-site. There are different ways to generate both aqueous and gaseous ClO_2. The chosen method is based on its applications (such as size and type of surfaces and/or materials), desired concentration, and environmental factors. As each of these concerns is important, scientists must determine the appropriate treatment conditions for applying ClO_2. Traditionally, ClO_2 generation requires either a reaction with acid or on-site equipment such as an applicator or generator. Easy-to-use commercial systems exist on the market for on-site ClO_2 generation. They generally consist of two sachets containing a precursor for ClO_2 generation, which takes place upon mixing two components (Lee et al. 2004; Sy et al. 2005a; Wu and Kim 2007; Wu and Rioux 2010).

One common method of gaseous ClO_2 generation takes place in an isolator and is commonly used for the sterilization of industrial supplies. This method of generation is similar to those that use humidity or moisture along with specific gas for sporicidal treatments. The generation of gas within the isolator is performed by using solid sodium chlorite in small cartridges. After the addition of a chlorine-nitrogen (2:98 %) gas mixture, pure ClO_2 will result in the generation of nitrogen without any form of by-product (Czarneski and Lorcheim 2005). **The reaction is as follows:**

$$Cl_2(g) + 2NaClO_2(s) \rightarrow 2ClO_2(g) + 2NaCl(s)^*$$

*The letter (g) stands for gas and (s) stands for solid.

Sieman's Water Technologies has put out a Wallace and Tieman Millenium III™ CUBE batch generator that is made for smaller scale ClO_2 decontamination. This system generates gas using two methods. The first method is by the reaction of molecular chlorine gas and a 25 % aqueous sodium chlorite solution. The reaction is as follows:

$$2NaClO_2 + Cl_2 \rightarrow 2ClO_2 + 2NaCl.$$

The second method consists of two steps. The first step is to produce chlorine gas by the reaction between a 12.5 % solution of sodium hypochlorite and a 15 %

solution of hydrochloric acid. In the second step, the chlorine gas reacts with 25 % sodium chlorite solution to produce ClO_2. The reactions are as follows:

Step 1: $NaOCl + 2HCl \rightarrow Cl_2 + 2NaCl + H_2O$

Step 2: $2NaClO_2 + Cl_2 \rightarrow 2ClO_2 + 2NaCl$

Another method for generating gaseous ClO_2 is using a tablet called Aseptrol® manufactured by Engelhard (Iselin, New Jersey, USA). Aseptrol® allows for the delivery of ClO_2 in a stable form. An Aseptrol® tablet consists of proprietary activators that react with chlorite salt within the tablet to generate ClO_2 when in contact with water or moisture from the air. Aseptrol® is available in forms of loose powder, dissolvable tablets, hot melts, and sachets for the application of deodorizing and disinfecting (Cochrane 2005).

Other methods have also been used to generate aqueous ClO_2. For example, Purogene® and Purogene Professional® are premium blends of ClO_2 produced by Bio-cide International (Norman, OK, USA). Purogene® is scientifically proven to be a broad-spectrum antimicrobial agent used to eliminate both Gram-negative and Gram-positive bacteria, yeasts, mold, and fungi. Purogene® (2 % ClO_2 solution) is used pre-storage on potatoes as a mist at concentrations between 200 and 400 ppm. Purogene® is shipped as sodium chlorite and activated by a small amount of food grade acid to produce ClO_2. Purogene Professional® offers a 5 % ClO_2 solution that uses citric acid and sodium chlorite to produce ClO_2. One method uses 45 g/L of citric acid dissolved in a 5 % concentrated sodium chlorite solution. This product is then diluted to 2000 ppm and further diluted to obtain the desired solution concentration (Anonymous 2015). Anthium AGP® (5 % ClO_2 solution), manufactured by International Dioxcide (North Kingstown, RI, USA), generates aqueous ClO_2 similarly to Purogene Professional® and is also used on potatoes during storage to prevent spoilage organisms from contaminating the storage facility.

Vibrex®, a form of germicide produced by Agranco Corp. (Coral Gables, FL, USA), is an advanced formulation of ClO_2 and oxygen in an aqueous solution. Vibrex®, a colorless, odorless, nontoxic, noncorrosive, and nonflammable liquid, is a wide spectrum bactericide, fungicide, virucide, and algaecide. It is 160 % more effective than chlorine, 10 times more stable, and will not dissipate. It has been approved by the US FDA as a disinfectant in many applications, especially for fresh fruits and vegetables (Agranco Corp. 2007). In the potato industry, Vibrex® at 50 ppm is used as part of a humidification system for storage and a germicide for controlling common potato bacteria, such as *Pseudomonas aeruginosa* and *Erwinia carotovora*, fungi, and viruses. At this concentration, Vibrex® completely inactivates *Pseudomonas* spp. after 10 min and inactivates 99.9 % of *E. carotovora* after 60 s (Agranco Corp. 2007).

A simple, small, and dry chemical pouch method was developed for generating ClO_2 (Intellectual Capital Associates [ICA] TriNova, LLC, Forest Park, GA, USA). This method, generating chlorine dioxide from sachets, is easy to use comparing with machinery systems. Gaseous ClO_2 is generated by combining an equal amount of sodium chlorite and activating acids in a sachet without adding any solution. After activation, the sachet is placed in the application area. Different concentrations of gaseous ClO_2 may be generated by controlling the amounts of compounds

without the need for expensive equipment (Wu and Rioux 2010). For generation of aqueous ClO_2, a ClO_2 solution sachet consisting of two components is shaken to mix the chemicals (sodium chlorite and acid). The mixed sachet is placed in distilled water in a container to allow the complete release of ClO_2 into the water. The stock solution can be further diluted to obtain various concentrations of aqueous ClO_2 prior to use (Wu and Kim 2007).

Applications of Chlorine Dioxide as a Sanitation Method on Produce

Numerous studies have proven that ClO_2 is effective in reducing foodborne pathogens such as *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* spp. as well as spoilage organisms on fruits and vegetables surfaces. In general, the level of decontamination achieved through gaseous treatment is higher than those with aqueous washes, and a greater than 8-log reduction has been reported (Han et al. 2000, 2001; Wu and Kim 2007; Gómez-López et al. 2009; Wu and Rioux 2010). However, results were various depending on factors such as the design of the studies, sample variety, and treatment scale.

Han et al. (2001) tested the reduction of *L. monocytogenes* on injured and non-injured green pepper surfaces by water washing, aqueous ClO_2, and gaseous ClO_2 treatments. Gaseous ClO_2 was generated by a laboratory generator using 4 % chlorine in nitrogen gas. The gas was then collected in sampling bags. A sampling syringe was used to add a certain amount of gas into the Plexiglas cylinder where the samples were placed. Results were positive for gaseous ClO_2 as it showed a significantly higher log reduction than treatments with aqueous ClO_2 and water for both injured and uninjured surfaces (Han et al. 2001). Lee et al. (2004) studied gaseous ClO_2 by the use of a dry chemical sachet against *L. monocytogenes*, *E. coli* O157:H7, and *S.* Typhimurium on lettuce leaves in a model 20-L gas cabinet. After microbial inoculation, lettuce leaves were treated with ClO_2 at 4.3, 6.7, and 8.7 mg for 30 min, 1 h, and 3 h. The gaseous ClO_2 reduced 3–6 log of the population of each bacterium without altering the sensory quality of the leaves (Lee et al. 2004).

Gaseous ClO_2 was also studied on strawberries using batch and continuous ClO_2 generating treatments (Han et al. 2004). Both *L. monocytogenes* and *E. coli* O157:H7 decreased over time after 15 min and 30 min using 0.2 mg/Land 0.6 mg/L of gaseous ClO_2. After 15 min with 0.2 mg/L, *E. coli* O157:H7 showed a 1.2-log CFU/strawberry reduction and a 2.4-log CFU/strawberry reduction after 30 min. *L. monocytogenes* showed a 1.8-log CFU/strawberry reduction after 15 min and a 2.8-log CFU/strawberry reduction after 30 min. At the 0.6 mg/L treatment, *E. coli* O157:H7 showed a 1.9-log CFU/strawberry reduction after 15 min, and a 3.0-log CFU/strawberry reduction after 30 min. *L. monocytogenes* showed a 2.6-log CFU/strawberry reduction after 15 min and a 3.6-log CFU/strawberry log reduction after 30 min. In general, *L. monocytogenes* had a greater log reduction than *E. coli* O157:H7 for each treatment (Han et al. 2004). The results also indicated that the

continuous gas treatment was more effective as it showed more than a 3-log reduction after 10 min at a 0.6 mg/L treatment, whereas the batch gas treatment showed more than a 1.5-log reduction with the 0.6 mg/L treatment after 15 min (Han et al. 2004).

In a study by Sy et al. (2005a), gaseous ClO_2 was generated using a Plexiglas desiccator to determine its effectiveness as a sanitizer for killing *Salmonella*, yeasts, and molds on blueberries, strawberries, and raspberries, as small fruits have been associated with foodborne illnesses. Their results showed that the 8.0 mg/L treatment (for 120 min) significantly reduced the *Salmonella* population on blueberries (a reduction of 2.4–3.7 log CFU/g), strawberries (a reduction of 3.8–4.4 log CFU/g), and raspberries (a reduction of 1.5 log CFU/g). Results from 4.1 to 8.0 mg/L from 30 min to 120 min for yeasts and molds on blueberries, strawberries, and raspberries were 1.4 to 2.5, 1.4 to 4.2, and 2.6 to 3.0 log CFU/g reductions, respectively (Sy et al. 2005a).

Reducing populations of yeast and molds as well as spoilage microorganisms on produce is important especially during storage. As produce generally maintains a high population of yeast and mold, it is best that these microorganisms are controlled as produce may contain weakened or damaged points which become sites for microbial contamination. Gaseous ClO_2 has been shown to have a variable effect on produce shelf life, either remaining the same or increasing the shelf life (Gómez-López et al. 2009). A shelf life extension of 8–16 days was seen with treated strawberries (Mahmoud et al. 2007). Kleinkopf et al. (2001) used a humidifier as a method of ClO_2 application at 50–200 ppm against late blight in potato storage. The experiment showed an unacceptable 30 % reduction on the surface infection.

Wu and Rioux (2010) studied gaseous ClO_2 generated by combining an equal amount of impregnated sodium chlorite and activating acids in a sachet without using any solution or equipment. After activation by mixing, the sachet was placed in the application area. They reported that gaseous ClO_2 was effective for natural microbiota, showing over a 5-log CFU/potato reduction. For *P. aeruginosa*, there was almost a 6-log CFU/potato reduction after treatment. Gaseous ClO_2 did not affect the overall visual quality of the potato. The residue of ClO_2 decreased to <1 mg/L after 14 days for each treatment, indicating ClO_2 dissipated naturally over time.

Gaseous ClO_2 has been known to cause browning or bleaching in treated samples. Mahmoud and Linton (2008) found that lettuce samples all turned brown and white after 10 min treatment with ClO_2 at 5 mg/L. On the other hand, some studies did not find that ClO_2 altered the sensory quality of produce (Sy et al. 2005a, b; Mahmoud et al. 2008).

Compared to the amount of information available on chlorine as an aqueous antimicrobial for fresh produce, much less is available for aqueous ClO_2 on the antimicrobial effectiveness for fresh and fresh-cut produce. Zhang and Farber (1996) found that a 10-min exposure of shredded lettuce or cabbage to 5 ppm ClO_2 caused a 1.1-log reduction of *L. monocytogenes*, while Rodgers et al. (2004) reported a 4-log reduction of *L. monocytogenes* on shredded lettuce using 5 ppm for 5 min. The difference in results from these studies is likely due to the variation in the methods employed. Singh et al. (2002) reported that washing shredded lettuce

inoculated with *E. coli* O157:H7 for 5 min with 10 mg/L ClO_2 reduced counts by 1.20 log CFU/g and a second washing step of 5 min resulted in additional reduction, although a third one did not show additional effect. Rodgers et al. (2004) reported that aqueous ClO_2 at 5 ppm concentration was able to achieve greater than 5 log reductions of *L. monocytogenes* and *E. coli* O157:H7 on apples, lettuce, and cantaloupe. Pao et al. (2007) reported significant sanitizing ability of 20 mg/L ClO_2 against *S. enterica* and *E. carotovora* inoculated onto the surface of tomato only when the fruit was freshly inoculated with these microorganisms, reaching a 5-log CFU/cm^2 reduction. Once the inoculation suspension was dried out on the fruit surface, no significant reduction was observed in comparison with regular tap water. Wu and Kim (2007) studied the effect of aqueous ClO_2 on controlling foodborne pathogens, yeasts, and molds on blueberries. They found that ClO_2 was most effective in reducing *L. monocytogenes* (4.88 log CFU/g) as compared to the other pathogens. *Pseudomonas aeruginosa* was reduced by 2.16 log CFU/g after 5 min when treated with 15 ppm of ClO_2. The most effective treatment for *S.* Typhimurium was 15 ppm ClO_2 for 20 min that reduced *S.* Typhimurium by 3.32 log CFU/g. The highest reduction (4.56 log CFU/g) of *Staphylococcus aureus* was achieved with 15 ppm of ClO_2 for 30 min. When treated for 2 h with 5 ppm of ClO_2, *Yersinia enterocolitica* was reduced by 3.49 log CFU/g.

Aqueous ClO_2 has been reported to reduce native microflora on food products and extend the shelf life (Gómez-López et al. 2009). Rodgers et al. (2004) found a 4.2-log reduction of mesophilic bacteria treated with a 5 mg/L solution and Wu and Kim (2007) reported a 2.82 log reduction of yeasts and molds on blueberries treated with 15 mg/L. Depending on products, shelf life extension ranges from no increase to significantly increased have been reported (Gómez-López et al. 2009; Chen et al. 2010, 2011). The effect of aqueous chlorine treatment on the sensory degradation of treated products is rarely reported and is usually considered minor (Gómez-López et al. 2009).

Based on the results from these studies, scientists have been working to continue to determine if ClO_2 is effective enough to actually penetrate produce. Researchers are also interested in developing methods that will allow ClO_2 to be effective for the entire produce production from preharvest management to postharvest process and in implementing ClO_2 as one of the treatments (hurdles) combined with other interventions to achieve effective control of microbial contamination.

Conclusions

As a strong oxidizing agent, ClO_2 has a potential to be used to maintain the postharvest quality and microbial safety of fresh produce. More research is needed for the effects of ClO_2 on the produce physiology, toxicology, and nutrients' stability. A better understanding of the interactions between ClO_2 and produce and microorganisms will provide insight into finding means to improve the efficacy of decontamination, including enhancing the antimicrobial activity against pathogens that

are adhered to or entrapped in structures and tissues of produce surfaces. Incorporating ClO_2 in a multiple intervention approach throughout the supply chain could be considered to enhance the safety of fresh and fresh-cut produce.

Acknowledgements The author would like to thank Amanda Rioux and David Bridges for assistance with collecting information.

References

Agranco Corporation (2007). Vibrex: Technical summary. http://www.agranco.com/vibrex.htm.

Anonymous (1995). 60 CFR, Part 173. Secondary direct food additives permitted in food for human consumption. U.S. Code of Federal Registrations.

Anonymous (1998). 21 CFR, Part 173.300. Secondary direct food additives permitted in food for human consumption. Chlorine Dioxide. U.S. Code of Federal Registrations.

Anonymous (2000a). 40 CFR, Part 141.64. Maximum containment level for disinfection by products. U.S. Code of Federal Registrations.

Anonymous (2000b). Environmental health criteria 216: Disinfectants and disinfectant by-products. World Health Organization.

Anonymous (2015). Bio-cide International. http://www.bio-cide.com/.

Benarde, M. A., Snow, W. B., Olivieri, V. P., & Davidson, B. (1967). Kinetics and mechanism of bacterial disinfection by chlorine dioxide. *Applied Microbiology, 15*, 257–265.

Beuchat, L. (1998). Surface decontamination of fruits and vegetables eaten raw: A review. World Health Organization.

Beuchat, L. R., Pettigrew, C. A., Tremblay, M. E., Roselle, B. J., & Scouten, A. J. (2004). Lethality of chlorine, chlorine dioxide, and a commercial fruit and vegetable sanitizer to vegetative cells and spores of *Bacillus cereus* and spores of *Bacillus thuringiensis. Journal of Food Protection, 67*, 1702–1708.

Chen, Z., Zhu, C., Zhang, Y., Niu, D., & Du, J. (2010). Effects of aqueous chlorine dioxide treatment on enzymatic browning and shelf-life of fresh-cut asparagus lettuce (*Lactuca sativa* L.). *Postharvest Biology and Technology, 58*, 232–238.

Chen, Z., Zhu, C., & Han, Z. (2011). Effects of aqueous chlorine dioxide treatment on nutritional components and shelf-life of mulberry fruit (*Morus alba* L.). *Journal of Bioscience and Bioengineering, 111*, 675–681.

Cochrane, M. (2005). Redefining chlorine dioxide delivery systems. http://www.idspackaging.com/Common/Paper/Paper_56/Redefining%20Chlorine%20Dioxide%20Delivery%20Systems.htm.

Czarneski, M. A., & Lorcheim, P. (2005). Isolator decontamination using chlorine dioxide gas. *Pharmaceutical Technology, 29*, 124–133.

Gómez-López, V. M., Rajkovic, A., Ragaert, P., Smigic, N., & Devlieghere, F. (2009). Chlorine dioxide for minimally processed produce preservation: A review. *Trends in Food Science & Technology, 20*, 17–26.

Han, Y., Linton, R., Nielsen, S., & Nelson, P. (2001). Reduction of *Listeria monocytogenes* on green peppers (*Capsicum annuum* L.) by gaseous and aqueous chlorine dioxide and water washing and its growth at 7 °C. *Journal of Food Protection, 64*, 1730–1738.

Han, Y., Selby, T., Schultze, K., Nelson, P., & Linton, R. (2004). Decontamination of strawberries using batch and continuous chlorine dioxide gas treatments. *Journal of Food Protection, 67*, 2450–2455.

Han, Y., Sherman, D., Linton, R., Nielsen, S., & Nelson, P. (2000). The effects of washing and chlorine dioxide gas on survival and attachment of *Escherichia coli* O157:H7 to green pepper surfaces. *Food Microbiology, 17*, 521–533.

Herdt, J., & Feng, H. (2009). Aqueous antimicrobial treatments to improve fresh and fresh-cut produce safety. In X. Fan, A. N. Brendan, C. J. Doona, F. E. Feeherry, & R. B. Gravani (Eds.), *Microbial safety of fresh produce* (pp. 169–190). Iowa, USA: Wiley-Blackwell and the Institute of Food Technologists.

Kleinkopf, G. E., Olsen, N. L., Woodell, L. K. (2001). Efficacy of chlorine dioxide in potato storage environments. http://www.cals.uidaho.edu/potatoes/Research&Extension/Topic/Storage/EfficacyOfChlorineDioxideInPotatoStorageEnvironments-01.pdf.

Lee, S.-Y., Costello, M., & Kang, D.-H. (2004). Efficacy of chlorine dioxide gas as a sanitizer of lettuce leaves. *Journal of Food Protection, 67*, 1371–1376.

Lee, S.-Y., Dancer, G. I., Chang, S.-S., Rhee, M.-S., & Kang, D.-H. (2006). Efficacy of chlorine dioxide gas against *Alicyclobacillus acidoterrestris* spores on apple surfaces. *International Journal of Food Microbiology, 108*, 364–368.

Lenntech (2007). Disinfection: Chlorine dioxide. http://www.lenntech.com/water-disinfection/disinfectants-chlorine-dioxide.htm#Ontdekking%20chloordioxide.

Mahmoud, B. S., Bhagat, A., & Linton, R. (2007). Inactivation kinetics of inoculated *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella enterica* on strawberries by chlorine dioxide gas. *Food Microbiology, 24*, 736–744.

Mahmoud, B., & Linton, R. (2008). Inactivation kinetics of inoculated *Escherichia coli* O157:H7 and *Salmonella enterica* on lettuce by chlorine dioxide gas. *Food Microbiology, 25*, 244–252.

Mahmoud, B., Vaidya, N., Corvalan, C., & Linton, R. (2008). Inactivation kinetics of inoculated *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Poona on whole cantaloupe by chlorine dioxide gas. *Food Microbiology, 25*, 857–865.

Pao, S., Kelsey, D., Khalid, M., & Ettinger, M. (2007). Using aqueous chlorine dioxide to prevent contamination of tomatoes with *Salmonella enterica* and *Erwinia carotovora* during fruit washing. *Journal of Food Protection, 70*, 629–634.

Richardson, S., Thruston, A., Caughran, T., Collette, T., Patterson, K., & Lykins, B. (1998). Chemical by-products of chlorine and alternative disinfectants. *Food Technology, 52*, 58–61.

Rodgers, S. L., Cash, J. N., Siddiq, M., & Ryser, E. T. (2004). A comparison of different chemical sanitizers for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes* in solution and on apples, lettuce, strawberries, and cantaloupe. *Journal of Food Protection, 67*, 721–731.

Singh, N., Singh, R., Bhunia, A., & Stroshine, R. (2002). Effect of inoculation and washing methods on the efficacy of different sanitizers against *Escherichia coli* O157:H7 on lettuce. *Food Microbiology, 19*, 183–193.

Sy, K. V., McWatters, K. H., & Beuchat, L. R. (2005a). Efficacy of gaseous chlorine dioxide as a sanitizer for killing *Salmonella*, yeasts, and molds on blueberries, strawberries, and raspberries. *Journal of Food Protection, 68*, 1165–1175.

Sy, K. V., Murray, M. B., Harrison, M. D., & Beuchat, L. R. (2005b). Evaluation of gaseous chlorine dioxide as a sanitizer for killing *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and yeasts and molds on fresh and fresh-cut produce. *Journal of Food Protection, 68*, 1176–1187.

Wu, V. C., & Kim, B. (2007). Effect of a simple chlorine dioxide method for controlling five foodborne pathogens, yeasts and molds on blueberries. *Food Microbiology, 24*, 794–800.

Wu, V. C., & Rioux, A. (2010). A simple instrument-free gaseous chlorine dioxide method for microbial decontamination of potatoes during storage. *Food Microbiology, 27*, 179–184.

Young, S., & Setlow, P. (2003). Mechanisms of killing of *Bacillus subtilis* spores by hypochlorite and chlorine dioxide. *Journal of Applied Microbiology, 95*, 54–67.

Zhang, S., & Farber, J. (1996). The effects of various disinfectants against *Listeria monocytogenes* on fresh-cut vegetables. *Food Microbiology, 13*, 311–321.

Index

M.W. Siddiqui et al. (eds.), *Postharvest Management Approaches for Maintaining Quality of Fresh Produce*, DOI 10.1007/978-3-319-23582-0