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Microbes in Food and Health

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Contents

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Non-dairy Functional Foods: Potential of Probiotics

Rama Bhadekar and Priyanka Parhi

1 Introduction

Nowadays concern about one's health and well-being is growing due to realization of importance of co-relation between diet, nutrition, and healthy lifestyle. Consumers demand for safe and varied food products which will ensure longevity and reduce risk of diseases. This is mainly due to increased incidences of obesity and overweight in men and women, chronic and non-communicable diseases, and mental health problems like depression, poor memory, and loss of memory. Majority of them are the result of increased urbanization, lack of physical exercise, and inclusion of high calorie foods in diet. Hence, foods which play significant role in various disorders or diseases are gaining importance. This has led to commercialization of functional foods. These are the foods that positively affect health and can be defined as foods containing significant levels of biologically active components that provide specific health benefits beyond the traditional nutrients they contain (Drozen and Harrison 1998). Such bioactive components include probiotics, antioxidants, omega-3-fatty acids, or synthetic food ingredients like prebiotics, vitamins, minerals, amino acids, proteins, etc. Another important reason for increasing interest in functional foods is the increase in healthcare cost.

Functional food may help to prevent or reduce risk of developing diseases and enhance human health. Noteworthy benefits of functional foods are in reducing risk of cardiovascular disease, cancer, and osteoporosis. Also, they play an important role in the improvement in general health and mental health. Thus, they have both protective and remedial effects (Stanton et al. 2001). Majority of the immunomodulatory effects of functional foods are due to probiotic microorganisms

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conventionally carried through milk-based products like yogurt, curd, cheese, etc. However, nowadays there is growing interest in non-dairy functional foods fortified with probiotics although traditionally they have been consumed in various countries for a long time. This chapter focuses on various non-dairy probiotic foods, their nutritional value, technological aspects, and challenges in developing such food types.

2 Global Market

The world market of functional foods is predicted to reach $130 billion after 2015 (http://www.reportlinker.com/ci02036/Functional-Food.html). Among the functional foods most important are probiotic yogurts, plant sterol spreads, functional waters, juices, deserts, and cheeses (Granato et al. 2010; Stanton et al. 2001). Major factors affecting their market potential are government support, consumer demand, consumer confidence in products, and health awareness. Functional foods have to compete with organic foods and foods with low fat, low sugar, and low salt labels. Thus, the prerequisites to increase their market are communication of their health benefits in simple language, good taste, convenience, and affordable price. In addition, brand name, loyalty, advertising and promotion, quality control, competitors, and economics are also important (Euromonitor 2009). In Europe, huge market exists for pro-, pre-, and synbiotics (Bhadoria and Mahapatra 2011).

3 Probiotics

3.1 Health Benefits

Probiotics are defined as "live microorganisms which when administered in adequate amount confer health benefits on the host by improving the properties of indigenous microflora" (Tabbers and Benninga 2007). Earlier research has shown that certain strains of probiotic bacteria have many health benefits. These microorganisms

(a) enhance immune response by (1) improving innate and acquired immunity, (2) changing cytokine profiles, and (3) increasing levels of immunoglobulins.
(b) reduce severity of constipation and improve bowel moment frequency (Ouwehand et al. 2003)
(c) control urogenital infections in women (Dani et al. 2002)
(d) inhibit effect on *Helicobacter pylori* (Hamilton-Miller et al. 2003)
(e) reduce the risk of bladder cancer (Rafter 2004)
(f) decrease LDL cholesterol levels (Pereira and Gibson 2002)
(g) prevent fungal outgrowth and allergic reactions

(h) produce vital nutrients like vitamin K and act as antioxidants (Crittenden et al. 2005; Anonymous 2010).

3.2 *Lactobacilli and Bifidobacteria*

The probiotic bacteria used today mainly belong to the genera *Lactobacillus* and *Bifidobacterium*. Commonly used strains are *L. acidophilus, L. casei, L. rhamnosus, L. johnsonii*, etc. Bifidobacteria strains include *B. bifidum, B. longum, B. infantis*, etc. (Reuter 1997; Holzapfel et al. 1997; Huis in't Veld and Havenaar 1997; Bonaparte and Reuter 1997). Both lactobacilli and bifidobacteria are important microorganisms in the gastrointestinal tract (GIT) and urogenital tract of humans and higher animals (Sgorbati et al. 1995). Many different environmental factors like pH, O_2 availability, specific substrates, and bacterial interactions affect distribution of lactobacilli, while age and diet are the main deciding factors for bifidobacteria. The latter are predominantly present in infants; however, with increasing age their number decreases (Finegold et al. 1983). Thus, they belong to the category of generally regarded as safe (GRAS) microorganisms and act as health promoters.

3.3 *Strain Selection*

The probiotic strain selection for food application depends on their technologic properties, besides the health benefits. The main selection criteria include (1) their survival during transit through stomach and small intestine, (2) adhesion to human GIT, (3) tolerance to oxygen, acid, bile, and salt, and (4) genetic stability (Karovicova et al. 1994, 1999; Holzapfel 2002; Aukrust et al. 1994; Ausco et al. 1998). Moreover, they should produce final product with good taste and acceptable texture, be produced on a large scale, be nonpathogenic and nontoxic, and be safe for technological uses (Adams and Marteau 1995; Donohue and Salminen 1996). Also, changes in food due to microbial metabolism should not affect their stability and functional properties. In addition, their survival throughout manufacturing process, storage, and distribution also must be regularly controlled and monitored. In order to achieve maximum benefits, the selected strain of microorganisms must be present in high numbers, i.e., 10^9 cells/daily ingested dose. Moreover, the minimum dose must be indicated on the product to confer specific health benefits (Guarner and Schaafsma 1998). Thus, their functional and technological properties are equally important. Nevertheless, food production process affects properties of probiotics, indicating importance of their interactions with other microorganisms as well as with food components. The latter depends on the time when probiotics are added to it, their physiological state, and treatment of probiotics during and after harvesting (Ross et al. 2005).

Synergism of probiotics with other food microorganisms normally results in increased acidification and increase in the number of organisms (Driessen et al. 1982; Radke-Mitchell and Sadine 1986; Perez et al. 1991; Zourari et al. 1992), while inhibition of other microorganisms results due to (1) competition for available nutrients, (2) decrease in redox potential, (3) organic acid production, (4) decrease in pH, and (5) production of bacteriocins, H_2O_2, biogenic amines, benzoic acid, etc. Their ability to produce bacteriocins helps to extend shelf life and safety of the product (Kalantzopoulos 1997). These biopreservatives can be destroyed by digestive enzymes which is an advantage over classical antibiotics and chemical preservatives. Such strains can be used along with starter cultures to improve the quality of food (Caplice and Fitzerland 1999). However, their antagonistic activity may hinder the development of probiotic food with starters (Joseph et al. 1998). Thus, in order to produce marketable probiotic products, the most important prerequisites are (1) survival of microorganisms in sufficient number in the product, (2) their physical and genetic stability during storage of the product, and (3) expression of their beneficial health effects after consumption. Table 1 enlist commercial probiotic strains sold by different companies in the world.

4 Preference for Non-dairy Probiotic Food

Usually, health benefits of probiotics are achieved and maintained by consumption of milk-based products or dairy products. However, lactose intolerance, cholesterol content, and allergenic milk proteins are the major limitations of consuming milk-based products (Yoon et al. 2006). Almost 75 % of world population is lactose intolerant (http://www.pcrm.org/health/diets/vegdiets/what-is-lactose-intolerance). It is mainly due to deficiency of one or more enzymes required for lactose digestion. Unfortunately, there is no treatment to improve the enzyme levels. Hence, the symptoms have to be treated by changing the diet containing alternatives to dairy products (Schaafsma 2008). The starter culture in yogurt and cheeses can lessen lactose intolerance in those individuals consuming probiotics through dairy products. These microorganisms produce β-galactosidase in small intestine which assists in lactose digestion (Li et al. 2012). But the effectiveness depends on certain factors like number of cells in the product and amount of lactose produced. In case of milk-sensitive individuals, lactose indigestion results in bloating, cramping, and flatulence that affects the quality of life. Besides, these people are deprived of other health benefits resulting from consumption of probiotics. This emphasizes the need to develop non-dairy products with probiotic benefits. Additionally, worldwide trend of vegetarian diet and traditional and economic reasons in developing countries support the concept of using the substrates other than milk to deliver probiotics.

Table 1 Commercially available probiotic strains (*source*: http://www.nature.com/ajgsup/journal/v1/n1/fig_tab/ajgsup20127t2.html, Bhadoria and Mahapatra 2011)

S. No.	Strains	Sold by
1.	*Lactobacillus acidophilus* NCFM	Dupont Nutrition Biosciences ApS (Madison WI)
2.	*Saccharomyces cerevisiae boulardii*	Biocodex (creswell OR)
3.	*B. infantis* 35624	Procter & Gamble (Mason OH)
4.	*L. rhamnosus* R0011	Lallemand (Montreal, Canada)
5.	*B. lactis* Bb-12	Chr. Hansen (Milwaukee WI)
6.	*L. casei* Shirota	Yakult (Tokyo, Japan)
7.	*L. casei* DN-114 001	Danone (Paris, France)
8.	*B. animalis* DN-173 010	Dannon (Tarrytown, NY)
9.	*L. johnsonii* Lj-1	Nestle (Lausanne, Switzerland)
10.	*L. plantarum* 299V	Probi AB (Lund, Aweden)
11.	*L. rhamnosus* 271	NextFoods (Boulder, Colorado)
12.	*L. reuteri* ATCC 55730	Biogaia (Stockholm, Sweden)
13.	*L. rhamnosus* GG	Valio Dairy (Helsinki, Finland)
14.	*L. rhamnosus* LB21	Essum AB (Umea, Sweden)
15.	*L. salivarius* UCC118	University College (Cork, Ireland)
16.	*B. longum* BB536	Morinaga Milk Industry Co., Ltd. (Zama-City, Japan)
17.	*L. acidophilus* LB	Lacteol Laboratory (Houdan, France)
18.	*Bacillus coagulans* BC30	Ganeden Biotech Inc. (Cleveland, OH)
19.	*L. fermentum* VRI003 (PCC)	Probiomics, Eveleigh, Australia
20.	*L. rhamnosus* R0011, *L. acidophilus* R0052	Institut Rosell (Montreal, Canada)
21.	*L. salivarius* UCC118	University College (Cork, Ireland)
22.	*B. longum* BB536	Morinaga Milk Industry Co., Ltd. (Zama-city, Japan)
23.	*B. lactis* HN019 (DR10)	Danisco (Madison WI)
24.	*L. rhamnosus* HN001 (DR20)	Fonterra (Wellington, New Zealand)
25.	*L. paracasei* F19	Medipharm (Des Moines, lowa)
26.	*Bifidobacterium adolescentis*	Lichu Drug House, China
27.	*Bacillus licheniformis*	Shenyang First Drug House, China
28.	*Bifidobacterium bifidum*, *L. acidophilus*	Jilin Weite Group, China
29.	*Bacillus subtilis*	Harbin, China
30.	*Clostridium tyrobutyricum*	Chongqing Taipin Drug Co. Ltd., China

5 Traditional Non-dairy Probiotic Foods

As mentioned above, lactose intolerance and cholesterol content are the two major drawbacks associated with probiotic dairy products (Yoon et al. 2006). Therefore, traditional non-dairy fermented foods are being examined for their nutritional value as well as analyzed microbiologically. Current research is also focused on

developing new or innovative non-dairy probiotic products, their health-promoting effects, sensory qualities, and shelf life. Commercialization of research efforts has led to the manufacture of some of these products on industrial scale. The following section discusses traditional as well as new products and challenges in product development.

5.1 Cereals and Legumes

The traditional cereal-based beverages have been commonly consumed in various countries for a long time mainly because cereal grains are nutritionally important due to their content of proteins, carbohydrates, vitamins, minerals, water-soluble fibers, and oligosaccharides (which can act as prebiotics). However, they have only been recently studied for their health benefits. Their microbiological analyses revealed the presence of probiotic microorganisms in these products. Table 2 summarizes traditional probiotic beverages and foods based on cereals and pulses, the probiotic strains identified in them, and their preparation procedure. Besides, wheat, rye, millet, sorghum, and oats have also been used as substrates for fermentation (Angelov et al. 2006). Another widely accepted substrate is soybean. Fermented soymilk and yogurt are popular alternatives to dairy products (Fuchs et al. 2005). Fermentation by probiotic bacteria also helps in preservation of food

Table 2 Traditional cereal-based fermented products

S. No.	Name of the product	Source	Strains	References
1.	Boza	Wheat, rye, millet, and other cereals	*Lactobacillus plantarum*, *Lb. acidophilus*, *Lactobacillus fermentum*, *Lactobacillus coprophilus*, *Leuconostoc raffinolactis*, *Leuconostoc mesenteroides*, *Lactobacillus brevis*	Blandino et al. (2003)
2.	Bushera	Sourghum and millets	Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Streptococcus, *Lb. brevis*	Muianja et al. (2003)
3.	Mahewu	Maize, sorghum, millet malt, and wheat flour	*Lactococcus lactis* subsp. *lactis*	Blandino et al. (2003)
4.	Pozol	Maize		Wacher et al. (2000)
5.	Togwa	Maize flour and finger millet malt	Lactobacillus, Streptococcus, *Lb. plantarum* A6	Parada et al. (1996) and Giraud et al. (1993)
6.	Natto	Soybeans	*Bacillus subtilis* ssp.natto	13.pdf

such as rice wine/beer, rice cakes, fish (Bonaparte and Reuter 1997; Shah et al. 1995), etc., by producing organic acids which control spoilage microorganisms and pathogens as discussed in the previous section. In addition, they produce desirable flavors and improve nutritional value during fermentation of product.

5.2 *Fruits and Vegetables*

Other than cereals, fruits and vegetables or their juices are promising substrates to produce non-dairy probiotic foods. Fruit juices fortified with probiotics and prebiotics are gaining importance as fruits have additional advantage of being tasty, healthy, and refreshing and are rich in vitamins, mineral, fibers, and antioxidants (Luckow and Delahunty 2004). Traditionally consumed fruit-based products are Yan-Taozih (pickled peaches), Pobuzihi (fermented cummingcordia), etc. (Table 3). In Turkey, the popular traditional fermented beverage is hardaliye based on grape juice. It also contains mustard seeds which add to the flavor of product. Studies on hardaliye by Arici and Coskun (2001) indicated different strains of lactobacilli in this beverage viz. *L. casei, L. paracasei, L. brevis*, etc. (Table 3). Oranges, pineapples, grapes, and cranberry are also commonly used substrates. Studies on pineapple and cranberry probiotic juices were published by Sheehan et al. (2007). The authors have reported better survival of lactobacillus strains in orange and pineapple as compared to cranberry. However, the organisms were unable to withstand pasteurization (76 °C for 30 s) and high pressure treatment required for preservation.

Traditional vegetable-based fermented foods are Kimchi, Saurekraut Soidon, Gundruk, Dakguadong, etc. (Table 3). Mostly, the strains used are *L. casei, L. acidophilus, L. plantarum,* and *L. delbrueckii*. Table 4 summarizes recent studies carried out to produce probiotic non-dairy functional foods using vegetables and fruits as raw material.

6 Commercial Products

The technological advances are moving ahead to manufacture traditionally prepared beverages/foods by industrial processes. However, such foods differ in their sensory qualities as compared to the traditional ones, e.g., conventional orange juice is preferred by consumers over its probiotic-fortified counterpart due to its sensory properties; traditional rice wine has a deep and bounty flavor, while industrial wine has light simple flavor (Henneberg 1926). This difference may be attributed to a number of acid-forming bacteria, types of acids, and other

Table 3 Traditional fruit- and vegetable-based probiotic products (Swain et al. 2014)

S. No.	Name of product	Raw material	Strain
1.	Saurekraut	Cabbage	*Leuconostoc mesenteroides, Lactobacillus brevis, Lactobacillus plantarum*
2.	Kimchi	Korean cabbage, radish	*L. mesenteroides*, *L. citreum*, *L. gasicomitatum*, *Lactobacillus brevis*, *L. curvatus*, *L. plantarum*, *L. sakei*, *L. lactis*, *P. pentosaceus*, *W. confusa*, and *W. koreensis*
3.	Dhamuoi	Cabbage, various vegetables	*L. mesenteroides, L. plantarum*
4.	Dakguadong	Mustard leaf	*L. plantarum*
5.	Burong mustasa	Mustard	*L. brevis, Pediococcus cerevisiae*
6.	Fermented cucumber	Cucumber	*L. plantarum*
7.	Gundruk	Rayosag, mustard leaves, cauliflower leaves, and cabbages	*L. fermentum*, *L. plantarum*, *L. casei*, *L. casei* subsp. *pseudoplantarum*, and *Pediococcus pentosaceus*
8.	Sinki	Radish tap root	*L. plantarum*, *L. brevis*, *L. casei*, and *Leuconostoc fallax*
9.	Khalpi	Cucumber	*L. plantarum*, *L. brevis*, and *Leuconostoc fallax*
10.	Soidon	Tip of mature bamboo shoots	*L. brevis*, *Leuconostoc fallax*, and *Lactococcus lactis*
11.	Inziangsang	Mustard leaves	*L. plantarum*, *L. brevis*, and *Pediococcus*
12.	Goyang	Leaves of *maganesaag*	*L. plantarum*, *L. brevis*, *Lactococcus lactis*, *Enterococcus faecium,* and *Pediococcus pentosaceus*, yeasts *Candida* spp.,
13.	Paocai	Cabbage, celery, cucumber, and radish	*Lactobacillus plantarum*, *L. brevis*, *L. paracasei*, *L. buchneri*, and *Pediococcus pentosaceus*
14.	Yan-Taozih	Pickled peaches	*Leuconostoc mesenteroides*, *L. lactis, Weissella cibaria*, *W. paramesenteroides*, *W. minor*, *Enterococcus faecalis,* and *Lactobacillus brevis*
15.	Pobuzihi	Cummingcordia	*Lactobacillus pobuzihii*, *L. plantarum*, *Weissella cibaria*, *W. paramesenteroides*, and *Pediococcus pentosaceus*
16.	Yan-Dong-Gua	Wax gourd	*Weissella cibaria* and *W. paramesenteroides*

(continued)

Table 3 (continued)

S. No.	Name of product	Raw material	Strain
17.	Tempoyak	Pulp of the durian fruit	*Lactobacillus brevis*, *L. mali*, *L. fermentum*, *L. durianis*, *Leuconostoc mesenteroides*, and an unidentified *Lactobacillus* sp.
18.	Sayur Asin	Fermented mustard cabbage leaf	*Leuconostoc mesenteroides*, *Lactobacillus confusus*, *Lactobacillus curvatus*, *Pediococcus pentosaceus*, and *Lactobacillus plantarum*
19.	Salam Juice	Mixture of turnips, black carrot bulgur (broken wheat) flour, salt	*Lactobacillus plantarum*, *L. brevis*, *L. paracasei*, *L. buchneri*, and *Pediococcus pentosaceus*
20.	Nozawana-Zuke	Nozawana, a leafy turnip plant	*Lactobacillus curvatus*
21.	Yan-Jiang	Ginger	*Lactobacillus sakei* and *Lactococcus lactis* subsp. *Lactis* and this species are replaced by *Weissella cibaria* and *L. plantarum* at the final stages of fermentation
22.	Jiang-Gua	Cucumber	*Weissella cibaria*, *W. hellenica*, *L. Plantarum*, *Leuconostoc lactis*, and *Enterococcus casseliflavus*

metabolites produced. In spite of this, the advantages of probiotics and vegan diets are becoming more appealing. Hence, new plant-based probiotic products are coming in the market, e.g., grainfields whole grain liquid containing beans, oats, maize, rice, alfalfa seed, pearl, barley, linseed, mung beans, wheat, rye grains, and millet. It is fermented with lactobacilli and yeast (Saarela et al. 2006). Other non-dairy probiotic products include vita Biosa, Proviva, Malted barley, Gefilus fruit drinks, etc. Table 5 describes commercially available non-dairy probiotic products and their manufacturers. Majority of their health claims include improvement in digestive system and immune response. Also, they are claimed to be safe for everyday use.

7 Sensory Qualities

Most of the probiotics containing non-dairy fermented foods prepared from cereals, fruits, or vegetables usually have favorable texture, flavor, and aroma, e.g., salty taste, fresh carbonated sensation, and crispy texture of kimchi (Dodd and Gasson 1994). Its optimum taste and high vitamin C content of the latter are attained when pH decreases (4–4.5) due to acid fermentation (Metchnikoff 1908). Normally, fermentation results in pleasant acid taste and characteristic aroma due to esters

Table 4 Innovative non-dairy probiotic functional foods

S. No.	Non-dairy functional foods	Raw material	Strain used	Fermentation conditions	Viability during refrigeration	References
Vegetables and fruits						
1.	Carrot juice	Carrot juice	*Bifidobacterium lactis* Bb-12 *Bifidobacterium bifidum* B7.1 *Bifidobacterium Bifidum* B3.2			Kun et al. (2008)
2.	Beet juice	Red beets	*Lactobacillus plantarum* *L. casei* *L. delbrueckii* *L. acidophilus*	30 °C for 48 h	10^6–10^7 CFU/ml after 4 weeks of cold storage at 4 °C	Yoon et al. (2005)
3.	Fermented pomegranate juice	Pomegranate juice	*L. plantarum* *L. delbrueckii* *L. acidophilus* *L. paracasei*	30 °C for 72 h under microaerophilic conditions		Mousavi et al. (2011)
4.	Fermented cashew apple juice	Cashew apple juice	*L. casei*	30 °C for 16 h	8.0 Log CFU/ml for 42 days	Pereira et al. (2011)
5.	Fermented cabbage juice	Cabbage	*L. plantarum* *L. casei* *L. delbrueckii*	30 ° C for 48 h	4.1×10^7 CFU/ml for *L. plantarum* and 4.5×10^5 CFU/ml for *L. delbrueckii*	Yoon et al. (2006)
6.	Fermented radish	Radish	Lactobacillus	2.5 % salt at a temperature of 25 ± 1 °C for 16–18 days	15 days at 4 °C	Joshi and Sharma (2009)

7.	Fermented Garlic	Blanched garlic	*Lactobacillus plantarum*		30 °C in an acidified brine	
8.	Fermented carrot slice	Carrot	*Lactobacillus sakei*			
Cereals and legumes						
1.	Fermented oat product	Oat	*Pediococcus damnosus*	28 and 37 °C for 24 h	$>10^8$ CFU/ml	Martensson et al. (2002)
2.	Fermented beverages	Cassava flour	*L. plantarum* *L. casei Shirota* *L. acidophilus*	35 °C for 16 h	28 days at 4 °C	Santos (2001)
3.	Symbiotic functional drink	Oats and barley	*L. plantarum*	8 h	7.5×10^{10} CFU/ml 21 days	Angelov et al. (2006)

Table 5 Commercial non-dairy probiotic products

S. No.	Product name	Manufacturer	Strains
1.	Active balance High Potency Probiotic	Active Balance	*L. acidophilus, B. bifidum*
2.	Align probiotic	Align	*Bifidobacterium infants*
3.	Enzyme Probiotic complex	American Health	*L. acidophilus, B. bifidum, L. bulgaricus, L. brevis, B. lactis*
4.	Syntol AMD	Arthu Andrew Medical	*B. subtilis, L. helveticus, S. boulardii, L. acidophilus, L. rhamnosus, L. plantarum, L. lactis, B. bifidum*
5.	Bacid with *Lacto-bacillus acidophilus*	Bacid	*L. acidophilus, L. bulgaricus, B. bifidum, S. thermophilus*
6.	TruBiotics	Bayer	*L. acidophilus, B. animalis*
7.	Bio-K + Extra Strength Probiotic	Bio-K plus	*L. acidophilus, L. casei*
8.	Nexabiotic	Bioprosper Labs	*Saccharomyces boulardii, S. thermophilus, L. fermentum, L. acidophilus, L. brevis, L. bulgaricus, L. casei, L. helveticus, L. paracasei, L. pantarum, L. rhamnosus, L. salivarius, L. lactis, Bacillus coagulans, B. bifidum, B. breve, B. infantis, B. lactis, B. longum, Pediococcus acidilactici*
9.	Digestive Health	Culturele	*Lactobacillus GG*
10.	Adult Probiotic	CVS Pharmacy	*B. breve, B. longum, L. acidophilus, L. casei, L. rhamnosus, L. plantarum, L. lactis, S. thermophilus*
11.	Digestive probiotic	CVS Pharmacy	*B. infantis*
12.	Probiotic Blend	Daily Essentials	*L. acidophilus, L. rhamnosus, L. rhamnosus (Type B, Bifidus), B. lactis, B. longum, B. bifidum, S. thermophilus*
13.	D. L. ULTRA-DOPHIULUS	Douglas Laboratories	*L. acidophilus*
14.	Complete probiotics	Dr. Mercola	*L. casei, L. plantarum, L. salivarius, L. rhamnosus, L. brevis, B. lactis, B. longum, B. bifidum, S. thermophilus*
15.	Pearls Elite	Enzymatic	*L. acidophilus, B. longum*
16.	Pro-Bio	Enzymedica	*Bacillus subtilis, L. paracasei, L. acidophilus, L. casei, L. bulgaricus, L. plantarum, L. rhamnosus, L. salivarius*
17.	Propolis Plus	Essential Formulas	*B. breve, B. infantis, B. longum, Enterococcus faecalis, L. acidophilus, L. brevis, L. bulgaricus, L. casei, L. fermentum, L. helveticus, L. plantarum, Streptococcus thermophilus*
18.	Flora Q	Flora Q	*L. acidophilus, Bifidobacterium, L. paracasei, S. thermophilus*
19.	Florajen 3	Florajen	*L. acidophilus, B. lactis, B. longum*

(continued)

Table 5 (continued)

S. No.	Product name	Manufacturer	Strains
20.	Mega Probiotic-ND	Food science	*L. acidophilus, L. rhamnosus, B. lactis, L. casei, B. breve, B. longum, B. bifidum, S. thermophilus*
21.	Daily Probiotic	Ganaden Sustenex	*Bacillus coagulans*
22.	Primal defense	Garden of life	*L. plantarum, L. brevis, B. bifidum, L. salivarius, B. lactis, L. acidophilus, Bacillus subtilis, B. breve, L. paracasei, L. casei, B. longum, L. rhamnosus*
23.	HMF Neuro	Genestra	*Lactobacillus acidophilus, Bifidobacterium bifidum, Bifidobacterium lactis, Lactobacillus rhamnosus*
24.	Flora 5	Global Health Trax	*B. longum, L. acidophilus, L. rhamnosus, L. casei, B. lactis*
25.	Super 10 Probiotic Complex	GNC	*L. salivarius, L. rhamnosus, B. bifidum, B. longum, B. infantis, L. acidophilus, B. lactis*
26.	Probiotic Acidophilus	Good 'N Natural	*L. acidophilus, B. bifidum, L. salivarius, L. bulgaricus*
27.	Friendly Force	HealthForce Nutritionals	*L. plantarum, L. paracasei, L. rhamnosus, L. salivarius, S. thermophilus, L. bulgaricus*
28.	Healthy Origins 30 Billion	Healthy Origins	*L. acidophilus, Bifidobacterium lactis, L. casei, Bifidobacillus breve, L. salivarius, L. plantarum, Bifidobacillus longum, Bifidobacillus rhamnosus*
29.	Flora 50–14	Innate response	*Bifidobacterium longum, L. acidophilus, Bifidobacterium infantis, L. lactis, L. acidophilus DDS-1, L. reuteri, L. salivarius, L. brevis, L. plantarum, Streptococcus thermophilus, Bifidobacterium bifidum, L. bulgaricus, L. casei, L. rhamnosus*
30.	Flora 20–14	Innate response	*Bifidobacterium longum, L. acidophilus, Bifidobacterium infantis, L. lactis, L. acidophilus DDS-1, L. reuteri, L. salivarius, L. brevis, L. plantarum, Streptococcus thermophilus, Bifidobacterium bifidum, L. bulgaricus, L. casei, L. rhamnosus*
31.	Jarro-Dophilus EPS	Jarrow Formulas	*L. rhamnosus, Pediococcus acidilactici, Bifidobacterium longum, Bifidobacterium breve, L. helveticus, L. casei, L. plantarum, L. lactis* ssp. *Lactis*
32.	Probiotic Powerhouse	Jay Kordich	*L. plantarum, L. paracasei, L. rhamnosus, L. salivarius, S. thermophilus, L. bulgaricus*
33.	ACTIFlora	Kendy	*L. bulgaricus, L. acidophilus, S. thermophilus*
34.	Pro-Bio Gold	Kirkman	*L. rhamnosus, L. acidophilus, B. bifidum/lactis, L. casei, L. plantarum, S. thermophilus*

(continued)

Table 5 (continued)

S. No.	Product name	Manufacturer	Strains
35.	LactoPrime Plus	Klaire Labs	*L. rhamnosus, L. acidophilus, L. brevis, L. casei, L. plantarum, L. salivarius, L. paracasei, B. bifidum, B. infantis, B. longum, B. breve, B. lactis*
36.	Kyo-Dophilus	Kyolic	*L. gasseri, B. bifidum, B. longum*
37.	Good Flora	Mt Angel Vitamins	*B. longum, B. bifidum, L. acidophilus*
38.	Healthy Trinity	Natren	*L. acidophilus, B. bifidum, L. bulgaricus*
39.	Life Start 2	Natren	*B. infantis*
40.	Triple Probiotic	Nature Made	*L. gasseri, B. bifidum, B. longum*

produced from organic acids and alcohols which are produced from sugars (e.g., fructose) (Biacs 1986). In acid-fermented vegetables, a rapid decrease in pH at the beginning of fermentation is of great importance for the quality of end product (Viander et al. 2003). In addition, CO_2 produced in acid-fermented vegetables by *Leuconostoc mesenteroides* inhibits growth of unwanted microorganisms, preventing undesirable softening of vegetables. Also, the anaerobic conditions produced by CO_2 help in stabilization of ascorbic acid and natural colors of vegetables (Metchnikoff 1908). Moreover, exopolysaccharides produced by LAB also help to improve rheological and textural properties of food products (Leroy et al. 2002).

Fruit juice-based functional beverages fortified with probiotic microorganisms are dairy free, soy free, wheat free, and vegan. Hence, they are preferred by a large portion of the population. However, it is important to get rid of the off flavors in fermented fruit juices and enhance their sensory acceptability (Granato et al. 2010). For this purpose, addition of fruit juices like pineapple, mango, or passion fruit was found to be useful to improve the aroma and flavor of the final product masking the probiotic off flavors (Luckow and Delahunty 2004). On the other hand, undesirable aromas/flavors might gain consumers' assertion as indicators of probiotic ingredients or their action (Juttlestad 1998). Alternative to adding probiotic microorganisms directly to the foods is by using them in previously immobilized form which is discussed in the following section. Iron-fortified soy yogurt is found to have suitable hedonic scores for creaminess and flavor. It is also observed that selection of probiotic strains for fermentation purpose affects sensory qualities of soy beverages, e.g., fermented soy beverages produced by using bifidobacteria have better acceptability as compared to that of *L. casei*. Besides, addition of prebiotics like oligofructose and inulin to soy yogurt was found to increase acceptance index more than 70 % (Hauly et al. 2005). Thus, elevating the palatability of probiotic products is one way to make them more appealing to the consumers, while another means can be making people aware of probiotic health benefits of the product.

8 Additional Health Benefits of Non-dairy Probiotic Functional Foods

8.1 Increase in Nutritional Quality

The nutritional value of a particular food depends on its digestibility and its content of essential nutrients. Both are improved by fermentation, since fermentation increases its nutrient density, the amount, and bioavailability of nutrients. The latter may be achieved by degradation of antinutritional factors, by predigestion of certain food components, and by improving the absorption and uptake of nutrients by the mucosa (Svanberg and Lorri 1997). In addition, fermentation results in the (1) increase in protein solubility and availability of scarce amino acids by as much as 50 %, (2) increase in micronutrient availability because of reduction of phytates and reduction in tannins by as much as 50 %, and (3) decrease in oligosaccharides by as much as 90 % (Nout and Ngoddy 1997) concentration and increase in the availability of proteins and vitamins like thiamine, folic acid, riboflavin, etc. Hence, such types of foods have direct curative effects on consumers of such foods (Steinkraus 1997). LAB also help in increase in iron uptake (Venkatesh 1998). Besides, fermentation may reduce the content of non-digestible plant foods like cellulose, hemicelluloses, polygalacturonic, and glucuronic acids. Breakdown of these compounds may lead to increase in bioavailability of minerals and trace elements (Kalantzopoulos 1997).

8.2 Soy-Based Products

Soy-based products flavored with fruit juices have become very popular as alternatives to dairy products (Champagne et al. 2005). Global market for soy-based probiotic yogurts is increasing annually by 10 %. Various health benefits of such products include (1) weight management, (2) decrease in risk of heart diseases and some cancers (Larkin et al. 2007), (3) good protein source, (4) help in immunomodulation, reduction in levels of –CHO causing gas production in intestine, (5) increase in isoflavone levels, and (6) beneficial to bone health. Besides, soy is in itself a source of vitamins and minerals, antioxidants, and some isoflavones. Hence, traditional soy-based products, e.g., miso, natto, tempe, etc., have better antioxidant activity than the non-fermented ones (Esaki et al. 1994).

8.3 Fruit- and Vegetable-Based Products

Probiotic foods prepared using fruits, vegetables, and cereals also have additional health benefits since fruits and vegetables are themselves considered as health foods

due to vitamins, minerals, antioxidants, phenolics, and fibers present in them. Moreover, many products contain additional ingredients used to enhance their flavoring and/or taste. These flavoring agents may also increase nutritional benefits of such foods, e.g., antitumor activities of cabbage and garlic used in kimchi, inhibition of aflatoxin B1 due to red pepper extract (Park et al. 1991), etc. Currently, there are more than 21 different commercial vegetable fermentations in Europe along with a large number of fermented vegetable juices and blends. The fermentations of olives, cucumbers, and cabbage are economically most significant (Caplice and Fitzerland 1999). Nonetheless, inhibitory effects of fermentation metabolites such as bacteriocins are well known (Dave and Shah 1997). Several bacteriocin strains have been isolated from kimchi. The inhibitory effects were observed not only in bacteria but also in fungi, e.g., antifungal compound 3,6-bis (2-methylpropyl)-2,5-piperazinedione was identified as being produced by *L. plantarum* strain from kimchi (Yang and Chang 2010). Bacteriocins along with organic acids (e.g., lactic acid) play a key role in controlling growth of undesirable and pathogenic organisms, thus avoiding costly treatments and packaging. The large amount of raw material is processed in this way in food industry because of nutritional, physiological, and hygienic aspects of the process (Karovicova et al. 1999).

9 Product Development and Challenges

New product development is always a challenge for both basic and applied research. It is an expensive process and requires detailed knowledge of the product, procedure, and consumers. In food sector, many parameters have to be considered such as sensory and physicochemical properties, extended shelf life, stability, and reasonable price. Careful selection of strains used and good monitoring throughout the manufacturing process are required to control the metabolic products and hence the final pH. So, use of mixed probiotic cultures is preferred which help to increase the growth rates, decrease the fermentation time, and eliminate certain sensory and texture defects and above all improve the nutritional value of the product (Gomes and Malcata 1999).

Another means to use probiotic strains is by adding them to beverages directly into the finished product which may help to retain better viability and functionality (Prado et al. 2008). Probiotic soy products or the products prepared from cereals, vegetables, and fruits are excellent substitutes for dairy products as a source of probiotics. Not only probiotic fermented vegetables but also their by-products have commercial value, e.g., during sauerkraut production, pH of final product is 3.5–3.8, at which cabbage or other vegetables can be preserved for a long time. Sauerkraut brine is an important by-product of cabbage fermentation industry and can be used as a substance for production of carotenoids by *Rhodotorula rubra* or for β-glucosidase production by *Candida wickerhamii* (Sim and Hang 1996) for commercial applications.

10 Quality Control

It is evident from the above discussion that non-dairy probiotic beverages are recognized as health drinks with a lot of scope in product development. However, use of probiotic organisms depends on many parameters like processing, storage, chemical composition of food, growth phase of organism, pH, water activity and salt content, interaction with starter culture, food matrix, etc. which affect their ability to survive in the product as well as in the consumers' GI tract. Hence, quality control of probiotic strains is an important issue especially for monitoring their adhesion, gastric stability, and viability during manufacture, storage, and distribution. For these, some recommended practices are (1) appropriate culture maintenance procedures to reduce the number of passages, (2) use of more than one model to examine stability of the strain, (3) attempts for in vivo quantitative extrapolation of in vitro assays, and (4) studies on interspecies variation with respect to functional properties (Lee and Salminen 1995).

If adhesion properties of probiotic strains are altered during industrial processes their technological traits may also alter. Hence, they should be monitored carefully. Adhesion of probiotic bacteria varies in different in vitro models within the same strains and also shows difference between the strains (Lehto and Salminen 1996, 1997; Tuomola and Salminen 1998). This may be due to difference in surface properties required for adhesion to epithelial cells. Commonly used system is Caco-2 cell line and human ileostomy glycoprotein (Lehto and Salminen 1996, 1997; Tuomola and Salminen 1998).

Viability is another important characteristic as the strain should be viable during manufacture, storage, and after consumption. It is essential that fermented products should contain satisfactory number of active cells at the time of consumption (10^6 CFU/ml) since minimum therapeutic daily dose is 10^8–10^9 viable cells which is equivalent to 100 g of food intake containing 10^6–10^7 viable cells/ml (Rasic and Kurmann 1983). The organism should survive in sufficient numbers without any adverse effects on sensory properties of the product and should not increase acidification of product during shelf life (Shah et al. 1995; Roy et al. 1997). Such products must be consumed regularly to achieve and maintain the desirable effects on intestinal microflora. Unfavorable water activity (e.g., cereals, honey, marmalade, chocolate, etc.), pH, bile and salt concentration, and oxygen tension lead to death of bacteria (Vasudha and Mishra 2013). Therefore, acquiring the data for long-term stability to acid/bile is essential (Lee and Salminen 1995; Lee and Wong 1998).

Among the health benefits, hypocholesterolemic potential of probiotics is well known. However, there is lack of dosage response studies to determine minimal effective dosage of probiotics and prebiotics to reduce blood cholesterol levels (Kun et al. 2008). A review of previous studies represents that it depends on strains used and clinical characters of patients. It is necessary to establish clinically effective dosage based on human studies (Larkin et al. 2007). It is clear from the above discussion that viability, stability of probiotic strains (Bonaparte and Reuter

1997; Joseph et al. 1998), and determination of effective dosage are main challenges in research and development of probiotic products. In order to overcome the challenges, microencapsulation technologies have been developed and used successfully in various foods, which increase their viability in cereal- and fruit-based matrices.

11 Technological Innovations

The markets of probiotic products and supplements are increasing worldwide (Playne 1997). Today, there are >70 bifidus and acidophilus containing products worldwide (Shah 2000). Probiotic survival in products is affected by a range of factors including pH, hydrogen peroxide production, oxygen toxicity, storage temperatures, stability in dried or frozen form, and compatibility with traditional starter culture during fermentation (Dave and Shah 1997; Kailasapathy and Rybka 1997). Oxygen plays a major role in the poor survival of probiotic bacteria (Brunner et al. 1993). Hence, it is necessary to prevent their exposure to adverse external conditions. Therefore, the research in the past decade was focused on replacing their carrier food or by improving the protection of acid-sensitive strain via microencapsulation with cellulose acetate phthalate (Rao et al. 1989), n-carrageenan (Dinakar and Mistry 1994), or Ca-alginate (Kim et al. 1996). The former appears to be technologically and commercially most feasible since food carrier has buffering/protective effect and helps in survival in gastric juice.

11.1 Microencapsulation

Microencapsulation helps to separate a core material from its environment until it is released. It protects the unstable core from its environment, thereby improving its stability and viability, extends the core's shelf life, and provides a sustained and controlled release in specific parts of the gut. The size of capsules may vary from submicron to several millimeters and they can be of different shapes (Franjione and Vasishtha 1995). Most commonly applied technologies are emulsification, coacervation, spray drying, spray cooling, freeze drying, fluid bed coating, and extrusion technologies. More expensive techniques are liposome encapsulation and cyclodextrin encapsulation. Sensitivity of the system to mechanical stress, pH, or different microbial enzymes in the gut facilitates targeted and sustained release.

Microencapsulation using the gentle and nontoxic matrices helps to enhance survival of the bacteria in acid and bile as well as heat. For this, alginate is commonly used due to (1) its non-toxicity, (2) its ability to form gentle matrices with calcium chloride to trap probiotic bacteria, (3) the viability of bacteria following encapsulation, and (4) reversibility of immobilization (Shah and Ravula 2000). Microencapsulation techniques are commonly used for probiotics, vitamins,

minerals, antioxidants, etc. Further research in this line will help to develop co-encapsulation technique so as to combine two or more bioactive components for collective effect.

Stress response mechanism in LABs for industrial applications has been studied at the molecular level (Prasad et al. 2003). In *L. acidophilus*, the genes identified are FiFo-ATPase in acid stress response (Kullen and Klaenhammer 1999) and molecular chaperones groESL and dnaK in heat stress (Shah 2002). Thermotolerance was found to be better in heat-adapted strains of *L. paracasei* NFB338 (Desmond et al. 2001). Also, *Lactobacilli* were found to acquire cross-stress tolerance to heat when exposed to mild osmotic stress (Desmond et al. 2001). The authors have also demonstrated enhanced viability of salt-adapted culture as compared to the control one when dried under same conditions. In addition to acid and heat, organisms in fermented foods are also exposed to oxygen stress (Shah 2002). Oxygen content in the product and oxygen permeation through package may affect viability of probiotics in fermented products. Hence, it is necessary to measure oxygen tolerance of probiotic bacteria. Talkwalker and kailashpathy (2004) have published modified Relative Bacterial Growth Ratio (RBGR) method for quantitative measurement of oxygen tolerance, which will help in screening more oxygen-tolerant strains (Talkwalker and kailashpathy 2004). Osp protein was found to be upregulated in O_2-tolerant *Bifidobacterium* strain (Ahn et al. 2001).

11.2 Spray Drying and Freeze Drying

Conventionally, freeze drying or spray drying of probiotic strains is used to make them available in the powder form on a large scale (Holzapfel et al. 2001). Spray drying is the most commonly used microencapsulation method in the food industry since it is economical and flexible, easy to scale up, produces a good quality product, and can be operated on continuous basis with simple equipmentation (Dzieazak 1988). However, further research in this area showed that retaining viability is the challenge due to temperature and osmotic extremes used in this process (Silva et al. 2002). This problem can be overcome either by optimizing the drying technology to reduce the harshness of the treatment or by improving the strains by gene manipulation or mutation. The former has been achieved by proper control and monitoring of processing conditions to produce viable encapsulated cultures of desired particle size, e.g., O'Riordan et al. (2001) have found that 100 °C inlet temperature and 45 °C of outlet temperature are suitable to produce microspheres of bifidobacteria with gelatinized modified starch as coating material. Meng et al. (2006) have reviewed that the stress responses in probiotic strains can have a remarkable effect on their ability to survive processing such as freeze drying, spray drying, and during gastric transit. In such cases, overexpressing heat shock proteins like GroEsL or addition of thermoprotectants to drying medium is advantageous (Desmond et al. 2004). Stress induced by temperature changes, phase changes, drying, or a combination tends to damage cell membrane and proteins. To

overcome cell injury or death due to heat and dehydration, thermoprotectants have been added to media prior to drying, e.g., trehalose (Conrad et al. 2000), non-fat milk solids (Corcoran et al. 2004), prebiotics (Corcoran et al. 2005), granular starch (Crittenden et al. 2001), gum acacia (Desmond et al. 2004), etc. The addition of gum acacia in the drying medium resulted in 1000-fold increase in stability of dried *L. paracasei* NF13C 338 during powder storage at 15 °C and 30 °C (Lian et al. 2002). Hundred fold increase in viability was also observed when exposed to porcine gastric juice compared to control spray-dried culture (Desmond et al. 2002). Similarly, addition of cryoprotectants like inulin improves viability during freeze drying. Freeze-dried *L. bulgaricus* survived better at −20 °C for more than 10 months when grown in the presence of fructose, lactose, or mannose. Incorporation of glucose, fructose, or sorbitol in drying medium also resulted in better survival at low temperature (Carvalho et al. 2004).

11.3 Emulsion and Phase Separation

Most of the literature reported on the encapsulation of probiotic bacteria has used the emulsion technique to produce small amount of capsules. The capsules or beads are formed in a two-step procedure involving dispersion and hardening. The dispersion can be performed either by extrusion or by emulsification (Groboillot et al. 1994). The former involves projecting an emulsion core and coating material through a nozzle at high pressure. If the droplets are formed in a controlled manner, the technique is known as prilling. Beads can be produced on a large scale by using multinozzle systems, rotating disk atomizers, or by the jet cutting technique (Heinzen 2002).

11.4 Other Technologies

Another technology reported is vacuum impregnation to have beneficial effects of probiotics with fruits and vegetables. In this, apple cylinders were impregnated either with commercial apple juice containing *Saccharomyces cerevisiae*, or with whole milk or apple juice containing 10^7 or 10^8 cfu/ml of *Lactobacillus casei*. Impregnated apple samples were air dried at 40 °C and stored at room temperature for 2 months to increase stability and to assure fruit preservation. The number of *L. casei* viable cells in dried and stored product was more than 10^6 CFU/g which is similar to that in commercial dairy products (Vos et al. 2010).

12 Future Perspectives

Non-dairy probiotic products have a huge potential for food industry. These functional foods may be further explored through the development of new ingredients, processes, and technologies in order to improve their nutritional and sensory appeal. The key areas for research and development in non-dairy food products are raw materials used, sensory qualities, microencapsulation technologies, and strain improvement. The studies on novel or different matrices/carrier foods will help to increase the variety of non-dairy probiotic foods to offer a wide choice to consumers. The main reason for this is occurrence of non-dairy allergies linked to soya, gluten, and vegetables. Enhancement in sensory appeal of probiotic foods will ensure its consumption in the quantity and frequency to achieve anticipated health benefits. Also, consumers must be convinced by clear and trustworthy health claims so as to further increase the market of these functional foods.

In order to achieve the claimed health benefits, sustained viability and stability of the culture are significant. Hence, the technology of microencapsulation needs to develop with more precise machinery, capsule, and better delivery systems to protect the strains from external stress. In this regard, nano-encapsulation may get importance in near future to develop designer probiotic bacterial preparations for delivery to certain parts of GIT where they can interact with specific receptors. Such probiotic preparations may act as de novo vaccines and help in immunomodulation. Thus, research and development in this area will improve the delivery and sustained release of viable cells. Moreover, this has to be coupled with in vivo studies using human subjects (Kaliasapathy 2002).

Efforts for strain development are required to obtain mildly acidifying, less fastidious strains with sustained probiotic potential for application as starters or as supplements for food fortification. Besides, the advances in genetic engineering techniques will lead to the designing of more efficient strains and also introduce new functions in same strains. Earlier examples of such studies are expression of *S. mutans* surface protein Ag in *L. lactis* (Iwaki et al. 1990), murine interleukin 10 (IL-10) in recombinant *L. lactis* (Steidler et al. 2000), etc. Research and development in this area will result in live recombinant vaccines (Seegers 2002) using food grade bacteria (GRAS). However, studies on safety and efficacy of engineered strains and original strains are highly desirable. This is particularly necessary for children, pregnant women, elderly people, and immunocompromised people.

In addition to the technological aspects, future efforts should be directed to understand in detail the mechanisms of their health-promoting effects, increase in public awareness, and their applications in human and veterinary foods so as to gain their health benefits as per the recommended dosage, thereby reducing the health care cost.

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Impact of Probiotics and Gut Microbiota on Host Behavior

Sarabjit Singh Kanwar, Sohini Walia, and Sakshi Sharma

Abstract Probiotic bacteria are living organisms that inhabit the gut and contribute towards the health of the host. The idea that implanting the intestines with probiotic bacteria may improve quality of life and mental health is not a new one. Accumulating clinical evidences suggest that probiotics can modulate the stress response and improve mood and anxiety symptoms in patients with chronic fatigue and irritable bowel syndrome. One such organism is *Lactobacillus rhamnosus* (JB-1), which has shown antidepressant and anxiolytic-like properties in mice as observed recently. Probiotic supplementation can also lead to significant improvement in motor coordination and spontaneous locomotor activity, in addition to reduction in anxiety and cognitive behavior in rats. There are increasing, but largely indirect, evidences to point out the effect of commensal gut microbiota on the central nervous system. Microbes in gastrointestinal (GI) tract which constitute normal gut microbiota are represented by a wide variety of bacterial species. Emerging studies have shown that probiotic bacteria can directly communicate with the central nervous system by way of the vagal sensory nerve fibers and the peripheral immune system. Indeed, experimental studies have shown that even minute doses of these bacteria within the gastrointestinal tract are capable of influencing neurotransmission. Probiotic bacteria and gut microbiota can exert numerous effects on the intestinal neuroimmune system and influence a variety of host functions such as metabolic activity, immune response, and physiological functions. Thus, the emerging concept of probiotics on "microbiota–gut–brain axis" provides a novel insight for improved understanding of their potential role in psychological disorders.

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1 Introduction

In the last few years, evidences from studies in rodents have demonstrated that the gut microbiota can influence neural development, brain chemistry, and a wide range of behavioral phenomena, including emotional behavior, pain perception, and response to stress system. Researchers have found a balance between beneficial and disease-causing bacteria in an animal's gut which can alter its brain chemistry to make it either bolder or more anxious. The brain can also exert a powerful influence on gut bacteria; as many studies have shown, even mild stress can tip the microbial balance in the gut, making the host more vulnerable to infectious diseases and triggering a cascade of molecular reactions that provide feedback to the central nervous system.

Such findings offer the possibility of using beneficial or probiotic bacteria to treat mood and anxiety disorders, either by administering beneficial microbes themselves or by developing drugs that mimic their metabolic functions. The new research also hints at new ways of managing chronic gastrointestinal (GI) disorders that are commonly accompanied by anxiety and depression and that also appear to involve abnormal gut microbiota. Microbes in the gastrointestinal (GI) tract are represented by a wide variety of bacterial species. They can exert numerous effects on the intestinal neuroimmune system and influence a variety of host functions such as metabolic activity, immune response, and physiological functions (O'Hara and Shanahan 2007). The gut microbiota (whose genes represent the intestinal microbiome) composition and activity is influenced by host physiology, immunology, diet, antibiotic usage, and enteric infections. Microbial dysbiosis is associated with gastrointestinal and metabolic disorders. A growing body of evidences suggests that the host–microbial interaction may result in deregulated neuroimmune functions, thus impacting behavior (Bercik et al. 2012; Grenham et al. 2011). Probiotic bacteria are "live microorganisms which when administered in adequate amounts confer a health benefit on the host." Dietary prebiotics, "selectively fermented dietary ingredients that result in specific changes on consumption and/or alter activity of the gastrointestinal microbiota, thus conferring benefit (s) upon host health," have been used in animal studies and human clinical trials to improve peripheral (gastrointestinal) and central (psychological) symptoms.

2 Probiotics

Probiotics are defined as live microorganisms which beneficially affect the host by improving its intestinal microbial balance (Fuller 1989). The probiotics recommended for human applications are primarily two classes of lactic acid producing microorganisms: the bifidobacteria and lactic acid bacteria (LAB) including species of *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Vagococcus*, *Aerococcus*, *Carnobacterium*, *Tetragenococcus*,

Streptococcus, and *Weisella* (Felis and Dellaglio 2007; Weiss et al. 2011). Some yeast strains such as *Saccharomyces cerevisiae* and *Saccharomyces boulardii* have also emerged as probiotics due to their influence on the human health (Bao et al. 2010; Sourabh et al. 2011). Most LAB are Generally Regarded As Safe (GRAS) for human consumption due to their ubiquitous appearance in food and their contribution to the healthy microflora of human mucosal surfaces (Donohue et al. 1993; Sanders 1993; Marteau and Rambaud 1993). They have been of scientific and commercial interest due to a range of health-promoting attributes, including suppression of growth of pathogens, control of serum cholesterol level, modulation of the immune system, improvement of lactose digestion, synthesis of vitamins, ability to adhere to gut tissue, increase in bioavailability of minerals, antigenotoxicity, and possible anticarcinogenic activity (Kailasapathy and Chin 2000; Kullisaar et al. 2002; Chan and Zhang 2005; Wagar et al. 2009; Foligné et al. 2010; Todorov et al. 2012; Walia et al. 2014).

Probiotics have multiple mechanisms of action, including prevention of pathogenic bacterial growth, prevention of penetration of pathogens to mucosal surfaces, stimulation of mucosal barrier function, production of antimicrobial agents or altering immunoregulation, decreasing proinflammatory, and promoting protective molecules (Novak and Katz 2006; Sartor 2006). The intake of probiotics in humans has also been shown to enhance cytokine production in vivo and by peripheral blood mononuclear cells in vitro. Probiotic intake has been reported to be effective in restoring the age-related decline in phagocyte function (Gill 2003). Some of the clinical evidences suggest that probiotics can modulate the stress response and improve mood and anxiety symptoms in patients with chronic fatigue and irritable bowel syndrome (Rao et al. 2009; Silk et al. 2009). LAB such as *L. rhamnosus* (JB-1) can also modulate depression and anxiety-like behavior in healthy mice (Bravo et al. 2011). Moreover, there are some clinical evidences to support a role of probiotic intervention in reducing anxiety, decreasing stress, and improving mood in individuals with irritable bowel syndrome and with chronic fatigue (Logan and Katzman 2005; Rao et al. 2009).

In a recent study, ingestion of *Lactobacillus rhamnosus* (JB-1) has decreased anxiety and despair-like behavior and has reduced the stress-induced increase of plasma corticosterone levels in mice (Bravo et al. 2011). Moreover, this potential probiotic has also altered the mRNA expression of both GABAA and GABAB receptors in several brain regions (with a complex pattern of region- and receptor-specific increases and decreases). Alterations in these receptors have been associated with anxious and depression-like behaviors in animal models. Interestingly, these effects are vagus dependent as vagotomy prevented the anxiolytic and antidepressant effects.

3 The Gut Microbiota

The gut microbiota consists of a complex of microorganisms that live in the digestive tracts of animals and is the largest reservoir of microorganisms commensal to humans. The gut microbiota is a population of estimated 100 trillions of microbes that reside within the GI tract. This population of microbes harbors 100-fold more genes than are found in the human genome. The human gastrointestinal tract is inhabited by 10^{13}–10^{14} microorganisms—more than 10 times that of human cells in our bodies and containing 150 times more genes as our own genome (Gill et al. 2006; Qin et al. 2010; O'Hara and Shanahan 2006). Bacteria make up most of the flora in the colon and up to 60 % of the dry mass of feces. The estimated number of species in the gut microbiota varies greatly, but it is generally accepted that the adult microbiota consists of more than 1000 species (Qin et al. 2010) and more than 7000 strains (Ley et al. 2006). Most bacteria that make up the gut microbiota belong to the genera *Bacteroides*, *Clostridium*, *Fusobacterium*, *Eubacterium*, *Ruminococcus*, *Peptococcus*, *Peptostreptococcus*, and *Bifidobacterium*. Other genera, such as *Escherichia* and *Lactobacillus*, are present to a lesser extent. Species from the genus *Bacteroides* alone constitute about 30 % of all bacteria in the gut, suggesting that this genus is especially important in the functioning of the host (Eckburg et al. 2005). The currently known genera of fungi which harbor gut flora include *Candida*, *Saccharomyces*, *Aspergillus*, and *Penicillium*. Archaea constitute another large class of gut flora which are important in the metabolism of the bacterial products of fermentation (Guarner and Malagelada 2003).

It is becoming clear that the microbiota normally has a balanced compositional signature that confers health benefits and that a disruption of this balance confers disease susceptibility (Cryan and O'Mahony 2011). Diet is one of the key factors that can substantially affect microbiota composition. The composition of the microbiota plays an important role in the maintenance of intestinal homeostasis and host health. Other factors, including infection, disease, and antibiotics, may transiently alter the stability of the natural composition of the gut microbiota and thereby have a deleterious effect on the well-being of the host (Forsythe et al. 2010). Given the overarching influence of gut bacteria on health, it is perhaps not surprising that a growing body of literature focuses on the impact of enteric microbiota on brain and behavior. A brief account of effect of microbiota and probiotics on brain and behavior is given in Table 1.

Microbiota provides the significant protection against incoming bacterial pathogens. It has been shown that microbiota helps and protects the host against the viruses. There is a growing appreciation of the critical role played by the commensally microbiota, both in our general well-being and in the specific functioning of the brain–gut axis. Interestingly, bacteria may respond directly to stress-related host signals because of interplay between stress and gut microbiota.

Table 1 Summary of animal studies evaluating the effect of the gut microbiota and probiotics on host behavior

Animal model	Gut microbiota/probiotics	Effect(s)	References
Balb/c male mice	GF vs. SPF vs. gnotobiotic mice (with *Bifidobacterium infantis*; with enteropathogenic *Escherichia coli* with/without intimin receptor gene)	GF male mice had a decrease in brain-derived neurotrophic factor (BDNF)—a key neurotrophin involved in neuronal growth and survival—compared with SPF mice. Effect was reversed in gnotobiotic animals colonized with *B. infantis*, but not with *E. coli*	Sudo et al. (2004)
Urethane anaesthetized rats	*Lactobacillus johnsonii* La1	Intraduodenal injection of *Lactobacillus johnsonii* La1 reduced renal sympathetic nerve activity (RSNA) and BP and enhanced gastric vagal nerve activity (GVNA)	Tanida et al. (2005)
Sprague dawley rats	*Lactobacillus reuteri* ATCC 23272	Live, killed probiotic bacteria, or conditioned medium inhibited the constitutive cardioautonomic response to colorectal distension in rats through effects on enteric nerves	Kamiya et al. (2006)
Male Balb/c mice or AKH mice infected with *Trichuris muris*	*Lactobacillus rhamnosus* NCC4007 and *Bifidobacterium longum* NCC3001	Infection with *Trichuris muris* induced mild to moderate colonic inflammation and anxiety like behavior. However, treatment with *B. longum* reversed the effect and normalized brain-derived neurotrophic factor level	Bercik et al. (2010)
Rat maternal separation model	*Bifidobacterium infantis*	Probiotic treatment resulted in normalization of the immune response, reversal of behavioral deficits, and restoration of basal noradrenalin concentrations in the brainstem	Desbonnet et al. (2010)
Balb/c mice	*Lactobacillus rhamnosus* (JBI)	*L. rhamnosus* (*JB-1*) reduced stress-induced corticosterone and anxiety- and depression-related behavior and also increased $GABA_{A\alpha 2}$ expression	Bravo et al. (2011)

(continued)

Table 1 (continued)

Animal model	Gut microbiota/probiotics	Effect(s)	References
Balb/c mice and NIH mice	SPF + antibiotic vs. GF mice	Administration of oral antimicrobials to SPF mice transiently altered the composition of the microbiota and increased behavior and hippocampal expression of brain-derived neurotrophic factor. Interspecies gut microbiota transplantation changed species specific associated phenotype	Bercik et al. (2011)
Swiss Webster female mice	GF vs. SPF mice	GF mice have a more pronounced anxiolytic behavior and increased expression of BDNF mRNA in the hippocampus compared with SPF mice. They have reduced serotonin 1A receptor mRNA expression in the hippocampus	Neufeld et al. (2011)
NMRI mice	GF vs. SPF	GF mice have increased motor activity and reduced anxiety compared with SPF with a normal microbiota. GF mice exposed to gut microbiota early in life display similar characteristics as SPF mice	Diaz Heijtz et al. (2011)

GF germ-free, *SPF* specific pathogen free

4 Mechanisms by Which Microbiota Affect CNS Function

4.1 Activation of Vagus Nerve

The vagus nerve plays a major role in communicating changes in the gastrointestinal tract to the CNS. Information from the heart, lungs, pancreas, liver, stomach, and intestine is delivered to the brain via sensory fibers in the vagus nerve (Browning and Mendelowitz 2003). Moreover, activation of the vagus nerve has been shown to have a marked anti-inflammatory capacity (Wang et al. 2003). Many of the effects of the gut microbiota or potential probiotics on brain function have shown to be dependent on vagal activation (Goehler et al. 2008; Bravo et al. 2011; de Lartigue et al. 2011). There is now strong evidence from animal studies that gut microorganisms can activate the vagus nerve and that such activation plays a critical role in mediating effects on the brain and, subsequently, behavior. However, the mechanisms through which gut microbiota activate the vagus nerve are

currently unclear. Therefore, considerable investigations are needed to be conducted to understand the molecular mechanisms at a microbiome level underlying the effects observed.

4.2 Alteration of Microbial Composition

Probiotics may restore the composition of the gut microbiome and introduce beneficial functions to gut microbial communities, resulting in amelioration of gut inflammation and other intestinal or systemic diseases. Exogenously administered potential probiotic bacteria or infectious agents can affect the composition of the gut microbiota in multiple ways (O'Toole and Cooney 2008). Mechanisms whereby probiotics impact on the intestinal microbiota include competition for substrates, direct antagonism by inhibitory substances, competitive exclusion, and potentially host-mediated effects such as improved barrier function and altered immune response, thereby altering intestinal properties for colonization and persistence (O'Toole and Cooney 2008). All of these can have marked effects on gut–brain signaling.

4.3 Immune Activation

Microbiota and probiotic agents can have direct effects on the immune system (Forsythe and Bienenstock 2010; Duerkop et al. 2009). It has been observed that decreased intestinal microflora increases antigen transport across gastrointestinal mucosa, which is the primary interface between the external environment and the immune system. This suggests that the normal gut microflora is important in maintaining gut defenses. The beneficial probiotic bacteria have been found to interact with gut epithelial cells, the M cells in the Peyer's patches, and allied immune cells to initiate immune responses. In addition to regulating immunoglobulin production, these bacteria are also involved in increasing the profiles of some cytokines (TNF-alpha, IFN-gamma, IL-10) which are known to regulate the immune responses and maintain intestinal homeostasis. Indeed, the innate and adaptive immune systems collaborate to maintain homeostasis at the luminal surface of the intestinal host–microbial interface, which is crucial for maintaining health (Duerkop et al. 2009). The immune system also exerts a bidirectional communication with the CNS (Sternberg 2006; Dantzer et al. 2008), making it a prime target for transducing the effects of bacteria on the CNS. The cytokine production and other immune changes can modulate the peripheral and central nervous system and are associated with altered mood and behavior (Dantzer et al. 2000; Vitkovic et al. 2000). In addition, indirect effects of the gut microbiota and probiotics on the innate immune system can result in alterations in the circulating levels of pro-inflammatory and anti-inflammatory cytokines that directly affect brain function.

4.4 Production of Microbial Metabolites

Gut bacteria modulate various host metabolic reactions, resulting in the production of metabolites such as bile acids, choline, and short-chain fatty acids that are essential for host health. Through the cooperative action of different functional microbial groups, the gut microbiota synthesizes essential amino acids and vitamins. In addition, by deploying an array of glycoside hydrolases and polysaccharide lysases, the microbiota facilitates utilization of otherwise indigestible food compounds. Short-chain fatty acids (SCFAs) are organic fatty acids with 1–6 carbon atoms and are the principal anions which arise from bacterial fermentation of polysaccharides, oligosaccharides, proteins, peptides, and glycoprotein precursors in the colon. Increase in SCFAs results in the decrease of pH which indirectly influences the composition of colonic microflora, decreases solubility of bile acids, increases absorption of minerals, and reduces ammonia absorption by protonic dissociation of ammonia and other amines. It has been suggested that short-chain fatty acid delivery through probiotic ingestion may be an exciting treatment option for neurodegenerative diseases. Microbial metabolites are indispensable for majority of the biological effects of gut microbiota. Under physiological conditions, soluble dietary fibers and resistant starch can be actively fermented by commensal microbiota in the large intestine. The fermentation products such as SCFAs have been appreciated for their beneficial effects on intestinal epithelium and the gut immune system and are also known to have neuroactive properties (Thomas et al. 2012; MacFabe et al. 2011).

4.5 Production of Neurometabolites

Probiotics may act via their ability to produce various biologically active compounds, such as peptides and mediators normally associated with mammalian neurotransmission. Several molecules with neuroactive functions such as gamma-aminobutyric acid (GABA), serotonin, catecholamines, and acetylcholine have been reported to be microbially derived, many of which have been isolated from bacteria within the human gut. It has been postulated that *Lactobacillus* spp. and *Bifidobacterium* spp. produce GABA; *Escherichia* spp., *Bacillus* spp., and *Saccharomyces* spp. produce noradrenalin; *Candida* spp., *Streptococcus* spp., *Escherichia* spp., and *Enterococcus* spp. produce serotonin; *Bacillus* spp. produce dopamine; and *Lactobacillus* spp. produce acetylcholine (Lyte 2011; Matur and Eraslan 2012; Barrett et al. 2012). Secreted neurotransmitters from bacteria in the intestinal lumen may induce epithelial cells to release molecules that in turn modulate neural signaling within the enteric nervous system and consequently signal brain function and behavior of the host. Consequently, neurochemical containing/producing probiotic bacteria may be viewed as delivery vehicles for neuroactive compounds, and as such, probiotic bacteria may possibly have the potential as a therapeutic strategy

in the prevention and/or treatment of certain neurological and neurophysiological conditions.

4.6 Cell Wall Polysaccharides

The health-promoting effects of probiotics are largely due to their outer exocellular polysaccharide coating of probiotic bacteria. The exocellular polysaccharide of the probiotic bacteria protects the bacteria from acid and bile in the gut and shields from the host immune response (Fanning et al. 2012). Such studies suggest possibility of nonviable bacterial components as microbial-based therapeutic alternatives to probiotics. Like neuroactive metabolites, cell wall components of microorganisms in the intestine are believed to induce epithelial cells to release molecules which modulate neural signaling (Forsythe and Kunze 2012).

Multiple potential direct and indirect pathways exist through which the gut microbiota can modulate the gut–brain axis. They include endocrine (cortisol), immune (cytokines), and neural (vagus and enteric nervous system) pathways (Fig. 1). The brain recruits these same mechanisms to influence the composition of the gut microbiota. The hypothalamus–pituitary–adrenal axis regulates cortisol secretion, and cortisol can affect immune cells (including cytokine secretion) both

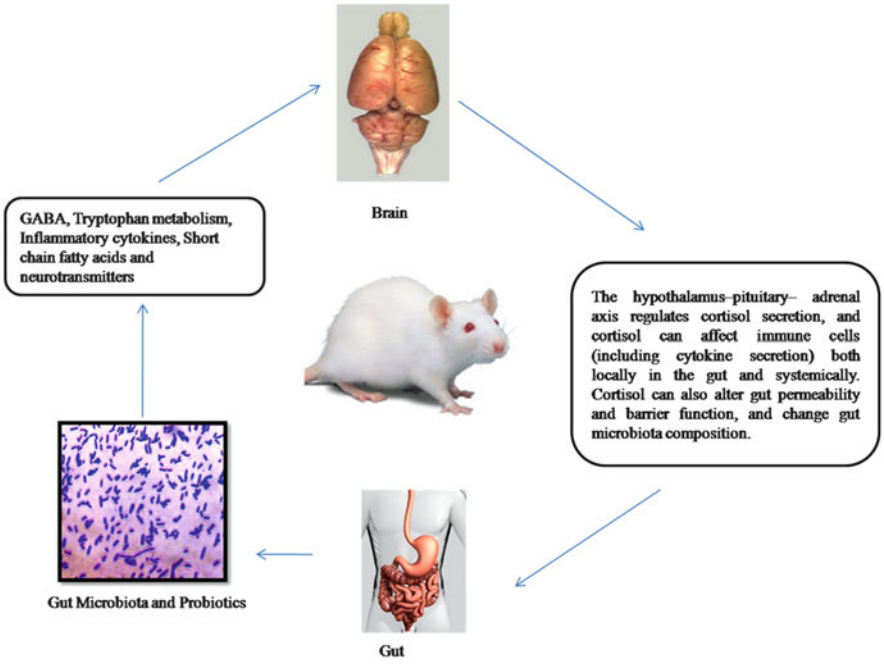

Fig. 1 Bidirectional communication between gut and brain

locally in the gut and systemically. Cortisol can also alter gut permeability and barrier function and change gut microbiota composition. Conversely, the gut microbiota and probiotic agents can alter the levels of circulating cytokines, and this can have a marked effect on brain function.

5 Conclusions

It is now established that there is a symbiotic interaction between gut microbiota and mental well-being and the integrity of both is essential to maintain the homeostasis. There is a growing body of experimental data and clinical observations which support the existence of the microbiota–gut–brain axis and suggest that it controls brain and behavior in health and disease. The knowledge gained in recent years about the ability of gut microbes to influence and contribute to host health and well-being opens new opportunity for nutritional and pharmacological tools to improve host–microbe symbiosis by using probiotics and prebiotics. It is essential that researchers should manipulate the microbial impact on gut–brain axis to elucidate the mechanisms by which microbiota communicate with the gut–brain axis which seems to be crucially important for the development of any microbiota-based and microbiota-specific therapeutic strategies for CNS diseases.

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Antioxidant and Antimicrobial Potential of Polyphenols from Foods

Anita Dua, Sharad Agrawal, Avtar Singh, and Ritu Mahajan

Abstract Change in the lifestyle is causing the overproduction of reactive oxygen species (ROS), free radicals, and decreasing the physiological antioxidant capacity. ROS can also cause protein and lipid oxidation, nucleic acid mutation, and further responsible for the development of various diseases including cancer, cardiovascular diseases, cataract, ageing, etc. Antioxidants can prevent the oxidation of biomolecules. Antioxidants present in food are now being preferred by consumers instead of synthetic antioxidants due to their non-toxic and non-carcinogenic effects. Antioxidants present in leafy vegetables, fruits, herbs, spices, seeds, alcoholic, and non-alcoholic beverages have the ability to reduce the damage caused by ROS. Polyphenols have redox potential high enough to scavenge or terminate ROS and also provide the environment favourable for inhibition of bacterial growth. Polyphenols also have metal ion chelating property which causes the deficiency of essential metal ions in the growth medium and ultimately responsible for antimicrobial effect. Binding of the polyphenols to the thiol groups at the active site of various microbial enzymes makes them inactive, thus inhibiting the growth of microbes. Polyphenols particularly phenolic acids and flavonoids have great potential as food additives with pharmaceutical, nutraceutical, and food preservative properties. The aim of this chapter is to present some valuable natural sources of polyphenols, structural characteristics, main classes of polyphenolic compounds, and extraction of polyphenols and also provide information on the most recent developments in the chemical investigation of polyphenols, emphasising their antioxidant and antimicrobial potential.

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1 Introduction

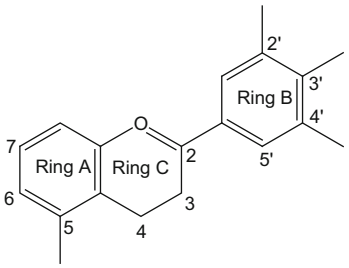
Phenolic compounds are secondary metabolites of plants, considered as important determinants in the nutritional and sensory quality of fruits, vegetables, other plant foods, and food preparations (Lapornik et al. 2005). These compounds possess an aromatic ring with one or more hydroxyl groups. The structures of phenolics from plants may range from a simple phenolic molecule to complex high-molecular mass polymer. As a large, most widely occurring group of bioactive phytochemicals, they have diverse physiological and biological functions. Phenolics may act as phytoalexins (Popa et al. 2008), antifeedants, attractants for pollinators, contributors to plant pigmentation, antioxidants, and protective agents against UV light (Naczk and Shahidi 2006). These bioactive properties made these compounds play an important role in plant growth and reproduction by providing an efficient protection against pathogens and predators (Popa et al. 2002). Phenolic compounds from plants have been reported to have excellent properties as food preservatives (Valenzuela et al. 1992). These compounds are reported to play an important role in the prevention and protection against a number of pathological disturbances, such as atherosclerosis, brain dysfunction, diabetes, and cancer (Gordon 1996). Phenolics from plants also contribute to the colour and sensory characteristics of fruits and vegetables (Alasalvar et al. 2001). These properties make them suitable for various industrial applications such as natural colourants and preservatives for foods or in the production of paints, paper, and cosmetics.

2 Structural Characteristics of Polyphenolic Compounds

Phenolic compounds include not only the polyphenols but also molecules with one phenol ring, e.g. phenolic acids and phenolic alcohols. Polyphenols are divided into several classes according to the number of phenol rings and based on the structural elements that bind these rings to one another. The main groups of polyphenols are flavonoids, phenolic acids, tannins, stilbenes, and lignans.

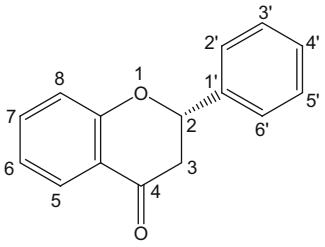
2.1 Flavonoids

Flavonoids are low-molecular-weight compounds, consisting of 15 carbon atoms, arranged in a C6–C3–C6 configuration. Essentially, the structure consists of two aromatic rings, A and B, joined by a three-carbon bridge, usually in the form of a heterocyclic ring, C. The aromatic ring A is derived from the acetate/malonate pathway, while ring B is derived from phenylalanine through the shikimate pathway (Merken and Beecher 2000).



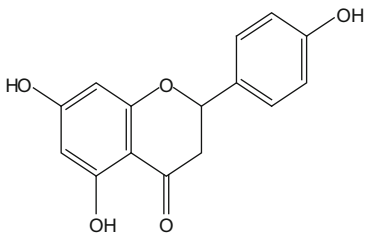
Variations in the substitution patterns of ring C result in the major flavonoid classes, i.e. flavonols, flavones, flavanones, flavanols (or catechins), isoflavones, flavanonols, and anthocyanidins, of which flavones and flavonols are the most widely occurring. Structural substitutions to rings A and B give rise to different compounds within each class of flavonoids. These substitutions may include oxygenation, alkylation, glycosylation, acylation, and sulphonation (Balasundram et al. 2006).

Flavanones are the flavonoids having a saturated three-carbon chain and an oxygen atom attached to C_4. They are generally glycosylated by a monosaccharide/disaccharide at C_7. Flavanones are present in high concentrations in citrus fruits, tomatoes, and some aromatic plants such as mint. The main aglycone in grapefruit is naringenin, a bitter tasting antioxidant, in oranges is hesperetin, and in lemons is eriodicitrin. Flavanones are compounds that give many plants colour, as well as affect their taste. Various foods and juices, as well as bee pollen, include such materials; in general, these can aid in the body's response to viruses, allergens, and even carcinogenic substances.



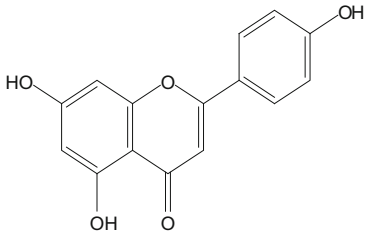
Isoflavones have hydroxyl groups in the C_7 and C_4 positions like estradiol molecule. They are phytochemicals that are found in many plants and plant-derived foods in both native ('aglycone') and acetyl- or malonyl-β-glucoside forms. Some physiological effects are attributed to their structural similarities to β-estradiols, and they are occasionally referred to as 'phytoestrogens' (Klejdus et al. 2007). With important health effects attributed to them, it has been suggested that they should be used for the prevention or cure of prevalent diseases such as atherosclerosis or cancer.

Flavanone



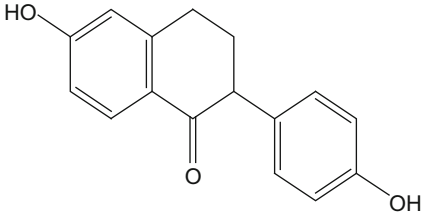
Naringenin

Flavone



Apigenin

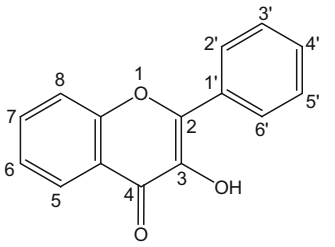
Isoflavones



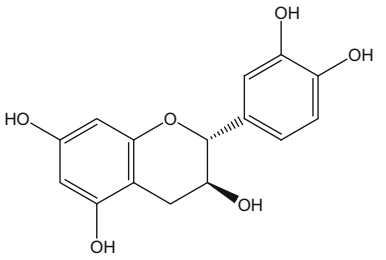
Daidzein

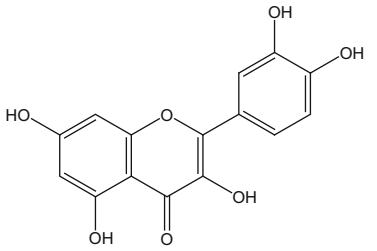
Flavonols are a class of flavonoids that have the 3-hydroxyflavone backbone. Their diversity stems from the different positions of the phenolic –OH groups. Flavonols are a class of flavonoids commonly found in many fruits and vegetables, their content varying widely, depending on environmental factors such as growing conditions, climate, storage, and cooking conditions (Caridi et al. 2007). The phenomenon of dual fluorescence (due to excited state intramolecular proton transfer or ESIPT) is induced by tautomerism of flavonols (and glucosides) and could contribute to plant UV protection and flower colour.

Flavonol

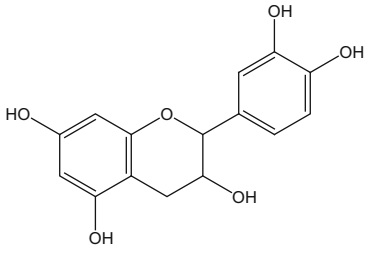


Flavanol





Quercetin



Catechin

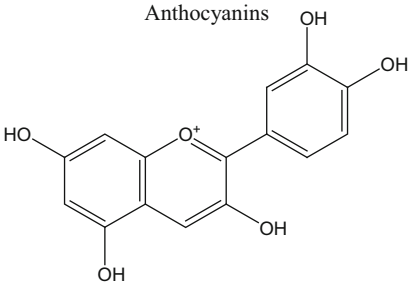
Anthocyanidins, the basic structures of the anthocyanidins, consist of an aromatic ring A bonded to an heterocyclic ring C that contains oxygen, which is also bonded by a carbon–carbon bond to a third aromatic ring B. When the anthocyanidins are found in their glycoside form (bonded to a sugar moiety), they are known as anthocyanins. These are water-soluble vacuolar pigments that may

appear as red, purple, or blue depending on pH. These are synthesised via the phenylpropanoid pathway. Anthocyanins have been reported in all plant tissues, including leaves, stems, roots, flowers, and fruits. Pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin are six anthocyanidins most frequently found in plants. The sugars commonly linked to anthocyanidins are monosaccharides: glucose, galactose, rhamnose, and arabinose and di- or tri-saccharides formed by combination of these monosaccharides (Bureau et al. 2009).

Their stability is affected by several factors such as pH, storage temperature, chemical structure, concentration, light, oxygen, solvents, and the presence of enzymes, flavonoids, proteins, and metal ions (Castaneda-Ovando et al. 2009). Anthocyanins possess well-known pharmacological properties and strong biological functions such as anti-inflammatory and antioxidant activities. Their antioxidant potential is dependent on the number and arrangement of the hydroxyl groups and the extent of structural conjugation, as well as the presence of electron-donating and electron-withdrawing substituents in the ring structure (Lapornik et al. 2005).

In recent years, synthetic food dyes have been banned in many countries because of their toxicity and carcinogenicity. Anthocyanins, coloured natural compounds easily obtained from fruits and vegetables, can be considered potential substitutes for the banned food dyes: they have, in fact, bright attractive colours, while their high solubility in water allows their easy incorporation into aqueous food systems. Moreover, the proved antioxidant activity of anthocyanins, related to the prevention of a number of degenerative diseases, provides additional benefits to the food supplemented with these natural substances (Bleve et al. 2008; Scalbert et al. 2005).

Flavonoids are especially important antioxidants due to their high redox potential, which allows them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelating potential. Flavonoids help to protect the plant against UV light, fungal parasites, herbivores, pathogens, and oxidative cell injury. Clinical trials indicate that when consumed regularly by humans, flavonoids have been associated with a reduction in the incidence of diseases such as cancer and heart disease (Scalbert et al. 2005; Liu et al. 2008). There is currently great interest in flavonoid research due to the possibility of improved public health through diet, where preventative health care can be promoted through the consumption of fruit and vegetables.



Cyanidin

2.2 Phenolic Acids

Phenolic acids or phenolcarboxylic acids are a type of aromatic acid compounds. Included in that class are substances containing a phenolic ring and an organic carboxylic acid function (C_6–C_1 skeleton). Phenolic acids consist of two subgroups: the hydroxybenzoic and hydroxycinnamic acids. Hydroxybenzoic acids include gallic, p-hydroxybenzoic, protocatechuic, vanillic, and syringic acids, which have in common the C_6–C_1 structure. Hydroxycinnamic acids are the aromatic compounds with a three-carbon side chain (C_6–C_3) represented by caffeic, ferulic, p-coumaric, and sinapic acids. About one-third of the dietary phenols are present as phenolic acids, which may be present in free and bound forms. Bound phenolics may be linked to other biomolecules through ester, ether, or acetal bonds (Zadernowski et al. 2009). Phenolic compounds, including anthocyanins, flavonoids, and phenolic acids, are known to be responsible for antioxidant capacities in fruits, the fruits with higher phenolic contents generally showing stronger antioxidant capacities (Fang et al. 2009).

2.3 Tannins

The third important group of phenolics is tannins. These are the high-molecular-weight compounds which may be hydrolysable or non-hydrolysable condensed tannins. Hydrolysable tannins are derivatives of gallic acid (3,4,5-trihydroxybenzoic acid). Gallic acid is esterified to a core polyol, and the galloyl groups may be further esterified or oxidatively cross-linked to yield more complex hydrolysable tannins. Condensed tannins are polymeric flavonoids. The biosynthetic pathways for flavonoid synthesis are well understood, but the steps leading to condensation and polymerisation have not been elucidated. Condensed tannins are based on flavan-3-ols (−)-epicatechin and (+)-catechin and are most widely studied. Tannins being potential metal ion chelators, protein precipitating agents, and biological antioxidants have diverse effects on biological systems. Tannins have great structural variation and can play varied biological roles, and therefore it has been difficult to predict structure function relationship for tannins.

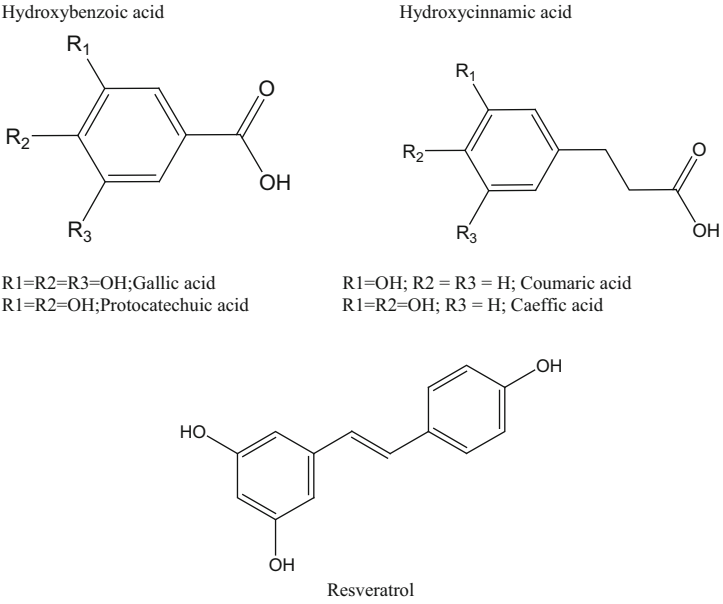
2.4 Stilbenes and Lignans

Stilbenes are 1,2-diarylethenes. Ring A usually carries two hydroxyl groups in the m-position, while ring B is substituted by hydroxy and methoxy groups in the o-, m-, and/or p-position. They are synthesised from cinnamic acid derivatives, and the substitution pattern of the cinnamic acid determines that of ring B of the stilbene.

Parent compounds of common stilbenes

Parent compound	Stilbene
Cinnamic acid	Pinosylvin
p-Coumaric acid	Resveratrol
Caffeic acid	Piceatannol
Isoferulic acid	Rhapontigenin

Low quantities of stilbenes are present in the human diet and the main representative is resveratrol that exists in both cis and trans isomeric forms, mostly in glycosylated forms (Delmas et al. 2006). It is produced by plants in response to infection by pathogens or to a variety of stress conditions (Bavaresco 2003). It has been detected in more than 70 plant species, including grapes, berries, and peanuts. Lignans are defined as compounds possessing 1,4-diarylbutane structure. Lignans are produced by oxidative dimerisation of two phenylpropane units; they are mostly present in nature in the free form, while their glycoside derivatives are only a minor form. These are widely distributed as minor constituents of some plant species. Several plants contain high concentrations of lignans; for example, flax seed is the richest identified source of the precursor seco-isolariciresinol. The interest in lignans and their synthetic derivatives is growing because of potential applications in cancer chemotherapy and various other pharmacological effects (Saleem et al. 2005).



Chemical structures of phenolic compounds

3 Extraction and Identification of Polyphenolics

Extraction is an important step in the isolation, identification, and use of phenolic compounds. Different extraction media and procedures are used for isolation and identification of polyphenols depending upon the characteristics of sources. Two most commonly used techniques for the isolation of phenolic compounds are solvent extraction (Bucić-Kojić et al. 2007) and extraction with supercritical fluid (Bleve et al. 2008). The phenolic compounds have been extracted by grinding, drying, or lyophilising fruits, vegetables, and herbs or only by soaking fresh plants with subsequent solvent extraction (Merken and Beecher 2000). Organic solvents such as ethanol, methanol, acetone, ethyl acetate, and their aqueous mixtures are commonly used for the extraction of polyphenols from plant material. These methodologies imply the co-extraction of non-phenolic substances, such as sugars, organic acids, and proteins, requiring subsequent purification processes (Castaneda-Ovando et al. 2009). Based upon the type and status of the source, solvent extraction may be liquid–liquid extraction or solid–liquid extraction. Conventional extraction procedures such as heating, boiling, or refluxing are used to extract natural phenolic compounds; however, these procedures have some disadvantages like the long extraction time, the loss of polyphenols due to ionisation, hydrolysis, and oxidation during extraction. To overcome these losses during extraction, various novel extraction techniques have been developed in recent years, including ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, and high hydrostatic pressure extraction (HHP) (Wang and Weller 2006).

A number of spectrophotometric methods have been developed for the quantification of plant phenolics. These assays are based on the principles to determine different structural groups present in phenolic compounds. The Folin–Ciocalteu assay (Lapornik et al. 2005) is widely used for determining total phenolics. Total flavonoid content can also be determined using a colorimetric method based on the complexation of the phenolic compounds with Al(III) (Huang et al. 2009; Naczk and Shahidi 2006).

The spectrophotometric assays cannot be used to separate or to analyse individual phenolic compound quantitatively. Chromatographic techniques are used to isolate and quantify polyphenols. Amongst the different chromatographic methods, for the separation, identification, and quantification of polyphenolics in fruits and herbs, HPLC is preferred. The chromatographic conditions of the HPLC methods include the use of, almost exclusively, a reversed-phase C18 column, UV–Vis diode array detector, and a binary solvent system containing acidified water (solvent A) and a polar organic solvent (solvent B). Reverse phase (RP) HPLC has become a dominating analytical tool for the separation and determination of polyphenols with different detection systems, such as diode array detector (DAD), mass spectrometry, or tandem mass spectrometry. Sakakibara et al. (2003) determined all polyphenols in foodstuffs simultaneously with HPLC–DAD and constructed a library comprising respective calibration curves for 100 standard chemicals.

Lower molecular mass polyphenols can be analysed by HPLC on reversed-phase or normal phase columns. However, these techniques are time-consuming and can have poor resolution as the polymer chain length and structural diversity increase. Counter current chromatography (CCC) uses a biphasic liquid system to separate the components of a mixture. The high advantage of the technique in preparative separation is the dual-mode capability of CCC. The role of the phases can be switched during a run. The mobile phase becomes stationary and vice versa. Then no injected material can be left in the machine. Combinations of these techniques are tried to get best output. Cao et al. (2009) applied two methods of separation and purification of polyphenols from apple pomace extract by combining gel chromatography with high-speed counter current chromatography (HSCCC) and solvent extraction with HSCCC, respectively.

Supercritical fluid chromatography (SFC) is a relatively recent chromatographic technique used in the separation and identification of phenolic compounds, where supercritical fluid is used as the mobile phase. Supercritical fluid chromatography is more versatile, more cost-efficient, user friendly, with higher output, better resolution, and faster analysis times than general liquid chromatographic methods. Capillary electrophoresis (CE) is especially suitable for the separation and quantification of low- to medium-molecular-weight polar and charged compounds. This is faster and more efficient than the corresponding HPLC separations (Caridi et al. 2007). Low sensitivity of CE as compared to GC or HPLC is the limitation of this technique (Liu et al. 2008). Nuclear magnetic resonance (NMR), mass spectroscopy (MS), and near-infrared (NIR) spectroscopy are some powerful, fast, accurate, and non-destructive analytical tools used for elucidating the chemical structures of polyphenolic compounds (Ignat et al. 2011).

4 Antioxidant Activity Measurement Methods

Antioxidants include a wide range of phytochemicals, which have redox potential intermediate to reduce ROS and get reduced by reducing power like glutathione in biological systems. Scavenging free radicals such as hydroxyl or superoxide radicals, terminating chain reaction, chelating metal ions, inhibiting ROS production, and donating electrons or hydrogen to terminate chain reactions are some of the ways by which antioxidants reduce oxidation (Schafer et al. 2003). The mode of action of natural antioxidants may be varied and could involve multiple mechanisms of action. Phenols act as primary antioxidants, while ascorbic acid may reductively regenerate oxidised primary antioxidants. The antioxidant activity of a natural source is generally related to either of these activities or as a synergist. Synergism between various antioxidants has been reported (Hudson and Lewis 1983). DPPH is a stable free radical which can absorb an electron or hydrogen to become a stable diamagnetic molecule. Scavenging of these free radicals by the antioxidants is observed as a decrease in optical density of the reaction mixture. Scavenging of free radicals generated by ABTS (2,2′-azino-bis-(3ethylbenzoline-6-

sulfonic acid)) diammonium salt with potassium persulfate is recorded at 645 nm (Matanjun et al. 2008). Superoxide scavenging activity in the reaction mixture containing NBT (Nitroblue tetrazolium), NADH, and PMS (Phenazine methosulphate) is recorded as a decrease in absorbance at 560 nm (Ani et al. 2006). Metal ions such as iron and copper can induce oxidation of lipids leading to the production of peroxy radicals, which in turn propagate chain reaction and accelerate lipid oxidation. Malonaldehyde produced by metal-induced oxidation of lecithin is determined as thiobarbituric acid reactive substances (Dua et al. 2013a, b, c, d). Reducing power is also estimated as the ferric ion reducing capacity (Ghorab et al. 2010). Oxidative stress generated by Fenton's reaction can cause breaks in calf thymus DNA and can uncoil the supercoiled DNA. Incubation of DNA with $FeSO_4$ and ascorbate has caused damage to DNA and damaged DNA moves to a greater extent in the gel. Protection of DNA against damage and protection of ribose oxidation is also used as an index of antioxidant activity (Ani et al. 2006).

5 Natural Source of Polyphenols

Polyphenols are widely distributed in plants such as fruits, vegetables, tea, olive oil, tobacco, herbs, medicinal plants, and others. The plant kingdom offers a wide range of natural antioxidants including polyphenols. Consequently, antioxidants have become an essential part of the preservation technology and contemporary health care. The potential toxicity of some synthetic antioxidants, however, has intensified research efforts to discover and utilise antioxidants from natural sources, such as fruits and vegetables (Zhang et al. 2009).

The most common sources of plant phenolics are presented in Table 1 (Pérez-Jiménez et al. 2010)

5.1 Spices and Herbs

Although spices and herbs have been added to foods since ancient times to improve or modify their flavours, the polyphenols present in them impart antioxidant, antimicrobial, and preservative properties to them. Cloves contain 10–13 % tannins. Eugenin and ellagitannin are the major phenolics present in conjugated form. Biflovin and isobiflovin are two flavonoids found in pyranoside form (Milind and Khanna 2011). Nakatani and Inatani (1984) have identified carnosol, rosmanol, and epirosmanol in rosemary and sage extracts. Baroty et al. (2010) have identified cinnamyl aldehyde, cinnamyl alcohol, eugenol, and ethylcinnamate as the major phenolics in cinnamon bark and leaves. Wojdylo et al. (2007) have analysed the extracts of 32 herbs for the presence of polyphenols and identified caffeic, coumaric, ferulic, and neochlorogenic acid as major phenolic acids, whereas

Table 1 Common sources of plant phenolics

Type of food	Food	Total polyphenols	Polyphenols as aglycone equivalents
Spice/herb	Cloves	15,188	16,047
	Peppermint, dried	11,960	980
	Mexican oregano, dried	2319	—
	Celery seed	2094	—
	Common sage, dried	1207	2920
	Rosemary, dried	1018	2519
	Common thyme, dried	878	1815
	Sweet basil, dried	322	4317
	Curry, powder	285	1075
	Ginger, dried	202	473
	Cumin	55	2038
	Ceylon cinnamon	27	9070
	Marjoram, dried	23	3846
	Curry, powder	285	285
Fruit/vegetable	Black chokeberry	1756	1432
	Black elderberry	1359	804
	Lowbush blueberry	836	496
	Blackcurrant	758	464
	Black olive	569	320
	Plum	377	285
	Green olive	346	233
	Sweet basil, dried	322	166
	Sweet cherry	274	145
	Globe artichoke heads	260	154
	Blackberry	260	180
	Strawberry	235	205
	Red chicory	235	131
	Red raspberry	215	107
	Black grape	169	124
	Red onion	168	99
	Green chicory	166	117
	Spinach	119	68
	Shallot	113	67
	Peach	59	54
	Broccoli	45	21
	Redcurrant	43	23
Non-alcoholic beverages	Cocoa powder	3448	3294
	Coffee, filter	214	110
	Black tea	102	90
	Green tea	89	82
	Pure apple juice	68	61

(continued)

Table 1 (continued)

Type of food	Food	Total polyphenols	Polyphenols as aglycone equivalents
	Pure pomegranate juice	66	37
	Pure blood orange juice	56	28
	Pure grapefruit juice	53	23
	Pure lemon juice	42	20
	Chocolate beverage with milk	21	21
	Soy milk	18	11
	Pure pummelo juice	18	7.9
Alcoholic beverages	Red wine	101	91
	White wine	10	8.6
	Rosé wine	10	7.8

quercetin, luteolin, apigenin, kaempferol, and isorhamnetin are the major flavonoids. Shan et al. (2005) have reported that phenolic acids, phenolic diterpenes, and flavonoids are the main phenolic constituents of extracts of 26 spices screened. Clove, cinnamon, and oregano were found to have high phenolic content and antioxidant activity. Zheng and Wang (2001) have reported a direct correlation between the total phenolic content and antioxidant activity of 27 culinary and 12 medicinal herbs. Rosmarinic acid, quercetin glycosides, and kaempferol glycosides have been found to be the major phenolic compounds. Ani et al. (2006) have also identified gallic acid, protocatechuic acid, caffeic acid, ellagic acid, ferulic acid, quercetin, and kaempferol in methanolic extract of cumin (*Cuminum nigrum*) after glycosidic bond breakage by HCl hydrolysis. The potential medicinal benefits of spices and herbs including possible roles in lowering the risk for atherosclerosis, cardiovascular diseases, cancer, and diabetes are well documented (Srinivasan 2005; Milind and Khanna 2011).

The recent studies done by Dua and coworkers indicated that polyphenol-rich methanolic extract had efficient free radical scavenging and metal chelating activity to protect the biomolecules (proteins, lipids, DNA) against oxidative stress (Table 2).

5.2 Agro-industrial By-Products

Along with fruits, vegetables, and different herbs, agricultural and industrial residues are also rich in natural antioxidants. By-products and residues after processing fruits and vegetables in the food processing industry still contain a huge amount of phenolic compounds, which could be potential sources of antioxidants. One of the richest sources are berry skins, which remain as husks during wine and juice making and are usually used to make compost (Lapornik et al. 2005). The olive

Table 2 Polyphenolic content of some important spices

S. No.	Spice	Type of extract	Polyphenolic content (mg gallic acid equivalent/g dry seeds)	Reference
1	Cumin	Methanolic extract	7.45 ± 0.10	Dua et al. (2012)
2	Fennel	Methanolic extract	16.506 ± 0.32	Dua et al. (2013a)
3	Fenugreek	Methanolic extract	9.47 ± 0.10	Dua et al. (2013b)
4	Coriander	Methanolic extract	18.696 ± 0.12	Dua et al. (2014a)

mill wastes and brassica seed meal are also a major potential source of phenolics. The phenolic content of the olive mill wastewater (OMWW) is reported to fluctuate between 1.0 % and 1.8 % depending on various factors and processing effects. Hydroxytyrosol, tyrosol, oleuropein, and a variety of hydroxycinnamic acids are the major components of OMWW (Obied et al. 2005). Olive leaves, another by-product of the industry, are also rich source of phenolics (Benavente-Garcia et al. 2000). Extract of mustard seed meal in 80 % methanol had comparatively higher amount of polyphenols (15.726 ± 0.15 mg GAE/g dry weight of seeds) (Dua et al. 2014c).

The citrus industry produces large quantities of peel and seed residues, which may account for up to 50 % of the total fruit weight. Peels of the citrus fruits have been found to contain higher amounts of total phenolics even as compared to the edible portions (Balasundram et al. 2006).

Citrus industry by-products, if utilised optimally, could be major sources of phenolic compounds. By-products obtained after artichoke, cauliflower, carrot, celery, and onion processing were investigated by Larrosa et al. (2002).

The peels of several other fruits have also been found to be rich in phenolics than the edible fleshy parts. Apple peels were found to contain up to 3300 mg/100 g dry mass of phenolics (Wolfe and Liu 2003), whereas 118 mg/g of phenolics could be recovered from apple pomace by lyophilisation (Schieber et al. 2003). The peels and seeds of tomatoes have been also found to be richer sources of phenolic compounds than the fleshy pulp. The efficiency of extraction is influenced by various process parameters such as solvent type and feed pre-treatment (crushing, removal of stems) (Lapornik et al. 2005).

5.3 Beverages

Beverages such as fruit juices, tea, and wines are important sources of phenolics in the human diet. Important dietary polyphenolic antioxidants include a large variety of both flavonoid (flavonol, flavan-3-ol, and anthocyanin) and non-flavonoid

compounds (phenolic acids, phenolic alcohols, stilbene, hydroxycinnamic acid) (Makris et al. 2007).

Fruit juices like grapefruit, orange, and apple are also abundant sources of natural phenolic compounds. Commercial or natural fruit juices contain vitamin C and an abundance of phytonutrients with antioxidant properties. Most of the data available on the phenolic contents of commonly consumed juices are for commercial samples.

Tea in different forms of non-fermented (green), semi-fermented (Oolong), and fermented (black) is widely used around the world. In addition, extracts of tea have become commercially available as antioxidants to control deterioration of lipids in foods. Fresh green leaves contain about 36 % of polyphenols on dry weight basis. The main phenolic compounds present in tea are catechins. Epigallocatechins (9–12 %) and epicatechin gallate (9–12 %) followed by epicatechin (5–7 %), catechin (0.3–0.6 %), and gallic acid (0.3–0.5 %) are identified in green tea leaves (Shahidi 2000). Green tea has been subjected to many scientific and medical studies to determine the extent of its long-purported health benefits, with some evidence suggesting that regular green tea drinkers may have lower chances of developing heart disease and certain types of cancer. Although the content of phenolics is quite diversified depending on the type of technology of its preservation, generally, green tea contains more phenolics than black or red tea and shows higher antioxidant activity (Sikora et al. 2008).

Red wine has been found to be more protective on health than other alcoholic beverages (Alen-Ruiz et al. 2009). Red wine helps in preventing oxidative stress-related diseases, possibly because of the polyphenols it contains. The polyphenolic profile of red wines is usually different from that of white wines due to differences in the composition of red and white grapes and also due to those in the vinification technology used (Alen-Ruiz et al. 2009).

Coffee also provides a significant source of dietary antioxidants. Roasted coffee contains phenolic compounds up to 8 % of the weight. Chlorogenic acid is the dominant phenolic in coffee, which is about 28 mg/g in this drink (Sikora et al. 2008). Klatsky et al. (2006) studied the interrelation between the consumption of coffee as a dietary source of polyphenolic compounds and the apparent reductions in the risks of Alzheimer's disease, Parkinson's disease, heart disease, diabetes mellitus type 2, and liver cirrhosis.

6 Measurement of Antimicrobial Activity

Different antimicrobial susceptibility testing methods are employed to confirm the antimicrobial activity of a chosen antimicrobial agent. The most widely used testing methods include:

- Tube dilution test
- Disc diffusion test

- Agar well diffusion test

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation.

Contamination of food due to microorganisms occurs during production, processing, sale, and distribution (Deak and Beuchat 1996), which ultimately results in microbial food-borne diseases (Mead et al. 1999) It is, therefore, necessary to emphasise the use of antimicrobial agents (Sagdıc and Ozcan 2003) to prevent food spoilage. The naturally occurring polyphenols present in vegetables and fruits have been shown to possess antibacterial activity and thus act as a source of antibacterial agents against food spoilage bacteria.

The antibacterial activity of different vegetable extracts such as broccoli, Brussels sprouts, and white cabbage was measured against *Listeria monocytogenes*, *Enterococcus faecalis*, *Salmonella abony*, and *Pseudomonas aeruginosa*, and extracts were effective against these microorganisms (Jaiswal et al. 2011a, b).

The *Listeria monocytogenes* growth is inhibited by the grape wine (Rodriguez-Vaquero et al. 2007) and the inhibition increased with the increase in concentration of polyphenols. Pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* are inhibited due to the antimicrobial activity exhibited by the extracts of alcohol-free red and white wine (Papadopoulou et al. 2005). A considerable number of white and red wine phenolic extracts inhibited the growth of *C. albicans*. However, in all tests, the diameter of the inhibition zone for *C. albicans* was smaller than the diameter measured for *S. aureus* and *E. coli* inhibition zones.

Green tea (*Camellia sinensis* var. *sinensis*) has a high content of polyphenol particularly flavonoids. Catechins are the main flavonoids found in green tea (Cabrera et al. 2006). Green tea also contains gallic acid (GA), chlorogenic acid, caffeic acid, and flavonols such as kaempferol, myricetin, and quercetin. Cho et al. (2007, 2008) demonstrated the antibacterial effect of tea polyphenols (TPP) on *E. coli* as well as methicillin-resistant *Staphylococcus aureus*. Starting with an inoculum of approximately 10^7 bacteria/ml, there were no *E. coli* CFUs after 30 h in the presence of 5000 μg/ml or after 18 h at 10,000 μg/ml TPP, whereas 50–180 μg/ml concentration of TPP was found to be minimal inhibitory for 30 clinical isolates of *Staphylococcus aureus*.

Fattouch et al. (2008) used aqueous acetone extracts of two apple cultivars, 'Golden Delicious' and 'Red Delicious'; a pear 'Williams' cultivar; and a local quince cultivar (*Cydonia oblonga* Miller) for the determination of antimicrobial activities against a wide range of microorganisms including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella* sp. strain. The MIC value of each phenolic extract ranged from 10^2 to 10^4 μg/ml among different tested microorganisms, but in case of *Salmonella*, a moderate antimicrobial activity was shown by 'Red Delicious' peel extract.

The antimicrobial activities of polyphenolic extracts of three wild red berry fruit species, namely, European cornel (*Cornus mass*), blackthorn (*Prunus spinosa*), and wild blackberry (*Rubus fruticosus*), were assessed by Radovanovic et al. (2013) using disc diffusion method. European cornel extract was found to contain maximum amount of total phenolic contents and the zone of inhibition was significant for the extracts against both the Gram-positive (*Clostridium perfringens*, *Bacillus subtilis*, *Staphylococcus aureus*, *Listeria inocula*, *Sarcina lutea*, *Micrococcus flavus*) and the Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Shigella sonnei*, *Klebsiella pneumonia*, *Proteus vulgaris*) tested bacterial strains.

Different solvent extracts of *Passiflora ligularis* fruit also exhibited antimicrobial activities against *Streptococcus fecalis*, *S. pyogenes*, *Bacillus subtilis*, *Klebsiella pneumonia*e, *Salmonella paratyphi*, and fungi *Candida albicans* and *Aspergillus niger* (Shanmugam and Thangaraj 2014), and the acetone extract of the fruit pulp showed maximum inhibitory effect.

Rahman et al. (2011) studied the antimicrobial effect of six crude plant extracts (*Allium sativum*, *Zingiber officinale*, *Allium cepa*, *Coriandrum sativum*, *Piper nigrum*, and *Citrus aurantifolia*) against five *Escherichia coli* isolated from potable water sources and observed that the aqueous extracts of *Allium cepa*, *Piper nigrum*, and *Coriandrum sativum* seeds alone did not exhibit any in vitro antibacterial effect; however, the combination of *Allium cepa* + *Allium sativum* (1:1) and *Citrus aurantifolia* + *Zingiber officinale* + *Allium sativum* (1:1:1) showed inhibition zones.

Antibacterial activities of methanolic extract of dried spice seeds were determined against pathogenic bacteria by determining cell damage, growth inhibition zone diameter, and minimum inhibitory concentration. Incubation of bacterial cultures with these spice extracts caused damage to their cell membranes and release of intracellular nucleotides and proteinaceous materials from the cells (Table 3).

Table 3 MIC value of some important spices against bacteria

S. No.	Spice	Type of extract	MIC value against bacteria equivalent to mg dry weight of spice/ml of extract	Reference
1	Cumin	Methanolic extract	*E. coli*—12.5 *P. aeruginosa*—6.25 *S. aureus*—25.0 *B. pumilus*—6.25	Dua et al. (2013d)
2	Fennel	Methanolic extract	*B. pumilus*—8.33 *S. aureus*—8.33	Dua et al. (2013c)
3	Coriander	Methanolic extract	*E. coli*—4.16 *P. aeruginosa*—4.16 *S. aureus*—4.16 *B. pumilus*—4.16	Dua et al. (2014b)

7 Conclusion

The studies carried out in the last few decades significantly demonstrate the antioxidant and antimicrobial role of polyphenols. The vegetable and fruit extracts can be utilised in food products for enhancing the quality and nutritive value of foods. The polyphenolic compounds including flavonoids, flavonols, as well as phenolic acids have a variety of bioactivities including antioxidant and antimicrobial activities against a wide range of microorganisms. The phenolic compounds could be utilised as preservatives and thus are better applicable in food preservation and also contribute as antioxidant supplements and antimicrobial agents for food.

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Oxidative Stress: Role of Natural Antioxidant Compounds

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Abstract Oxidative stress, an imbalance between the generation of reactive oxygen species and antioxidant defense capacity of the body, is induced by a wide range of factors including UV, pathogen invasion, or oxygen shortage. In oxidative stress, innate antioxidant deference system becomes circumscribed and thus this state becomes sole culprit for induction of highly prevalent diseases such as cancer, diabetes, hypertension, atherosclerosis, acute renal failure, and Alzheimer's and Parkinson's diseases. Currently, antioxidant supplementation derived from natural source, especially medicinal plants, is more of interest because of mutagenic or toxic effects of synthetic antioxidants like butylated hydroxytoluene (BHT). The implication of oxidative stress in the etiology of many chronic and degenerative diseases suggests that antioxidant therapy represents a promising avenue for treatment. This chapter thus includes basic understanding of oxidative stress phenomena and research reports with experimental studies on dietary antioxidants to mitigate the detrimental effects of oxidative stress.

1 Overview of Antioxidants, Free Radicals, and Oxidative Stress

Antioxidants are molecules capable of slowing or preventing the oxidation of other important molecules. Antioxidants are termed as reducing agents and affect cell differentiation and proliferation, block nitrosamine formation, stimulate the immune system, help to maintain the integrity of cell membrane and matrix, and aid in the maintenance of normal DNA repair (Khanam et al. 2004). Oxidation is a chemical reaction that transfers electron from a substance to an oxidizing agent. Oxidation reactions can generate toxic metabolite including free radicals, which

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start chain reactions that damage tissues/cells. Antioxidants terminate these chain reactions by removing free radical intermediates/derivatives and inhibit other oxidation reactions by being oxidized themselves. Although oxidation reactions are essential for human daily life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, natural vitamins including vitamin C and vitamin E, as well as enzymes such as superoxide dismutase and catalase, and various peroxidases. Low levels of these antioxidants, or inhibition of the antioxidants enzymes, cause oxidative stress and may cumulatively damage cells (Lesko and Lorentzen 1980).

Free radicals are generated during normal metabolism and exposure to environmental insults such as infection agents, pollution, UV light, radiation, and so on. These are highly reactive species capable of wide spread, indiscriminate oxidation and peroxidation of proteins, lipids, and DNA which can lead to significant cellular damage and even tissue and/or organ failure when these harmful free radicals cause damage to vital proteins, lipids, and DNA (Lesko and Lorentzen 1980). Free radicals can result in approximately 80 different age-related diseases. These include cancer, heart attack, stroke, rheumatoid arthritis, cataracts, and Alzheimer's disease. Antioxidants fight from free radicals and thus protect us from age-related diseases.

As oxidative stress might be an important cycle/part of many human disorders/diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for inflammation caused metabolic disorder and its complications like diabetes, cardio stroke, and other neurodegenerative diseases. Oxidative stress is defined in general as excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Turko et al. 2001; Maritim et al. 2003). ROS include free radicals such as superoxide (O_2^-), hydroxyl (OH), peroxyl (RO_2), hydroperoxyl (HRO_2^-), as well as non-radical species such as hydrogen peroxide (H_2O_2) and hydrochlorous acid (HOCl) (Evans et al. 2002). While ROS are generated under physiological conditions and are involved to some extent as signaling molecules and defense mechanisms as seen in phagocytosis, neutrophil function, and shear stress-induced vasorelaxation, excess generation in oxidative stress has pathological consequences including damage to proteins, lipids, and DNA (Johansen et al. 2005). In recent years, there has been increased interest in the therapeutic use of antioxidants in the treatment of disease associated with oxidative stress (Gupta and Sharma 2006; Rathore et al. 2011). Several studies reported that low antioxidant intake or low blood levels of antioxidants increases the risk of different diseases, infects low dietary intake of fruits and vegetables, and doubles the risk of cancer (Percival 1988). Therefore, wholesome antioxidant diet and natural antioxidant supplements as part of a healthy lifestyle are now being recognized to protect health from oxidative stress.

2 Role of Antioxidants in Controlling Oxidative Stress

Antioxidants are substances that neutralize free radicals or their actions. Nature has endowed each cell with adequate protective mechanisms against any harmful effects of free radicals: superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, thioredoxin, thiols, and disulfide bonding are buffering systems in every cell. Dietary antioxidants are potent solution to overcome the action of ROS. The most important dietary antioxidants are plant polyphenols, carotenoids, xanthophylls, and flavonoids. Many studies have suggested that they exhibit strong antioxidant activity as they can reduce free radical formation and scavenge free radicals (Adelman et al. 1988; Helbock et al. 1988). Antioxidant defense system against oxidative stress is composed of several lines, and the antioxidants are classified into four categories based on function. First line of defense comprises preventive antioxidants, which suppress formation of free radical (enzymes: glutathione peroxidase, catalase; selenoprotein, transferrin, ferritin, lactoferrin, carotenoids, etc.). Second line of defense is the radical scavenging antioxidants suppressing chain initiation and/or breaking chain propagation reactions: radical scavenging antioxidants. Third line of defense consists of repair and de novo antioxidant (some proteolysis enzymes, repair enzymes of DNA, etc.). A fourth line of defense is an adaptation where the signal for the production and reactions of free radicals induces formations and transport of the appropriate antioxidant to the right site (Kumari and Deshwal 2011).

Free radical-induced oxidative stress is now believed to be a fundamental mechanism underlying a number of human cardiovascular, neurologic, and other disorders. Antioxidants are our crucial defense against free radical-induced damage and are critical for maintaining optimum health and well-being. It has been estimated that ~5 % of inhaled oxygen is converted into several damaging ROS like superoxide, hydroxyl, and hydrogen peroxide by equivalent reduction of oxygen. In today's modern world, the risk of diseases due to oxidative stress is compounded by unhealthy lifestyle, exposure of chemicals, pollution, cigarette smoking, drugs, illness, and stress. Exogenous consumption of antioxidants from plant, animal, and mineral sources has proved beneficial to human health and effective to reduce the incidence of free radical-induced diseases.

Antioxidants can decrease oxidative stress-induced carcinogenesis by a direct scavenging of ROS and/or by inhibiting cell proliferation secondary to the protein phosphorylation. B-carotene may be protective against cancer through its antioxidant function, because oxidative products can cause genetic damage. Thus, the photoprotective properties of B-carotene may protect against ultraviolet light-induced carcinogenesis. Immunoenhancement of B-carotene may contribute to cancer protection. B-carotene may also have anticarcinogenic effect by altering the liver metabolism effects of carcinogens (Poppel and Golddbohm 1995). Vitamin C may be helpful in preventing cancer (Glatthaar et al. 1986). The possible mechanisms by which vitamin C may affect carcinogenesis include antioxidant effects, blocking of formation of nitrosamines, enhancement of the immune

response, and acceleration of detoxification of liver enzymes. Vitamin E, an important antioxidant, plays a role in immunocompetence by increasing humoral antibody protection, resistance to bacterial infections, cell-mediated immunity, the T-lymphocytes tumor necrosis factor production, inhibition of mutagen formation, repair of membranes in DNA, and blocking micro cell line formation (Sokol 1988). Hence, vitamin E may be useful in cancer prevention and inhibit carcinogenesis by the stimulation of the immune system. The administration of a mixture of the above three antioxidant revealed the highest reduction in the risk of developing cardiac cancer. We argue, therefore, that integration of antioxidant status as inclusion criterion in the interventional trials with antioxidants may represent another approach to better explore the role of oxidative stress and the potential efficacy of antioxidant treatment in patients at high risk for stress.

3 Sources of Antioxidants

Antioxidants are abundant in colorful fruits and leaf vegetables, as well as in other important functional foods, including nuts, grains and some meats, poultry, and fish. Here, we describe some food sources of common bioactive antioxidants. Beta-carotene is found in many foods that are orange in color, including sweet potatoes, carrots, cantaloupe, squash, apricots, pumpkin, and mangoes. Some of the green leafy vegetables, including collard greens, spinach, and kale, are also rich in beta-carotene. Lutein, best known for its association with healthy eyes, in plants (fruits, vegetables, medicinal herbs) may contain a wide variety of free radical scavenging molecules such as phenolic compounds (phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins, etc.), nitrogen compounds (alkaloids, amines, betalains, etc.), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites which are rich in antioxidant activity (Cai et al. 2003).

Natural and synthetic food antioxidants are used routinely in foods and medicine, especially those containing oils and fats to protect the food against oxidation. There are a number of synthetic phenolic antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) being prominent examples. These compounds have been widely used as antioxidants in food industry, cosmetics, and therapeutic industry. However, some physical properties of BHT and BHA such as their high volatility and instability at elevated temperature, strict legislation on the use of synthetic food additives, carcinogenic nature of some synthetic antioxidants, and consumer preferences have shifted the attention of manufacturers from synthetic to natural antioxidants (Papas 1999). In view of increasing risk factors of human to various deadly diseases, there has been a global trend toward the use of natural substance present in medicinal plants and dietary plants as therapeutic antioxidants. It has been reported that there is an inverse relationship between the dietary intake of antioxidant-rich food and medicinal plants and incidence of human diseases. The use of natural antioxidants in food, cosmetic,

and therapeutic industry would be a promising alternative for synthetic antioxidants in respect of low cost, highly compatible with dietary intake, and no harmful effects inside the human body. Many antioxidant compounds, naturally occurring in plant sources, have been identified as free radical or active oxygen scavengers (Brown and Rice-Evan 1998). Attempts have been made to study the antioxidant potential of a wide variety of vegetables like potato, spinach, tomatoes, and legumes (Furuta et al. 1997). There are several reports showing antioxidant potential of fruits (Wang et al. 1996). Strong antioxidant activities have been found in berries, cherries, citrus, prunes, and olives. Green and black teas have been extensively studied in the recent past for antioxidant properties since they contain up to 30 % of the dry weight as phenolic compounds (Lin et al. 1998). Apart from the dietary sources, Indian medicinal plants also provide antioxidants and these include *Acacia catechu* (Kair), *Aegle marmelos* (Bel), *Allium cepa* (Onion), *A. sativum* (Garlic), *Aloe vera* (Ghritkumari), *Amomum subulatum* (Greater cardamom), *Andrographis paniculata* (Kiryat), *Asparagus racemosus* (Shatavari), *Azadirachta indica* (Neem), *Bacopa monnieri* (Brahmi), *Butea monosperma* (Palas), *Camellia sinensis* (Green tea), *Cinnamomum verum* (Cinnamon), *Cinnamomum tamala* (Tejpat), *Curcma longa* (Turmeric), *Emblica officinalis* (Amlaki), *Glycyrrhiza glabra* (Yashtimudhu), *Hemidesmus indicus* (Anantamul), *Indigofera tinctoria, Mangifera indica* (Mango), *Momordica charantia* (Bitter gourd), *Murraya koenigii* (Curry leaf), *Nigella sativa* (Black cumin), *Ocimum sanctum* (Holy basil), *Onosma echioides* (Ratanjyot), *Picrorhiza kurroa* (Katuka), Piper beetle, *Plumbago zeylanica* (Chitrak), *Sesamum indicum, Sida cordifolia, Spirulina fusiformis* (Alga), *Swertia decussata*, *Syzigium cumini* (Jamun), *Terminalia arjuna* (Arjun), *Terminalia bellirica* (Beheda), *Tinospora cordifolia* (Heart leaved moonseed, Guduchi), *Trigonella foenum-graecium* (Fenugreek), *Withania somnifera* (Ashwangandha), and *Zingiber officinalis* (Ginger) (Devasagayam et al. 2004). Lycopene is a potent natural antioxidant found in tomatoes, watermelon, guava, papaya, apricots, pink grapefruit, blood oranges, and other functional foods. Estimates suggest that 85 % of American dietary intake of lycopene comes from tomatoes and tomato-related products (Xianquan et al. 2005). Selenium is an important mineral and not an antioxidant nutrient. However, it is a component of antioxidant enzymes. In addition, plant foods like rice and wheat are the major dietary sources of selenium in most developing and developed countries (Palanisamy et al. 2012).

4 Natural Polyphenolic Compounds as Antioxidants

Plant polyphenols are well-known antioxidants. Phenolics have been reported to have a capacity to scavenge free radicals. They are commonly found in both edible and non-edible plants and have multiple biological effects, including antioxidant activity. The antioxidant activity of phenol is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelating potential (Rice-Evans et al. 2005). Phenolics,

such as flavonoids, phenolic acids, lignin, and tannins, are especially common in leaves, flowering tissues, fruits, and woody parts, such as stems and barks. Venukumar and Latha (2002) reported antioxidant activity of methanol extract of rhizome of *Curculigo orchioides* using carbon tetrachloride-intoxicated rat liver. Javanmardi et al. (2003) evaluated Iranian *Ocimum basilicum* for its antioxidant activity and total phenolic content and found that a linear positive relationship existed between the antioxidant activity and total phenolic content.

Damintoti et al. (2005) screened *Combretum micranthum*, *Khaya senegalensis*, *Pterocarpus erinaceus,* and *Sida acuta* for antioxidant and antibacterial activities of polyphenols; different polyphenolic contents showed potent antioxidant activity. Kadifkova et al. (2005) investigated the chemical composition and antioxidant activity of different extracts obtained from *Teucrium* species and found the presence of flavonoids, luteolin, apigenin, and diosmetin. *Teucrium* species possess free radical and hydroxyl radical scavenging as well as antioxidant activity in vitro (Kadifkova et al. 2005).

Siddhuraju and Manian (2007) observed the antioxidant and free radical scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum*) seeds. Ozsoy et al. (2008) evaluated the antioxidant activity of water, infusion, ethanol, and ethyl acetate extracts of *Smilax excelsa* leaf. All extracts had good total phenolic and flavonoid contents inhibiting lipid peroxidation, radical scavenging, and iron-chelating activities and considered as a significant natural antioxidant source. Manian et al. (2008) evaluated *Ficus benghalensis* (aerial root) and *F. racemosa* (stem bark) for their antioxidant and radical scavenging capacity and compared with *Camella saneness*, where all extracts exhibited dose-dependent reducing power activity. All the extracts exhibited antioxidant activity against the linoleum acid and emulsion system (34–38 %).

5 Oxygen and Oxidative Stress

Although oxygen is potentially dangerous in excess, it is vital and essential element for all living cells whether neuronal or other kinds of cells taking part in tissue formation. Hence, oxygen is kept under crucial observation of complex system that regulates and monitors the usage and uptake of the important molecule. Oxygen takes part in glucose breakdown in Mt through oxidative phosphorylation and generates energy currency of cell called ATP (Harvey et al. 1999). Oxidative stress appears due to disturbed equilibrium between pro-oxidant/antioxidant homeostasis that further takes part in generation of ROS and free radicals, potentially toxic to various body cells. The reason for neuronal cell hypersensitivity toward oxidative stress arises due to anatomic and metabolic factors. In the brain, various types of glial cells are present and these are involved in anatomic support and metabolic requirement. The endothelial cells surrounding these glial cells are less permeable for uptake of various molecules and protective cells viz. macrophages compared to other endothelial cells in the body. Moreover, glial cells require more oxygen and

glucose consumption to generate continuous ATP pool for normal functioning of brain as it is one of the important organs responsible to activate and control other body organs. These consequences make these cells susceptible toward oxygen over load, resulting in more free radical formation (Lepoivre et al. 1994). Under physiological condition, 1–2 % of oxygen consumed is converted to ROS; however, in mature person, higher levels of percentage consumption of oxygen are achieved due to low regenerative capacity of aged brain and reduced surveillance of antioxidants (Lepoivre et al. 1994).

6 Concept of Oxidative Stress

The term best suits to describe the condition of oxidative damage resulting when the critical balance between free radical generation and antioxidant defenses is unfavorable (Rock et al. 1996). Oxidative stress, arising as a result of an imbalance between free radical production and antioxidant defenses, is associated with damage to a wide range of molecular species including lipids, proteins, and nucleic acids (Mc Cord 2000). However, short-term oxidative stress may occur in tissues injured by trauma, infection, heat injury, hypertoxia, toxins, and excessive exercise. The injuries caused in these tissue fragments produce increased radical generating enzymes such as xanthine oxidase, lipogenase, and cyclooxygenase, activation of phagocytes, release of free iron, copper ions, and a disruption of the electron transport chains of oxidative phosphorylation, resulting in the excess production of ROS formation. The initiation, promotion, and progression of cancer, as well as the side-effects of radiation and chemotherapy, have been linked to the imbalance between ROS and the antioxidant defense system. ROS have been implicated in the induction and complications of diabetes mellitus, age-related eye disease, and neurodegenerative diseases such as Parkinson's disease (Rao et al. 2006).

7 Oxidative Stress and Its Role in Human Health

Oxidative stress is a harmful condition that occurs when there is an excess of ROS and/or a decrease in antioxidant levels; this may cause tissue damage by physical, chemical, psychological factors that lead to tissue injury in human and causes variety of severe human diseases. Living creatures have evolved a highly complicated defense system and body defends against free radical-induced oxidative stress involved by various defense mechanisms such as preventative mechanisms, repair mechanisms, physical defenses, and antioxidant defenses (Valko et al. 2007).

Oxygen-derived free radical reactions have been implicated in the pathogenesis of many human diseases/disorders, including neurodegenerative disorders (Alzheimer's disease, Parkinson's disease), cardiovascular disorders (atherosclerosis, ischemic heart disease, cardiac hypertrophy, hypertension, shock, and trauma),

pulmonary disorders (inflammatory lung diseases such as asthma and chronic obstructive pulmonary disease), autoimmune disorders (rheumatoid arthritis), renal disorders (glomerulonephritis and tubulointerstitial nephritis, chronic renal failure, proteinuria, and uremia), gastrointestinal disorders (peptic ulcer, inflammatory bowel disease, and colitis), cancers (lung cancer, leukemia, breast, ovary, and rectum cancers), eye diseases (cataract and age-related maculopathy and ageing process of retina), diabetes, skin lesions, immune depression, liver disease, pancreatitis, AIDS, and infertility (Valko et al. 2007; Gupta et al. 1997; Sen et al. 2009).

8 Natural Protection Against Free Radical-Induced Oxidative Stress

Reactive species can be eliminated by a number of enzymatic and non-enzymatic antioxidant defense mechanisms. In enzymatic antioxidant system, SOD immediately converts superoxide to H_2O_2, which is then detoxified to water either by catalase in the lysosomes or by glutathione peroxidase in the mitochondria. Glutathione reductase, another important enzyme, regenerates glutathione, a hydrogen donor, which is used by glutathione peroxidase during the elimination of H_2O_2. Some other researchers reported that diabetes has multiple effects on the protein levels and activity of such antioxidant enzymes, which further augment oxidative stress by causing a suppressed defense response (Maritim et al. 2003). In diabetes, heart is an important target organ and prone to diabetic cardiomyopathy leading to chronic heart failure, expression of SOD, and glutathione peroxidase with decreased activity, whereas catalase is increased in various experimental models of diabetes (Maritim et al. 2003; Hayden and Tyagi 2003). In patients with chronic heart failure, all these enzymes are decreased in the smooth muscle (Linke et al. 2005) and the expression and activity of antioxidant enzymes can be upregulated by physical workout. Increased isoprostane levels in diabetic patients with chronic heart failure are correlated with antioxidant status and disease severity (Polidori et al. 2004). Thus, modulation of these enzymes in target organs prone to diabetic complications such as heart and kidney may prove beneficial in the prevention and management of heart- and kidney-related disorders.

On the other hand, vitamins A, C, and E, glutathione, α-lipoic acid, carotenoids, trace elements like copper, zinc and selenium, coenzyme Q10 (CoQ10), and cofactors like folic acid, uric acid, albumin, vitamins B1, B2, B6, and B12 are considered as non-enzymatic antioxidants. Changes in the antioxidant defense system in diabetes have been reported previously (Vega-Lopez et al. 2004). A tri-peptide, glutathione (GSH), acts as a direct scavenger as well as a co-substrate for GSH peroxidase. It is a major intracellular redox tampon system. Vitamin E is a fat-soluble vitamin that prevents lipid peroxidation. It exists in eight different forms, of which α-tocopherol is the most active form in humans. Hydroxyl radical reacts with tocopherol forming a stabilized phenolic radical which is reduced back

to the phenol by ascorbate and NAD (P) H-dependent reductase enzymes (Hensley et al. 2004). The coenzyme Q10 is an endogenously synthesized compound that acts as an electron carrier in the complex II of the mitochondrial electron transport chain, which is the site of superoxide generation under hyperglycemic conditions (Nishikawa et al. 2000; Brownlee 2001). The coenzyme Q10 is a lipid-soluble antioxidant, and in higher concentrations, it scavenges superoxide and improves endothelial dysfunction in diabetes (Hodgson and Watts 2003; Watts et al. 2002). Ascorbic acid increases nitric oxide (NO) production in endothelial cells by stabilizing NOS cofactor BH4 (Heller et al. 2001). The α-lipoic acid is a hydrophilic antioxidant and can therefore exert beneficial effects in both aqueous and lipid environments. This α-lipoic acid is reduced to another active compound dihydrolipoate which is able to regenerate other antioxidants such as vitamins C and E and reduces glutathione through redox cycling (Heller et al. 2001). These findings utilized naturally occurring antioxidants, especially vitamins C and E and α-lipoic acid, in order to delineate the role of oxidative stress/damage in the development of complications of diabetes.

9 Future Perspectives

Many randomized trial data are necessary to evaluate definitively the potential role of antioxidants in DNA damage. Various in vivo experiments should be conducted to prove the antioxidant properties of plants against DNA damage. Not only this, but genetic variation studies should also be performed in order to find if there is any difference in protective role of antioxidants in vivo. Recent research on the effect of antioxidants on DNA repair enzymes suggests that they remove oxidized purines, whereas mRNA levels of the relevant DNA repair genes appears to be unaffected by an antioxidant-rich diet. In the future, intervention studies and considerations of genotypes of defense enzymes as well as DNA repair capacity should be kept in mind. Fruits and vegetables are rich in number of phytochemicals that act as antioxidants, but which component is responsible for the most beneficial effects is still unknown. Various factors are still not known; hence, their protective role should be identified against DNA damage. There are many methods used to measure antioxidants and DNA damage. A minor change in methodology can vary the results to a great extent. Hence, consistent results with best suitable methods should be developed. Dietary antioxidants are not very effective due to their poor solubility, inefficient permeability, and instability due to storage of food. Novel drug delivery systems such as liposomes, microparticles, nanoparticles, and gel-based practices would also help in the oral delivery of these antioxidants, as this oral supplementary route is of prime importance when antioxidants are intended for prophylactic purpose against DNA damage. Some components of plants may also act as pro-oxidants if taken at higher dose in the presence of free transition metals. Future experiments should be aimed at purifying and characterizing the specific

components in order to describe a precise mechanism of action of various plant-based natural antioxidants.

10 Conclusion

The oxidative stress and free radicals are not measured by defense mechanism of body and they may cause damage to vital proteins, lipids, and DNA molecules. Therefore, we need antioxidants to ensure our defense mechanism for neutralizing harmful radicals. Hopefully, further research into the pathophysiology of oxidative stress and the role of antioxidant therapy will lead to appropriately designed clinical trials in which the promise of antioxidant therapy will be realized. All antioxidants have a chemical element referred to as a "redox" potential, which is the measurement of their ability to be oxidized. Considering the fact that the redox equilibrium is important to the body's coping mechanism, it follows that antioxidants can influence many health conditions.

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Medicinal Importance of Mangrove Plants

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Abstract In recent years, research on medicinal plants has attracted a lot of attention due to their importance and possibility for treatment of human diseases. Mangroves are unique group of vascular plants that occur in saline coastal habitats and are known to tolerate extreme environmental conditions. Some mangrove plants are used for a wide range of conditions, including bacterial, fungal, and viral diseases. The rise of antibiotic-resistant microorganisms is one of the severe problems in healthcare systems of the world, and infectious diseases are the second most serious cause of death worldwide. Therefore, new drugs have to be found in order to combat such diseases and it is essential to find new compounds that have antimicrobial properties. Medicinal-plant extracts, known to produce certain bioactive molecules which react with other organisms in the environment, are known to be less toxic to humans and are environmentally friendly due to the less pollutant released during production. Antimicrobial properties of medicinal plants are being increasingly reported worldwide. Mangroves are biochemically unique and produce a wide array of novel natural products and are considered a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids, and tannins. Extracts from the leaves, stems, barks, and roots of mangrove species have shown positive results for antioxidant activity tests. Effects of mangrove extracts on some microorganisms, including *Shigella* sp., *Staphylococcus* sp., and *Pseudomonas* sp. have been reported in some studies in the area of pharmacology.

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1 Introduction

The name "mangrove" originated from a combination of the Portuguese word "Mangue" that means tree and an English word "grove" that means orchard or garden (Shelar et al. 2012). Mangrove plants include approximately 12 families and more than 50 species. Other synonymous terms suggested include "Mangrove community," "Mangrove swamp," "Mangrove ecosystem," and "coastal woodland." Mangrove generally refers to a group of salt-tolerant and evergreen woody plants that have morphological adaptations. Mangrove plant extracts have been used for centuries to treat several health disorders. Plant-derived substances have recently become of great interest owing to their versatile applications (Shelar et al. 2012). As antibiotics are increasingly used and misused, the bacterial strains become resistant to antibiotics rapidly. Therefore, there is a need to search for new infection-fighting strategies to control microbial infections. The rise of antibiotic resistant microorganisms is one of the severe problems in healthcare systems of the world, and infectious diseases are the second most serious cause of death worldwide. Plants are rich in a wide variety of phytochemicals like tannins, terpenoids, alkaloids, flavonoids, and antimicrobial peptides that have been found to have antimicrobial activities (Panda et al. 2009). In addition, medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic values.

Mangrove plants have been used in medicinal fields and their extracts have proven to possess inhibitory activity against human, animal, and plant pathogens. These specialized plants are known to tolerate extreme environmental conditions. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself (Shelar et al. 2012). However, many phytochemicals also exhibit a protective effect on humans against different diseases. Mangrove plants are a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids, flavonoids, as well as fatty acids, quinones, and tannins. Extracts from different mangrove plants are reported to possess diverse medicinal properties such as antibacterial and vermifuge effect (Ravikumar et al. 2010). Mangrove-leaf extracts are nontoxic to humans and are environmentally friendly due to less pollutants' production. Mangrove extracts can also be a possible source against mosquito larvicides and show anticancer and antidiabetic effects (Ktahiresan and Thangam 1991). *Rhizophora stylosa* leaves contain 17 kinds of amino acids that concluded 7 kinds of essential amino acids accounting for 26 % in total amino acid (Song et al. 2008). Mangrove leaves contain several kinds of trace elements which are essential for human beings (Laurent et al. 2013). Many species of mangroves are being used to treat diseases such as rheumatism, small pox, ulcers, hepatitis, leprosy, asthma, snake bites, and toothache, and as purgatives (Prabhakaran and Kavitha 2012). Since mangrove plants offer a wide array of molecules with therapeutic value, there is an urgent need to discover new compounds with diverse chemical structures and novel mechanisms of action. This chapter reveals and evaluates the antimicrobial activity and

medicinal importance of some mangrove plants against some human bacterial and fungal pathogens.

2 Mangrove Trees

Mangrove trees grow where no tree has grown before because they are able to survive saltwater conditions and soil which is unstable and poor in oxygen (anaerobic). Mangroves are a group of trees and shrubs that are capable of growing in marine, estuarine, and, to a limited degree, freshwater (Colin Field 1995). They occupy the fringe of intertidal shallows between the land and the sea (Fig. 1) (Mastaller 1997). Mangrove trees are not able to grow in freshwater as fast as other freshwater plants and may also be unable to cope with the bacteria and fungi found in freshwater (Mastaller 1997; Tan 2001). Most mangrove trees lack a heartwood and instead have narrow vessels that are densely and evenly distributed throughout the wood. Thus, they are able to withstand damage to the bark and outer trunk (Tan 2001). About 70 species of trees and shrubs are considered principal or true mangrove forms. These belong to 19 families, but of these only 2 are exclusively mangroves. The highest diversity of mangroves is found in the region from Malaysia to New Guinea. Eighty percent of these are found in the Indo-Pacific (India to Australia), 9 % in East Africa, 6 % in West Africa, 5 % in the Caribbean, and 5 % in South America (Tan 2001).

Mangrove forests are fascinating and complex ecosystems (Feller et al. 2010). They serve coastal populations worldwide by protecting shorelines from storm surge and erosion, through filtration/remediation of terrestrial runoff, and as nurseries for important fisheries, among other useful roles (Laurent et al. 2013). Yet these marine margin communities are under constant threat of clearing from real estate development, fish-pond farming, and even for their wood to support

Fig. 1 Mangrove plants grow as a shrub or high trees that live along shores, rivers, and estuaries in the tropics and subtropics and characterized by survival in salt water by abundant roots. Mangrove roots provide support in unstable soils, provide structural support in the soft mud, and withstand currents and storms. Roots for absorbing nutrients are tiny and emerge near the muddy surface (Mastaller 1997)

cooking hearths in poor, rural communities (Laurent et al. 2013). The World Wildlife Foundation (Mangrove Forest 2013) reports that 35 % of global mangrove communities have disappeared in the last two decades alone (Valiela et al. 2001).

2.1 Salt Tolerance Properties

To deal with salt, all mangrove trees exclude some salt at the root level, and all can tolerate more salt in their tissues than normal plants, often in quantities that would kill other plants. But some have more effective ultrafiltration at the root level to exclude more salt. Any salt that gets through are believed to be stored in old leaves which are later shed (Colin Field 1995). These include *Bruguiera*, *Sonneratia,* and *Rhizophora*. A few can tolerate high levels of salt in their tissues and their sap can be up to one-tenth as salty as sea water. They then secrete the excess salt through special cells on their leaves. Although mangrove trees are adapted to grow in saltwater, they require regular flushing with freshwater; and will die if immersed in saltwater all the time (Patra and Mohanta 2014).

As a group of plants, mangroves share several highly specialized adaptations that have allowed them to colonize and thrive in intertidal areas. In particular, they have developed special ways of dealing with concentrations of salt. These include: (a) salty sap and removing salt by concentrating it in branches and leaves before dropping them, (b) leaves with a waxy coating that limits saltwater penetration, and (c) salt-secreting pores on the leaves that allow the plant to get rid of excess salt (Stewart and Fairfull 2008). The most visible adaptation of mangrove plants, and the one which most distinguishes them from other terrestrial plants, is their root system.

2.2 Mangrove Leaves

Mangrove plants will die if immersed in saltwater all the time. Thus, freshwater is as precious to a mangrove trees as to a desert plant. They have to expend energy to get rid of the salt (Tan 2001). Mangroves have many water conserving features of desert plants. To minimize water loss through evaporation, they may have thick waxy leaves. Mangrove plants may also store water in juicy leaves and protect the plant parts from toxins with spiny leaves and waxy leaves. Mangrove plants are thus a precious resource of chemicals that have myriad potential uses for humans (Tan 2001). Mangrove leaves are eaten by all kinds of creatures. Leaves are rapidly broken down by microorganisms into useful minerals. Fallen leaves are an important source of nutrients both within the mangrove habitat and when it is flushed out to the coral reefs.

The mangrove leaves are useful contributors to the nutrient system of the mangrove environment. It is known that mangrove leaves contain sufficient

amounts of minerals, vitamins, and amino acids, which are essential for the growth and nourishment of marine organisms and livestock. Mangrove foliage plays an important role in the formation of detritus, which is utilized by several estuarine and marine detritivorous organisms (Bandaranayake 2002). More recently, an important study dealt with production of silage using mangrove leaves are performed (Mouafi et al. 2014). This is the first study to exploit mangrove leaves as a fodder. Mangrove leaves make a superior fodder due to their high salt, minerals, vitamins, amino acid, and iodine content. Moreover, the resultant silage possesses long shelf-life properties (Mouafi et al. 2014).

2.3 Mangrove Roots

Mangrove roots not only provide support in unstable soils and to withstand currents and storms but also breathe air. To avoid suffocation in the oxygen poor mud, mangrove trees snorkel for air. They develop aerial or air-breathing roots in above-ground air (Fig. 2) (Stewart and Fairfull 2008). All aerial tree roots have on their surface special tiny pores to take in air (lenticels), where only air can get through the lenticels, not water or salts (Tan 2001; Patra and Mohanta 2014). All aerial roots also contain large air spaces (aerenchyma). These not only transport air but also provide a reservoir of air during high tide when all the aerial roots may be under water. The function of aerial roots are to absorb air or/and to provide structural support in the soft mud (Colin Field 1995). Roots for absorbing nutrients are tiny and emerge near the muddy surface. Underwater, a huge number of filter-feeders are fastened on the tangle of roots: barnacles, sponges, and shellfish. These filter

Fig. 2 All mangrove trees exclude some salt at the root level and all can tolerate more salt in their tissues. Mangrove roots develop aerial or air-breathing roots (Stewart and Fairfull 2008)

feeders clean the water of nutrients and silt. As a result, clear water washes out into the sea, allowing the coral reef ecosystem to flourish (Tan 2001).

3 Importance of Mangrove Plants

In the past, mangrove forests have been considerably undervalued. The wetlands in which mangroves occur have been considered "wastelands" or breeding grounds for nuisance insects such as mosquitoes (Stewart and Fairfull 2008). As a result, many mangrove forests have been cleared, dredged, reclaimed, degraded, or otherwise lost. Our understanding of the value of this habitat has greatly improved over the past few decades and mangrove forests are now regarded as key fish habitats. Because of their importance as habitat for fish, mangroves are protected in New South Wales under the *Fisheries Management Act 1994* (Harty 1997). As mangrove plants are adapting a unique kind of habitat with low oxygen and limited salt intake, their role in synthesizing the high potential secondary metabolites is of major biotechnological as well as biomedical importance (Patra and Mohanta 2014).

Mangroves can be classified into three broad categories (Bandaranayake 2002):

1. True mangroves are mainly restricted to intertidal areas between the high water levels of neap and spring tides. Plant species from true mangroves belong to at least 20 different families. About 80 species of true mangrove trees/shrubs are recognized, of which 50–60 species make a significant contribution to the structure of mangrove forests.
2. Minor species of mangroves are distinguished by their inability to form conspicuous elements of the vegetation and they rarely form pure communities.
3. The mangal associates are salinity-tolerant plant species, which are not found exclusively in the proximity of mangroves and may occur only in transitional vegetation. However, they do interact with true mangroves (Bandaranayake 1998).

Mangroves (mangroves, mangrove minors, and mangal associates) are highly productive ecosystem with various important economic and environmental functions. The uses of mangroves are often quoted in scientific and popular articles (Bandaranayake 1998) and fall in two major categories,. Firstly, the indirect use of the mangrove ecosystem is in the form of vital ecological functions such as control of coastal erosion and protection of coastal land, stabilization of sediment, and natural purification of coastal water from pollution. Secondly, the economic benefits which are many and varied. Apart from prawn fisheries, many other species of economic importance are associated with mangroves; these include crabs, shrimp, oysters, lobsters, and fish (Bandaranayake 2002).

Mangroves serve three key functions:

A. Provide Habitat
Mangroves provide shelter for the juveniles and adults of many fish species, including commercially and recreationally important species such as mullet, bream, whiting, luderick, flathead, and shellfish such as prawns and crabs (Stewart and Fairfull 2008).

B. Provide Food
Mangrove trees produce large amounts of organic matter. The fallen leaves, seeds, and seedlings enter the waterway and are directly grazed by some small animals. The litter is further broken down by bacteria and fungi. Decaying pieces of debris are eaten by other aquatic animals called detritivores (e.g., crabs). These in turn provide food for larger fish and other animals (Stewart and Fairfull 2008).

C. Act as a Buffer
Mangroves act as a buffer; reducing erosion and maintaining water quality. A mangrove community also provides a buffer between the terrestrial and nearby marine environments; trapping and stabilizing sediment, nutrients, and contaminants from runoff, thus helping to maintain water quality. Mangroves protect coastal land by absorbing the energy of tidal currents and storm-driven wind and wave action, creating a natural breakwater that helps stop erosion. Evidence from major storm and wave events has shown the importance of mangrove forests in reducing storm damage (Stewart and Fairfull 2008).

Two basic factors justify the study of the chemical constituents of mangrove plants. (1) Firstly, mangroves are one of the easiest tropical forest types to generate. They have the ability to grow where no other vascular plants can. The mangroves exist under stressful conditions such as violent environments, high concentration of moisture and salt, high and low tides of water, and abundant living microorganisms and insects (Bandaranayake 2002). They thrive in a very peculiar environment and serve as a bridging ecosystem between freshwater and marine systems. These have imposed several modifications in these plants. They possess an unusual morphology and physiognomy, and the path of photosynthesis in mangroves is different from other glycophytes. They possess modifications to establish water and salt economy. There are modifications or alterations in other physiological processes such as carbohydrate metabolism or polyphenol synthesis. Due to these reasons, they may have chemical compounds which protect them from these destructive elements (Bandaranayake 2002). (2) The second reason is that numerous mangrove plants are being used in folklore medicine, and recently, extracts from mangroves and mangrove-dependent species have proven activity against human, animal, and plant pathogens but only limited investigations have been carried out to identify the metabolites responsible for their bioactivities (Bandaranayake 2002).

4 Economic Value of Mangroves

Several studies have attempted to quantify the economic value of mangroves to both commercial fisheries productivity and to the community in terms of their ecosystem services. In 1990, it was estimated that mangroves in Moreton Bay, south-east Queensland contributed approximately $8380 per hectare to commercial fisheries production based on the monetary value of catch rates of target fish caught in this habitat type (Morton 1990). In 2006, a report by the United Nations Environment Program (UNEP) estimates that mangroves contribute an annual value of US$200,000–900,000 per km^2 in services such as protecting foreshores, fisheries production and supply of building materials (e.g., timber), tourism and recreation, and improving water (UNEP-WCMC 2006).

5 Medicinal Importance of Mangrove Extracts

Emergence of human pathogenic microorganisms that show multiple-antibiotic resistance to major classes of antibiotics has increased in recent years, due to the random use of antimicrobial drugs. This has caused many clinical problems in the treatment of infectious diseases, and the antibiotics commonly used are sometimes associated with adverse effects such as hypersensitivity, allergic reaction, and immunosuppression in the host (Mukherjee et al. 2002). Thus, the search for the discovery of natural antimicrobial agents is an urgent need. There is an increasing demand for biologically active substances from plant origin which is the current interest and focus of new research approach. The synthetic chemical pharmaceuticals showed various side effects on the functioning of different parts of the body both internally and externally. Mangroves have long been a source of amazement and interest for scientists. For many people and layman living in the Indo-West Pacific and American-East Atlantic regions, the word mangrove will be a familiar one (Bandaranayake 2002).

5.1 Phytochemicals Identified from Mangroves

There is an increasing demand for biologically active substances from plant origin which is the current interest and focus of new research approach. The synthetic chemical pharmaceuticals showed various side effects on the functioning of different parts of the body, both internally and externally. Plant products have been shown to have no side effect and have good therapeutic potential due to the

presence of active pharmacologically important substances, i.e., phytochemicals, such as terpenes, alkaloids, flavonoids, phenolics, steroids, triterpenes, and glycosides (Farrukh and Ahamed 2003).

5.2 Primary and Secondary Metabolic Extracts

A diversity of chemical classes has been characterized from mangroves. Amino acids, alkaloids, carbohydrates, carotenoids, saponins, free fatty acids as well as steroids, triterpenes, glycosides, and tannins are among these classes (Bandaranayake 2002). Chemicals such as amino acids, carbohydrates, and proteins are products of primary metabolism and are vital for the maintenance of life processes, while alkaloids, phenolics, steroids, and terpenoids are products of secondary metabolism which have toxicological, pharmacological, and ecological importance (Bandaranayake 1998).

5.3 Medicinal Use and Bioactivity of Mangrove Extracts

Acanthus ilicifolius, a plant useful in the treatment of paralysis, asthma, rheumatic pains, and possessing analgesic, anti-inflammatory, and leishmanicidal activities, is a rich source of long-chain alcohols, triterpenes, steroids, and triterpenoidal saponins. Stigmasterol, a common plant steroid, abundantly present in *A. ilicifolius* and many other mangrove plants, has been shown to have hypercholesterolemic effects (Bandaranayake 2002). 2-Benzoxazoline (v), a synthetic compound used extensively as a central nervous system depressant, also exhibiting antipyretic, hypnotic, and muscle relaxant activity has been isolated from the plant (Kapil et al. 1994). Tricin, a flavonoid, is the metabolite common to most mangroves showing antifeedant activity. Known triterpenes, steroids, and a novel triterpenoid ester have been isolated from *Acrostichum aureum* and *Rhizophora apiculata*, a mangrove fern and tree, respectively (Kokpol et al. 1990). The extracts of these plants are being used in folklore medicine.

Metabolites belonging to different chemical classes have been identified as antifungal agents and in chemical narcosing of fish. Antifungal metabolites include alkaloids, flavonoids and related compounds, modified fatty acids, oxygen heterocyclics, proanthocyanidins, quinones, stilbenes, terpenoids, and triterpenoid saponins. The extracts of the bark and root of the mollucidal and piscicidal plant *Balanites aegyptiaca* are also used for the treatment of abdominal pains, as a purgative, and as an anthelmintic, while the bark is employed as a detergent, fish poison, and also as a remedy for malaria and syphilis (Marston and Hostettmenn 1985). The leaf is edible and has been once regarded as an effective medicine for sleeping sickness. The effects of oral administration of crude saponin extract of the plant caused myositis or peritonitis among chicks (Bandaranayake 2002). The

oleoresin from the bark of *Calophyllum inophyllum* (Guttiferae) is used as a cicatrisant, whereas an infusion or decoction of the leaves has been traditionally used for the treatment of eye diseases and as an ingredient in aromatic powders and liniments. Antibacterial, anti-inflammatory, and phagocytosis stimulant activities have been reported for this plant (Bandaranayake 1998).

The relationship between structures of the metabolites and activity has been investigated. Earlier phytochemical studies had revealed *C. inophyllum* to be a rich source of benzopyrans, coumarins, steroids, triterpenes, and xanthones. Plants of the genus *Clerodendron* are well known for their pesticidal properties (Madhu and Madhu 1997). They are used as armyworm antifeedants and to arrest bleeding from wounds, as well as for stopping post-partum hemorrhage. *Clerodendrum inerme*, a mangal associate, is a recognized medicinal plant having febrifugal properties as well as exhibiting larvicidal, antiviral, and uterine stimulant activity (Bandaranayake 2002). Extracts of *Clerodendrum inerme* were effective as surface protectants for cowpea seeds against pulse beetle infestation (Olivieri et al. 1996). The antiviral resistance-inducing protein isolated from the plant is a polynucleotide which showed antiviral activity against mosquito (Devi et al. 1997).

Neuropharmacological actions (including viper venom neutralization) of the shrub *P. indica* have been investigated (Sen et al. 1996; Thongpraditchote et al. 1996). The leaves and roots of the shrub have been reported to possess anti-inflammatory and antiulcer, astringent, and antipyretic properties and are used as a diaphoretic in fevers. Fresh leaves are used in the form of poultices against atonic and gangrenous ulcers and chemicals with novel structures have been isolated from the leaves. Cigarettes prepared from the chopped stem bark are smoked to relieve the pain of sinusitis, and in Indo-China, the leaves and young shoots are crushed, mixed with alcohol, and applied in the back, in cases of lumbago and are also used to relieve rheumatic pains and in baths to treat scabies. More recently, a study was performed to examine fresh leaves of *Avicenna marina* and *Rhizophora stylosa*. Results showed that *A. marina* and *R. stylosa* are excellent sources for a large number of phytochemicals (Mouafi et al. 2014). Triterpenoids from *R. mangle* possess insecticidal properties and has clinical use in the control of diabetes. Warm aqueous extract of the bark of *R. apiculata* is used as an astringent for diarrhea, nausea, and vomiting, and as an antiseptic. The extract is also used to stop bleeding in fresh wounds and for the treatment of chronic typhoid fever. The plant also has uses in the textile industry (Kokpol et al. 1990).

Flavonoid sulfates have been identified in land halophytes such *as Armeria maritima, Halophila ovalis, Limonium vulgare, Nypa frutican, Suaeda maritima* and species of *Atriplex, Frankenia*, and *Tamarix*. They also occur abundantly in sea-grasses such as *Thalassia, Zannichellia*, and *Zostera*. Arsenic is accumulated in the leaves of some mangroves (Harborne 1982). Cations such as Na^+, K^+, Ca^{2+}, Mg^{2+}, and NH_4^+ and anions Cl^-, Br^-, NO_2^-, NO_3^-, SO_4^{--}, etc., have been found in various parts of species of *Avicenna, Bruguiera*, *Rhizophora*, *K. candel, H. litoralis, E. agallocha,* and *A. corniculatum*. Organic acids such as citric, malic, oxalic, and tartaric and carbohydrates such as glucose and sucrose are abundant in the roots and seeds of many mangrove plants. Most mangrove species

directly regulate salts. They may also accumulate or synthesize other solutes to regulate and maintain osmotic balance (Bandaranayake 2002). For example, *Aegiceras corniculatum, Aegialitis annulata* and *Laguncularia racemosa* and the halophytes *Aster tripolium* and *Armeria maritima* accumulate mannitol and two nitrogen compounds, the protein imino acid proline and the quaternary nitrogen compound glycine betaine. *Avicenna marina* accumulates glycine, betaine, and asparagine and *Sonneratia alba* synthesizes purine nucleotides (Harborne 1982). It is proposed that the high levels of proline actually provide the basis of resistance to salt accumulation.

A number of review and research articles provide the information of the biological activities among the members of *Sonneratiaceae* family. Mangroves species such as *Sonneratia griffithii* were reported to show remarkable antihyperglycemic activity (Shahbudin et al. 2012). The extracts from the leaves, stems, barks, and roots of mangroves species such as *Sonneratia apetala* have shown positive result for antioxidant activity test (Feller et al. 2010). It has also been tested for plant growth regulators, growth hormone tests on plants, and antiviral activity test. *Sonneratia caseolaris* was found to be traditionally used to stop bleeding, check hemorrhages, and treat piles and it was also used as sprain poultices. This species was also tested for toxicity against mosquito larvae (Devi et al. 1997). Increasing attention is being focused on the use of tannins which are polyphenolic substances as antimicrobial agents or prevention of dental caries (Shahbudin et al. 2012). This compound proved to be rich in the members of *Sonneratiaceae* family. Mangrove plants were examined as a promising alternative for treatment of cigarette smoking hazardous. Chemicals in cigarette smoke are a leading cause of death to both smokers and non-smokers (Chinnappan and Kandasamy 2012). Mangroves are potential and novel source of anticancer drugs that regulate cancer pathways and stimulate the immune system. Medicinal research on mangroves for treatment of cancer has provided important methods for studying cancer therapy and mechanisms (Chinnappan and Kandasamy 2012).

6 Antimicrobial Activity of Some Medicinal Mangroves

The antibacterial activity of the leaves and bark of mangrove plants, *Avicennia marina, A. officinalis, Bruguiera sexangula, Excoecaria agallocha, Lumnitzera racemosa,* and *Rhizophora apiculata*, was evaluated against antibiotic resistant pathogenic bacteria, *Staphylococcus aureus* and *Proteus* sp. (Abeysinghe 2010). Extracts of petroleum ether, ethyl acetate, ethanol, and water were prepared and evaluated the antibacterial activity using agar diffusion method. Most of the plant extracts showed promising antibacterial activity against both bacterial species. However, higher antibacterial activity was observed for *Staphylococcus aureus* than *Proteus* sp. The highest antibacterial activity was shown by ethyl acetate of mature leaf extracts of *E. agallocha* for *Staphylococcus aureus*. All ethyl acetate extracts showed higher inhibition against *S. aureus*, while some extracts of

chloroform, ethyl acetate, and ethanol gave inhibition against *Proteus* sp. None of the petroleum ether and aqueous extracts showed inhibition against *Proteus* sp. (Abeysinghe 2010). All fresh plant materials did also show more antibacterial activity against both bacterial strains than did dried plant extracts. Antibacterial activity of fresh and dried plant materials reduced for both bacterial strains with time after extraction. Leaves of *L. racemosa* and *A. marina* gave the best inhibition for bacterial species. Charcoal-treated plant extracts of *L. racemosa* and *A. marina* were able to inhibit the bacterial strains more than those of untreated plant extract (Fig. 3) (Abeysinghe 2010). Phytochemical screening of mature leaf, bark of *L. racemosa*, and leaf extracts of *A. marina* has been carried out and revealed that leaf and bark contained alkaloids, steroids, triterpenoids, and flavonoids (Abeysinghe 2010). None of the above extracts indicate the presence of saponins and cardiac glycosides.

The antibacterial activity of fresh leaves of mangrove plants, *Avicennia marina* and *Rhizophora stylosa* against some pathogens, was also determined (Mouafi et al. 2014). Four extracts from *Avicenna marina* and *Rhizophora stylosa* leaves were prepared using water and different solvents including ethyl acetate, ethyl ether, and ethanol. Antimicrobial activities were tested against three bacterial pathogens (*Escherichia coli, Bacillus subtilis,* and *Staphylococcus aureus*) and three fungal species (*Penicillium digitatum*, *Fusarium oxysporum*, and *Candida albicans*). Ethyl acetate showed the best results as inhibition zones. Leaf extracts of *A. marina* and *R. stylosa* showed inhibition zones against most of the tested microorganisms, with the greatest effect being toward *Escherichia coli* and the lowest was against *Candida albicans* (Figs. 4 and 5) (Mouafi et al. 2014). The qualitative phytochemical analysis revealed that extracts of mature leaves were found to contain tannins, flavonoids, terpenoids, alkaloids, as well as steroids, phenolic flavonoids, and cardiac glycosides (Mouafi et al. 2014).

Antibacterial potential of some mangrove plants against isolated urinary tract infectious bacterial pathogens was reported (Ravikumar et al. 2010). Among the urinary pathogens, *E. coli* was predominant (41 %) followed by *P. aeruginosa* (25 %), *Klebsiella pneumoniae* (22 %), *Enterobacter* sp. (9 %), and *Staphylococcus*

Fig. 3 Growth inhibition by charcoal treated and untreated plant extracts (Abeysinghe 2010)

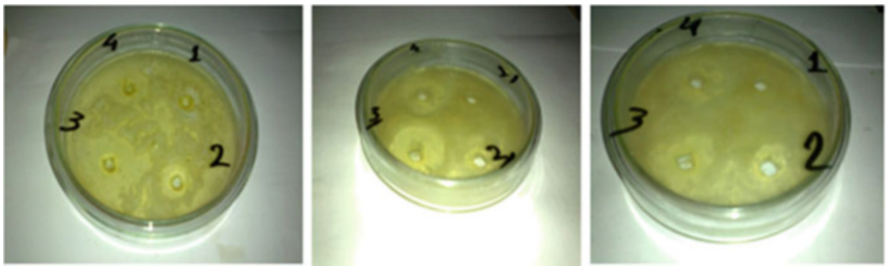

Fig. 4 Effect of water (1), ethyl ether (2), ethyl acetate (3), and ethanol (4) extracts on inhibition of: *E. coli* (*left image*), *S. aureus* (*middle image*), and *B. subtilis* (*right image*) (Mouafi et al. 2014)

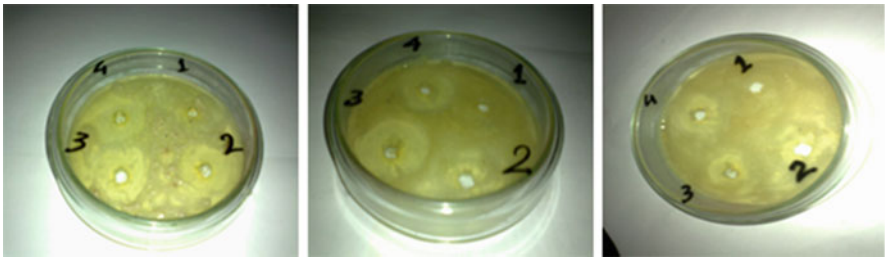

Fig. 5 Effect of water (1), ethyl ether (2), ethyl acetate (3), and ethanol (4) extracts on inhibition of: *F. oxysporum* (*left image*), *P. digitatum* (*middle image*), and *C. albicans* (*right image*) (Mouafi et al. 2014)

aureus (3 %). Bark extract of *A. marina* showed maximum zone of inhibition and hypocotyl of *B. cylindrica* and *R. apiculata* showed minimum zone of inhibition against *S. aureus*. The antibacterial activity of ethanolic extracts of five mangroves plants/parts was also evaluated (Ravikumar et al. 2010). Results revealed that *R. mucronata* showed greatest antibacterial activity (28 %) against isolated urinary tract infectious bacterial pathogens, followed by *A. marina* (27 %), *C. decandra* (18 %), *B. cylindrica* (16 %), and *R. apiculata* (11 %) (Ravikumar et al. 2010). On the other hand, hypocotyls (38 %) showed highest antibacterial activity followed by bark (34 %), collar (22 %) and least activity was recorded in flower (Ravikumar et al. 2010).

Phytochemicals such as alkaloids, triterpenoids, flavonoids, saponins, tannins, steroids, cardiac glycosides, and phenolic flavonoids are the major classes of antimicrobials and antioxidants in plants and are an indicative for such activities (Jadhave et al. 2013). A potent medicinal effect of plant materials may result from the combinations of antimicrobials, antioxidants, and other secondary products present in the plant, as well as phytochemicals (Jadhave et al. 2013). Such secondary products also play an important role in plant's defense through cytotoxicity toward microbial pathogens and this could prove the usefulness of these secondary products as antimicrobial medicines for humans (Patra and Mohanta 2014).

Table 1 Biological role of some phytochemicals included in the present study

Phytochemical	Biological role	References
Phenolic-flavonoids	Aggressively reacts with free radicals for prevention or treatment of skin aging in humans. Include a large group of several hundred chemical compounds that affect the taste, color, and mouthfeel of wine.	Podda and Grundmann-Kollmann (2001)
Alkaloids	Many alkaloids are still used in medicine, usually as antitumor, antihypertensive, muscle relaxant, and antiprotozoal agent.	Aniszewski (2007), Manske (1995)
Steroids	Have a potential effect as antioxidant and inflammatory drug. Have an ensure effect on hormonal balance.	Moss (1989)
Flavonoids	Have antioxidant properties that may lower the risk of some diseases.	Harborne (1984)
Tannins	Have diverse effects on biological system due to the potential metal ion chelators of tannins and biological antioxidants. Tannins are known to possess antimicrobial properties.	Tukiran (2013), Hagerman (2002)
Terpenoids	Terpenoids are used as a purgative for cough treatment and asthma.	Edeoga et al. (2005)
Cardiac glycosides	Drugs used in the treatment of congestive heart failure and cardiac arrhythmia. These glycosides are found as secondary metabolites in several plants.	McMurray and Pfeffer (2005)

Mangrove plants are biochemically unique, produce a wide array of novel natural products, and possess novel agrochemical products and compounds of medicinal value. For a long period of time in history, plants have been valuable and indispensable sources of natural products for the health of human beings and they have a great potential for producing new drugs (Patra and Mohanta 2014). Chemical components extracted from mangroves are used mainly in medicine (Table 1) as insecticides and pesticides (Bandaranayake 2002). Mangrove plants are widely used for herbal medicine, skin disorders, rheumatism, small pox, ulcers, hepatitis, leprosy, asthma, snake bites, toothache, diabetic, and as purgatives (Prabhakaran and Kavitha 2012). Investigations have led, so far, to the discovery of several novel compounds with prospective medicinal value as new chemotherapeutic agents. Chemical components of most mangrove plants still, however, need to be extensively studied.

7 Endophytic Fungi in Mangrove Plants

Pathogenic microorganisms are, increasingly, showing resistance against antimicrobial agents. As a consequence, search for antimicrobial metabolites from natural products are preferred as an alternative for many synthetic chemicals (Laurent et al. 2013; Shiv et al. 2014). In general, many antimicrobial compounds

are found in plants, and methods for extracting these compounds on large-scale production are reported. Endophytic fungi reside in the tissue of living mangrove plants and are a source of potential, valuable, and useful metabolites. Targeting endophytic fungi is, however, one of the most suitable resources and alternative approach for chemically synthesized antimicrobial compounds (Puri et al. 2006; Saravanakumar and Kathiresan 2014). Endophytic fungi exist mainly in roots, leaves, and stems of living tissues of different plants, establishing mutual relationship without showing in fact any symptom of diseases (Shiv et al. 2014). Over more than 20 years, the endophytic fungi have been explored as "biofactories" of novel bioactive substances, and they have not disappointed. Among the extracts and pure substances obtained from the culture broths or fungal biomass, some have exerted antibacterial activity ranging from moderate to powerful when tested on the bacterial strains resistant to the antibiotics (Natasa and Borut 2012).

Several hundred of fungi have been examined, and it is estimated that over one million endophytic fungi, in association of many plants, exist in nature (Natasa and Borut 2012). A total of 385 crude extracts from 150 fungal endophytes from mangrove plants were tested for antimicrobial activity (Buatong et al. 2011). The success for naturally occurring therapeutic agents depends on fractionation and purification procedures. Technique of careful fractionation of the culture broth and mycelium extract led to the isolation of the metabolite responsible for the antibacterial activity (Shiv et al. 2014). In this context, results of antibacterial testing of crude extracts and purified substances have been obtained from different endophytic fungi. Two new antimicrobial compounds (secondary metabolites) were obtained from extracts of the endophytic fungus *Alternaria* sp., isolated from the mangrove plant *Sonneratia alba* collected in China (Kjer et al. 2009). These metabolites showed activity against *E. faecalis*, *P. aeroginosa*, and *S. epidermidis*.

Mangrove endophytic fungi seem to play a major role in meeting the general demand for new biologically active substances. During the course of screening for biologically active secondary metabolites from marine microorganisms, an antibiotic compound containing an indole and a diketopiperazine moiety was isolated from the culture medium of *Penicillium chrysogenum* (MTCC 5108), an endophytic fungus on the mangrove plant *Porteresia coarctata* (Prabha et al. 2012). The cell free culture medium of *P. chrysogenum* showed significant activity against *Vibrio cholerae* (MCM B-322), a pathogen causing cholera in humans. Bioassay-guided chemical characterization of the crude extract led to the isolation of a secondary metabolite possessing a molecular formula $C_{19}H_{21}O_2N_3$. Its antibacterial activity was comparable with standard antibiotic, streptomycin.

A survey in leaves of mangrove plants (*Avicennia schaueriana, Laguncularia racemosa*, and *Rhizophora mangle*) was performed in Brazil (Isabella et al. 2012). Leaves were collected, during two seasons, dry and rainy, superficially sterilized and fragments maintained in Petri dishes with Potato dextrose agar at 28 ± 2 °C until isolation of the fungi. Leaves of *L. racemosa* hosted the highest number of colony-forming units. *Guignardia* sp. and *Colletotrichum gloeosporioides* were the most frequently isolated, while *Glomerella cingulata* was the only species found in association with the three host plants. The similarity of fungi species between the

two seasons reached only 4.2 %. *Chloridium virescens* var. *virescens, Microsphaeropsis arundinis, Penicillium pinophilum, Periconia cambrensis, Phoma herbarum, P. diachenii, P. obscurans,* as well as *Sphaerosporium, Sordaria prolifica,* and *Torula elisii* are reported for the first time as endophytic in tropical regions (Isabella et al. 2012).

A new compound named botryosphaerin F, along with other three known compounds, has been isolated from the mangrove fungus *Aspergillus terreus* (No. GX7-3B). The hypothetical biogenic relationship of four sesquiterpene analogues was described (Mangroves: tea mangrove Pelliciera rhizophorae). Furthermore, in the cytotoxicity assays, compound botryosphaerin F showed potent inhibiting activity toward MCF-7 and HL-60 cancer cell lines with 50 % inhibition of cell growth (IC_{50}) values of 4.49 and 3.43 μM, respectively (Deng et al. 2013).

8 Mangrove Tea

Mangrove Tea (*Pelliciera rhizophorae*) belongs to the "true mangroves" and is one of the most attractive, unique, and rarest mangroves ever. *Pelliciera rhizophorae* grows along the coast of Central America (Mangroves: tea mangrove Pelliciera rhizophorae). If the right conditions like nutritious soils, high humidity of 80–90 %, a lot of intense light, as well as air temperature of 25–30 °C are provided, *Pelliciera rhizophorae* grows which can reach up to 20 m in height (Fig. 6) (Mangroves: tea mangrove Pelliciera rhizophorae). The leather-like leaves of *Pelliciera rhizophorae* reach a length of 20 cm and more and the width is up to 5 cm. The surface and the bottom side are smooth, and only on the edges of the leaves are some small hairs (Fig. 6). The ripped brown fruit of *Pelliciera rhizophorae* is most of the time about 10 cm in diameter. The fruit of the Mangrove Tea contains exactly one viviparous seed which is just a little bit smaller than the fruit itself (Mangroves: tea mangrove Pelliciera rhizophorae). The seed is surrounded by a 3–5 mm thin protection layer (Fig. 6). The leaves of this mangrove contain tannins and other substances found in tea. Furthermore, its visual appearance resembles strongly to a leaf of a tea plant (Mangroves: tea mangrove Pelliciera rhizophorae). This is why the *Pelliciera rhizophorae* also called Mangrove Tea was considered part of the Tea Plant family for over than a century. Mangrove Tea grows in the intertidal zone in which the oxygen-poor soils offer high salinity and sometimes trace metals or other pollutants. Most of the time, these pollutants as well as the salt are excluded by the roots of the mangrove.

Black tea extracted from the mangrove plant *Ceriops decandra* has proved to possess potential oral anticancer effect (Ktahiresan and Thangam 1991). Effect of mangrove tea from *Ceriops decandra* plant on salivary bacterial flora in DMBA-induced hamster buccal pouch carcinoma was investigated (Natarajan et al. 2011). Tea mangrove was administered against DMBA-induced buccal pouch carcinoma in hamster rats and appropriate control animals were maintained. After 14 weeks of treatment, bacterial species in saliva were enumerated and tumor incidences and

Fig. 6 Mangrove tea plant: *Pelliciera rhizophorae*, height: up to 20 m (*left image*). The leather-like leaves of *Pelliciera rhizophorae* reach a length of 20 cm and more and the width is up to 5 cm. The surface and the *bottom side* are smooth, and only on the edges of the leaves are some small hairs (*middle image*) (Mangroves: tea mangrove Pelliciera rhizophorae)

volume were analyzed. The counts of beneficial and harmful bacteria were determined. Results revealed that the tea extract from *C. decandra* prevents the oral cancer incidences and maintain good health conditions of the animals (Natarajan et al. 2011). Salivary bacterial species of the test animals were detected. The predominant types of bacteria isolated from the saliva, tongue, dorsum, and buccal mucosa were *Streptococcus* spp., *Lactobacillus* spp., and *Bifidobacteria* (Natarajan et al. 2011). Lactic acid bacteria and *bifidobacteria* are two well-known groups of beneficial bacteria which constitute an integral part of the health condition. They impart nutritional and therapeutic benefits to their host. The vitamins and enzymes produced by the lactic acid bacteria contribute to host metabolism. The antimicrobial substances produced by these bacteria control the proliferation of undesired pathogens. Lactic acid bacteria produce a soluble compound which may interact directly with oral tumor cells in culture and inhibit their growth (Hirayama and Rafter 2000). Data from epidemiological and experimental studies indicate that ingestion of *lactobacilli* and *bifidobacteria* and their fermented products reduce the risk of certain types of cancer and inhibit tumor growth (Natarajan et al. 2011; Mitsuoka 1990).

Acanthus ilicifolius (Acanthaceae) has received considerable attention due to its wide range of secondary metabolites and its traditional usage in Indian and Chinese system of medicine (Singh and Aeri 2013; Joel and Valentin 2013). This plant is reported to be a rich source of steroids, glycosides, saponins, flavonoids, alkaloids, and tannins (Joel and Valentin 2013). Traditionally, the plant has been used for dyspepsia, paralysis, asthma, headache, skin diseases, and in medicine for rheumatic complaints (Singh and Aeri 2013; Sharma 2014). Recent developments in phytochemical and pharmacological studies explore the use of these plants like anticancer activity, antidiabetic activity, anti-inflammatory activity, etc. (Singh and Aeri 2013). This plant is promising to be used for production of a healthy tea "Mangrove tea", Fig. 7 (Sharma 2014), which contains natural compounds with

Fig. 7 The mangrove plant: *Acanthus ilicifolius* will be applied for a healthy tea drink "Mangrove Tea" (Sharma 2014)

free radical scavenging activity that combat diseases associated with stress. *Acanthus ilicifolius* plant is reported to possess antiulcer activity, antifungal activity, osteoblastic activity, antileishmanial activity, antimicrobial activity, anti-inflammatory activity, antidiabetic activity, anticancer activity, and antioxidant activities (Sharma 2014).

Conflict of Interest Statement The authors declare that there is no conflict of interest.

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Health Benefits and Possible Risks of Herbal Medicine

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Abstract Nowadays, attention is being focused on the investigation of the efficacy of plant in the traditional medicine because they are cheap and have little side effects. Synthetic preservatives, which have been used in foods for decades, may lead to negative health consequences. Moreover, the use of synthetic compounds has significant drawbacks, such as increasing cost, handling hazards, concerns about residues on food, and threat to human environment. As a good alternative, spices and herbs replace synthetic preservatives as natural, effective, and non-toxic compounds. Spices and herbs (garlic, mustard, cinnamon, cumin, clove, thyme, basil, pepper, ginger, rosemary, etc.) have been used as food additives since ancient times, as flavoring agents and natural food preservatives. A number of spices show antimicrobial activity against different types of microorganisms. The consumption of herbal medicines is increasing steadily throughout the world as an alternative treatment for alleviating a number of health problems including heart diseases, diabetes, high blood pressure, and even certain types of cancer. However, unlike drugs, herbal products are not regulated for purity and potency. Herbal drugs are considered as food integrators and readily available in the market without prescription. This chapter highlights potential benefits and possible risks associated with consumption of herbal products. Antimicrobial activity of spices and herbs as well as some essential oils against most common bacteria and fungi that contaminate food is also discussed.

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1 Introduction

Pain is the most important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause; generation of reactive oxygen species and other radicals can damage proteins, carbohydrates, polyunsaturated fatty acids, and DNA, and may thus lead to oxidative stress and to a variety of degenerative processes and diseases such as aging, immune deficiencies, neurologic disorders, inflammation, arthritis, ischemia, arteriosclerosis, coronary heart disease, stroke, diabetes mellitus, Parkinson's disease, Alzheimer's disease, and certain cancers (Kumpulainen and Salonen 1999). Reactive oxygen species are continuously produced during normal physiologic events and removed by antioxidant defense mechanisms (Halliwell and Gutteridge 1999). Therefore, the great interest has been recently focused on the natural foods, medicinal plants, and phytoconstituents due to their well-known abilities to scavenge free radicals (i.e., antioxidant power). Nowadays, attention is being focused on the investigation of the efficacy of plant in the traditional medicine because they are cheap and have little side effects.

In recent years, consumers have become more concerned about the processed food they eat. Synthetic preservatives, which have been used in foods for decades, may lead to negative health consequences. Besides, the use of synthetic compounds has significant drawbacks, such as increasing cost, handling hazards, concerns about residues on food, and threat to human environment. Therefore, there has been increasing interest to replace synthetic preservatives with natural, effective, and non-toxic compounds. Those are, in the first place, extracts and essential oils of spices and herbs (Smid and Gorris 1999). As natural foodstuffs, spices and herbs appeal to all who question safety of synthetic food additives and demand high-quality products that at the same time are safe and stable. Spices and herbs have been added to food since ancient times, not only as flavoring agents but also as folk medicine and food preservatives. Spices occupy a prominent place in the traditional culinary practices and are indispensable part of daily diets of millions of people all over the world. They are essentially flavoring agents used in small amounts and are reported to have both beneficial effect and antimicrobial properties. Nowadays, plenty of spices and herbs are valued for their antimicrobial activities and medicinal effects in addition to their flavor and fragrance qualities (Shan et al. 2007). This chapter highlights uses and adverse effects of herbal medicine as well as some possible risks. One should consider some herbal products for the possible risks while using in cure and treatments so that conventional treatments can be made more safe and effective. Some common herbs and their uses are discussed below.

Fig. 1 The Ebers Papyrus (*left image*, ca. 1550 BCE) from Ancient Egypt has a prescription for *Cannabis sativa* (marijuana) applied topically for inflammation (*left image*); Materia Medica (in Arabic) describes medicinal features of cumin and dill (*middle image*); Dioscorides De Materia Medica Byzantium fifteenth century (*right image*)

2 History

2.1 Ancient Times

In the written record, the study of herbs dates back over 5000 years to the Sumerians, who created clay tablets with lists of hundreds of medicinal plants such as myrrh and opium (Sumner 2000). Ancient Chinese and Egyptian papyrus writings describe medicinal uses of plants as early as 3000 BC. Ancient Egyptians wrote the Ebers Papyrus (Fig. 1, left), which contains information on over 850 plant medicines, including garlic, juniper, cannabis, castor bean, aloe, and mandrake (Sumner 2000).

2.2 Middle Age

Benedictine monasteries were the primary source of medical knowledge in Europe and England during the Early Middle Ages. However, most of these monastic scholars' efforts were focused on translating and copying ancient Greco-Roman and Arabic works, rather than creating substantial new information and practices (Arsdall 2002). Many Greek and Roman writings on medicine, as on other subjects, were preserved by hand copying of manuscripts in monasteries. The monasteries thus tended to become local centers of medical knowledge, and their herb gardens provided the raw materials for simple treatment of common disorders. At the same time, folk medicine in the home and village continued uninterrupted, supporting numerous wandering and settled herbalists. Medical schools known as Bimaristan began to appear from the ninth century in the medieval Islamic world among Persians and Arabs, which was generally more advanced than medieval Europe at the time (Fig. 1, middle). The Arabs venerated Greco-Roman culture and learning and translated tens of thousands of texts into Arabic for further study (Castleman

2001). As a trading culture, the Arab travelers had access to plant material from distant places such as China and India. Herbals, medical texts, and translations of the classics of antiquity filtered in from east and west. Muslim botanists and Muslim physicians significantly expanded on the earlier knowledge of materia medica. For example, al-Dinawari described more than 637 plant drugs in the ninth century (Moldão-Martins et al. 2000), and Ibn al-Baitar described more than 1400 different plants, foods, and drugs, over 300 of which were his own original discoveries, in the thirteenth century (Diane 2002).

The use of plants as medicines predates written human history. Ethnobotany (the study of traditional human uses of plants) is recognized as an effective way to discover future medicines. In 2001, researchers identified 122 compounds used in modern medicine which were derived from "ethnomedical" plant sources; 80 % of these have had an ethnomedical use identical or related to the current use of the active elements of the plant (Fabricant and Farnsworth 2001).

2.3 Early Modern Era

The fifteenth (Fig. 1, right), sixteenth, and seventeenth centuries were the great age of herbals, many of them available for the first time in English and other languages rather than Latin or Greek. The two best-known herbals in English were *The Herball or General History of Plants* (1597) by John Gerard and *The English Physician Enlarged* (1653) by Nicholas Culpeper. Gerard's text was basically a pirated translation of a book by the Belgian herbalist Dodoens and his illustrations came from a German botanical work. The Age of Exploration and the Columbian Exchange introduced new medicinal plants to Europe. The *Badianus Manuscript* was an illustrated Mexican herbal written in Nahuatl and Latin in the sixteenth century (Gimmel 2008).

2.4 Modern Herbal Medicine

The use of herbs to treat disease is almost universal among non-industrialized societies (Edgar et al. 2002). Development of chemical and phytochemical analysis has led to the increasing use of herbal medicine for the treatment of human diseases. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis, and quinine.

The World Health Organization estimates that 80 % of the populations of some Asian and African countries presently use herbal medicine for some aspect of primary health care. The use of, and search for, drugs and dietary supplements derived from plants has accelerated in recent years. Pharmacologists,

microbiologists, botanists, and natural products chemists are combing the Earth for phytochemicals and "leads" that could be developed for treatment of various diseases. In fact, according to the World Health Organization, approximately 25 % of modern drugs used in the United States have been derived from plants. Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80 % show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived (Fabricant and Farnsworth 2001). At least 7000 medical compounds in the modern pharmacopoeia are derived from plants. In many medicinal and aromatic plants, significant variations of plants characteristics have been correlated with varying soil nature. Great attention must be paid to choose soil and cropping strategies, to obtain satisfactory yields of high quality and best-priced products, respecting their safety and nutritional value (Carrubba and Scalenghe 2012).

3 Background Information

The use of plants for healing purposes predates human history and forms the origin of much modern medicine. Many conventional drugs originate from plant sources: a century ago, most of the few effective drugs were plant based (Vickers and Zollman 1999). Examples include aspirin (from willow bark), digoxin (from foxglove), quinine (from cinchona bark), and morphine (from the opium poppy). The development of drugs from plants continues, with drug companies engaged in large-scale pharmacological screening of herbs. Modern Western Herbalism emphasizes the effects of herbs on individual body systems (Vickers and Zollman 1999). For example, herbs may be used for their supposed anti-inflammatory, hemostatic, expectorant, antispasmodic, or immune-stimulatory properties.

3.1 Definition of Herbal Medicine

Herbal Medicine is called "Botanical or Phytotherapy," and it refers to using a plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. A botanical is a plant or plant part valued for its medicinal or therapeutic properties, flavor, and/or scent. Herbs are a subset of botanicals. Products made from botanicals that are used to maintain or improve health may be called herbal products, botanical products, or phytomedicines. They are sold as tablets, capsules, powders, teas, extracts, and fresh or dried plants. People use herbal medicines to try to maintain or improve their health. Many people believe that products labeled "natural" are always safe and good for them. This is not necessarily true. Herbal medicines do not have to go through the testing that drugs do. Some herbs, such as comfrey and ephedra, can cause serious harm. Some herbs can interact with prescription or over-the-counter medicines. People who are thinking about using

herbal medicine should first get information on it from reliable sources (Ernst 1998).

3.2 Herbal Medicine

Herbal medicine is also the study and use of medicinal properties of plants. Alternative medicine is a practice of consuming a medicine without the use of drugs. The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals and shells. Each type of medicine has many strengths and weaknesses. Herbal drugs are used widely for therapeutic purposes. However, herbal products are not regulated for purity and potency. Thus, some of the adverse effects reported could be caused by impurities. The potency of herbal products may increase the possibility of adverse effects. Potential benefits and possible risks associated with consumption of herbal product should be considered so that conventional treatments can be made more safe and effective (Kaur et al. 2013). Herbal medicines can be used as self-care options for sleep, stress, and mood disorders to digestive ailments, colds, and flu. Herbal medicine is also used to treat many conditions, such as asthma, eczema, premenstrual syndrome, rheumatoid arthritis, migraine, menopausal symptoms, chronic fatigue, irritable bowel syndrome, and cancer, among others. Herbal supplements are best taken under the guidance of a trained healthcare provider. For example, one study found that 90 % of arthritic patients use alternative therapies, such as herbal medicine. Nowadays attention is being focused on the investigation of the efficacy of plant in the traditional medicine because they are cheap and have little side effects. Herbal medicine is used to treat many diseases. For example, the bark of willow trees contains large amounts of salicylic acid, which is the active metabolite of aspirin (Lichterman 2004). Willow bark has been used for millennia as an effective pain reliever and fever reducer. Dandelion (*Taraxacum officinale*) contains a large number of pharmacologically active compounds and has been used for centuries as an effective laxative and diuretic and as a treatment for bile or liver problems (Schutz et al. 2006). Essential oil of Thyme (*Thymus vulgaris*) contains 20–54 % thymol, which possesses antiseptic and antifungal properties, used in a variety of products (Pierce 1999), and also used for the treatment of respiratory infections.

3.3 Biological Background

All plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into (1) primary metabolites such as sugars and fats, which are found in all plants; and (2) secondary metabolite compounds which are found in a smaller range of plants, serving a more specific function (Meskin 2002). For example, some secondary metabolites are toxins used to deter predation

and others are pheromones used to attract insects for pollination. Secondary metabolites and pigments can also have therapeutic actions in humans and which can be refined to produce drugs, examples are inulin from the roots of dahlias, quinine from the cinchona, morphine and codeine from the poppy, and digoxin from the foxglove (Meskin 2002).

Plants synthesize a variety of phytochemicals such as alkaloids, phenolics, glycosides, and terpenes (Springbob and Kutchan 2009):

1. Alkaloids
   Alkaloids are a class of chemical compounds containing a nitrogen ring. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products (also called secondary metabolites). Many alkaloids can be purified from crude extracts by acid–base extraction and they are toxic to other organisms. They often have pharmacological effects and are used as medications and as recreational drugs. Examples are the local anesthetic and stimulant cocaine, caffeine, and nicotine, the analgesic morphine, the anticancer compound vincristine, the antihypertension agent reserpine, the antiarhythmia compound quinidine, the antiasthma therapeutic ephedrine, and the antimalarial drug quinine. Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste.
2. Polyphenols
   Polyphenols are compounds that contain phenol rings and known also as phenolics. The anthocyanins that give grapes their purple color, the isoflavones, the phytoestrogens from soy, and the tannins that give tea its astringency all are phenolics.
3. Glycosides
   Glycosides are molecule in which a sugar is bound to a non-carbohydrate moiety, usually a small organic molecule. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides which can be activated by enzyme hydrolysis to break the sugars and making valuable chemicals available for use. Many such plant glycosides are used as medications. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body.
4. Terpenes
   Terpenes are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers, which are often strong smelling and thus may have had a protective function. They are the major components of resin and of turpentine produced from resin (the name "terpene" is derived from the word "turpentine"). Terpenes are major biosynthetic building blocks within nearly every living creature. Steroids, for example, are derivatives of the triterpene squalene. When terpenes are modified chemically, i.e., by oxidation or rearrangement of the carbon skeleton, the resulting compounds are generally referred to as *terpenoids*. Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and flowers. Essential oils are used

widely as natural flavor additives for food, as fragrances in perfumery, and in traditional and alternative medicines such as aromatherapy. Synthetic variations and derivatives of natural terpenes and terpenoids also greatly expand the variety of aromas used in perfumery and flavors used in food additives. The fragrance of rose and lavender is due to monoterpenes.

4 Healthy Herbs

The aim of herbal treatment is usually to produce persisting improvements in well-being. As indicated above, plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators such as insects, fungi, and herbivorous mam mals. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans and can be used to effectively treat human diseases. At least 12,000 such compounds have been isolated so far, a number estimated to be less than 10 % of the total (Tapsell et al. 2006). Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus, herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines but also gives them the same potential to cause harmful side effects (Lai and Roy 2004).

4.1 Differences Between Herbal and Conventional Drugs

Herbal medicine and pharmacotherapy have three important differences.

Use of Whole Plants Herbalists generally use unpurified plant extracts containing several different constituents. They claim that these can work together synergistically so that the effect of the whole herb is greater than the summed effects of its components. They also claim that toxicity is reduced when whole herbs are used instead of isolated active ingredients (“buffering”). Although two samples of a particular herbal drug may contain constituent compounds in different proportions, practitioners claim that this does not generally cause clinical problems (Vickers and Zollman 1999). There is some experimental evidence for synergy and buffering in certain whole plant preparations, but how far this is generalizable to all herbal products is not known.

Herb Combining Often, several different herbs are used together. Practitioners claim that combining herbs improves efficacy and reduces adverse effects. This contrasts with conventional practice, where polypharmacy is generally avoided whenever possible (Vickers and Zollman 1999).

Diagnosis Herbal practitioners use different diagnostic principles from conventional practitioners. For example, when treating arthritis, they might observe "under-functioning of a patient's systems of elimination" and decide that the arthritis results from "an accumulation of metabolic waste products." A diuretic, choleretic, or laxative combination of herbs might then be prescribed alongside herbs with anti-inflammatory properties (Vickers and Zollman 1999).

In contrast to conventional medications, unconventional treatments (such as herbs) have little or no actual scientific basis so doctors can guide their patients regarding proper usage or potential toxicity. There are no standardized references and most of the herbal formulations have not been analyzed, are not uniform, and have not been quality controlled. One batch can be very different from the next. Moreover, even if a given herb has a known toxicity, the manufacturer may or may not warn consumers. Manufacturers are not required to alert consumers to known dangers.

4.2 Differences Between Herbs and Spices

The terms "spice" and "herb" have both been used to describe parts of plants (possibly dried) that are used to enhance the flavor or taste of food. Spices and herbs are parts of plants (fresh or dried). In addition, herbs have been used to augment cosmetics, preserve foods, and cure illnesses. The difference between the two is where they are obtained from a plant. ***Herbs*** come from the leafy and green part of the plant, while ***Spices*** are parts of the plant other than the leafy bit such as the root, stem, bulb, bark, or seeds. Examples of herbs include basil, oregano, thyme, rosemary, parsley, and mint. Spices are usually dried before being used to season foods. Some examples for spices are cinnamon, cloves, ginger, and pepper. Unlike herbs, they are grown in more tropical countries. Spices have also been known to preserve foods and some have medicinal value, such as turmeric with its anti-inflammatory and antifungal properties.

Over time, the definitions for spices and herbs have changed a bit. In the past, spices have been categorized as fragrant, aromatic plant products like cinnamon, cloves, ginger, and pepper and are found in plants grown in tropical and subtropical regions of the world. While herbs have always been recognized as the most green, leafy products like mint, rosemary, and thyme are grown in more temperate areas. Some plants are both herbs and spices. The leaves of *Coriandrum sativum* are the source of cilantro (herb), while coriander (spice) is from the plant's seeds. Dill is another example. The seeds are a spice, while dill weed is an herb derived from the plant's stems and leaves. Examples of herbs are thyme, sage, oregano, parsley, marjoram, basil, rosemary, and mint; while examples of spices are cinnamon (bark of the cinnamon tree), ginger/root, cloves/flower bud, saffron—stigma (female reproductive part) of saffron crocus, nutmeg/seed, and cumin/seeds (Kaur et al. 2013).

4.3 Nutritive Value and Possible Side Effects

Herbs and spices are vital ingredients in many dishes. They add flavor, aroma, color, texture, and even nutrients. On other hand, however, the major perception for the use of herbal drugs is that "they are safe because they are natural and have fewer side effects than prescription drugs". Often, herbs may be used together because the combination is more effective and may have fewer side effects. However, various studies have highlighted their possible side effects, if taken irregularly, in excessive amounts or in combination with some medicines (Stickel et al. 2005). Interaction between drugs and herbs can cause undesired effects. Tables 1 and 2 represent the uses and side effects of some selected herbs and spices to be used safely.

5 Ingredients in Healthy Herbs

Different clinical tests revealed that many herbs have shown positive results in vitro, animal model, or small-scale clinical tests (Srinivasan 2005). In a 2010 survey of 1000 plants, 356 had clinical trials published evaluating their "pharmacological activities and therapeutic applications," while 12 % of the plants, although available in the Western market, had "no substantial studies" of their properties (Cravotto et al. 2010). Herbalists criticize the manner in which many scientific studies make insufficient use of historical knowledge, which has been shown useful in drug discovery and development in the past and present (Fabricant and Farnsworth 2001). They maintain that this traditional knowledge can guide the selection of factors such as optimal dose, species, time of harvesting, and target population. In many cases, scientists are not sure what specific ingredient in a particular herb works to treat a condition or illness.

Whole herbs contain many ingredients, and they may work together to produce a beneficial effect. Many factors determine how effective an herb will be. For example, the type of environment (climate, bugs, soil quality) in which a plant grew will affect it, as will how and when it was harvested and processed (Yarnell and Abascal 2002). Culinary herbs such as garlic and ginger, rosemary, and peppermint are a great addition to food, not just because they add special flavor and spicy taste to our food, but also they contain many antimicrobial and antioxidant substances that protect foods. Healthy herbs are used in small amounts; actually, they provide flavor rather than substance to food. Some herbal leaves and plant parts are increasingly been used as popular flavor drinks.

Healthy herbs possess unique features. Herbs contain (1) antioxidants, essential oils, vitamins, phytosterols, and many other plant-derived nutrient substances, which help equip our body to fight against germs and toxins and to boost immunity level. Herbs are, in fact, medicines in smaller dosages; (2) essential oils in herbs have been found to have anti-inflammatory function by inhibiting the enzyme *cyclooxygenase*, which mediates inflammatory cascade reactions in the body. The enzyme-inhibiting effect of essential oils in herbs makes it an important remedy for

Table 1 Benefits and possible side effects of some important herbs

Herb	Benefit	Side effect
Peppermint	Used for the cold, cough, sinus infections, and respiratory infections. It is also used for digestive problems including heartburn, nausea, vomiting, morning sickness, irritable bowel syn drome, cramps of the upper gastrointestinal tract and bile ducts, upset stomach, diarrhea, bacterial overgrowth of the small intestine, and gas. Pepper mint oil is applied to the skin for headache, muscle pain, nerve pain, toothache, inflammation of the mouth, joint conditions, itchiness, allergic rash, bacterial and viral infections, relaxing the colon during barium enemas	Peppermint can cause some side effects including heartburn and allergic reactions, including flushing, headache, and mouth sores. Large quantities of peppermint oil could damage the kidneys (Herbs: Benefits and Information. Herbwisdom.com)
Parsley	Parsley is used for urinary tract infec tions, kidney stones (nephrolithiasis), gastrointestinal disorders, constipation, jaundice, intestinal gas (flatulence), indigestion, colic, diabetes, cough, asthma, fluid retention (edema), osteo arthritis, "tired blood" (anemia), high blood pressure, prostate conditions, and spleen conditions. Protects against rheumatoid arthritis, antioxidant rich, fights cancer, and high in vitamin C and iron	In some people, parsley can cause allergic skin reactions. It is very high in oxalic acid, 1.70 mg per 100 g. Prolonged consumption of oxalate rich foods may result in gouty arthritis, kidney stones, and mineral nutrient deficiencies (Healthy herbs nutrition fact: www.nutrition-and-you.com)
Aloe vera	Anti-inflammatory, antiproliferative, antiaging, wound healing, recovery from burn injury, cell growth, and immune modulation	Hepatotoxicity (Yang et al. 2010), abdominal spasms, pain, allergic reactions, cramps, and kidney damage (Kaur et al. 2013)
Dill	Used for digestion problems, including loss of appetite, flatulence, and liver problems. It is also used for urinary tract disorders, including kidney disease and painful or difficult urination. Treatment of fever and colds, cough, hemorrhoids, infections, nerve pain, genital ulcers, and menstrual cramps	Dill can sometimes cause skin irritation. Fresh dill juice can also cause the skin to become extra sensitive to the sun (Herbs: Benefits and Information. Herbwisdom.com)
Rosemary	Accepted as a very powerful antioxidant, anti-inflammatory, antiviral, and antibacterial. Studies have also shown that carnosic acid in rosemary offers protection against harmful carcinogens and Alzheimer's disease	The undiluted oil is unsafe to take by mouth; taking large amounts can cause vomiting, uterine bleeding, kidney irritation, increased sun sensitivity, skin redness, and allergic reactions (Healthy herbs nutrition fact: www. nutrition-and-you.com)

(continued)

Table 1 (continued)

Herb	Benefit	Side effect
Celery	Celery is a functional food. Its leaves are rich source of flavonoid antioxidants which have been antioxidant, cancer protective, and immune-boosting functions. It is also rich in many vital vitamins, including folic acid, riboflavin, niacin, vitamins A and C, which are essential for optimum metabolism, as well as vitamin K which helps for increasing bone mass and has established role in Alzheimer's disease by limiting neuronal damage in the brain. A very good source of minerals and essential volatile oils that used for nervousness, osteoarthritis, and gouty arthritis conditions	It is very high in soluble and insoluble fiber contents. Eating recipes with too much of fibers may cause stomach pain, indigestion, and bloating and oftentimes complicates existing constipation condition (Healthy herbs nutrition fact: www.nutrition-and-you.com)
Dandelion	Fresh dandelion herb is one of the highest source of vitamin A among culinary herbs; it provides 10161 IU of vitamin-A per 100 g, that is antioxidant and required for maintaining healthy mucus membranes and skin. A good source of minerals and vital vitamins, including folic acid, riboflavin, pyridoxine, niacin, vitamins E and C, all are essential for optimum health. Rich in vitamin K which helps for increasing bone mass and has established role in Alzheimer's disease by limiting neuronal damage in the brain	In patients on potassium sparing diuretic therapy, it may aggravate potassium toxicity. Dandelion herb can also induce allergic contact dermatitis in some sensitive individuals (Healthy herbs nutrition fact: www.nutrition-and-you.com)

symptomatic relief in individuals with inflammatory health problems such as rheumatoid arthritis, osteoarthritis, and inflammatory bowel conditions like ulcerative colitis; (3) many unique compounds in the herbs have been found to reduce blood sugar levels in diabetics; (4) controlled-epidemiological studies have shown that certain compounds in garlic like thiosulfinates (*allicin*) can bring significant reduction in total cholesterol and in blood pressure and thereby help prevent coronary artery disease and stroke risk; (5) curcumin, together with other antioxidants in the turmeric, has been found to have antiamyloid and anti-inflammatory properties. Thus, it is thought to be effective in preventing or at least delaying the onset of *Alzheimer's disease*; (6) the volatile oils, vitamins, and antioxidants in the herbs have cytotoxicity action against prostate, pancreatic, colon, endometrial, and cancer cells; and (7) the chemical compounds in the herbs have been found to be antispasmodic, carminative, diaphoretic, analgesic, aphrodisiac, deodorant, digestive, antiseptic, lipolytic (fat and weight loss action), stimulant, and stomachic actions when taken in appropriate dosage (Healthy herbs nutrition fact: www.nutrition-and-you.com).

Table 2 Benefits and possible side effects of some important Spices

Spice	Benefit	Side effect
Turmeric	Antibacterial, anticancer, antifungal, antioxidant, hypoglycemic, colorant, antiseptic, and wound healer	Risk of bleeding or potentiate the effects of warfarin therapy (Heck et al. 2000).
Fennel	Carminative, aromatic, diuretic, and flavoring agent	Allergic reactions (Jensen-Jarolim et al. 1997), occupational rhinitis, asthma, conjunctivitis, and estrogenic activity
Cloves	Used in diarrhea, gas, bloating, intestinal spasms, nausea antioxidant, and effective pain reliever in toothache	Hemorrhagic pulmonary edema, pneumonia, bronchitis, hemoptysis central nervous system depression, and occupational allergic contact dermatitis (Kaur et al. 2013)
Nutmeg	Used as a flavoring agent for baked goods, desserts, and some beverages. Used medicinally as a digestive aid and to treat rheumatoid arthritis. Used for diarrhea, nausea, stomach spasms and pain, and intestinal gas. They are also used for treating cancer, kidney disease, and trouble sleeping (insomnia)	Side effects include nausea, vomiting, difficulty urinating, dizziness, constipation, dry mouth. More serious side effects might include hallucinations, seizures, and death (Venables et al. 1976)
Cardamom	Used for digestion problems including heartburn, intestinal spasms, irritable bowel syndrome, intestinal gas, constipation, liver and gallbladder complaints, and loss of appetite. It is also used for common cold, cough, bronchitis, sore mouth and throat, and tendency toward infection	Allergic contact dermatitis. The cardamom seed can trigger gallstone colic (Healthy herbs nutrition fact: www.nutrition-and-you.com)
Ginger	Useful in motion sickness, morning sickness, colic, upset stomach, gas, diarrhea, nausea caused by cancer treatment, arthritis or muscle soreness, menstrual pain, upper respiratory tract infections, cough, and bronchitis, chest pain, and stomach pain	Side effects include heartburn, diarrhea, and general stomach discomfort (Antoine 2007)
Thyme	Thyme is taken by mouth for whooping cough, sore throat, colic, arthritis, upset stomach, stomach pain (gastritis), diarrhea, intestinal gas (flatulence), parasitic worm infections, and skin disorders. It is also used to increase urine flow (as a diuretic), to disinfect the urine and as an appetite stimulant. Essential oil of thyme contains 20–54 % thymol which possesses antiseptic and antifungal properties, used in a variety of products, and used also for treatment of respiratory infections	It can cause digestive system upset. In some people, applying the oil to the skin can cause irritation. There is not enough information to know whether thyme oil is safe to take by mouth in medicinal doses (Moldão-Martins et al. 2000)

(continued)

Table 2 (continued)

Spice	Benefit	Side effect
Ginseng	Used for conditions of the heart and blood vessels. Used also for kidney disease, Alzheimer's disease, attention deficit-hyperactivity disorder, chronic fatigue syndrome, diabetes, rheumatoid arthritis, flu, colds, chronic bronchitis, and tuberculosis. It is also used for treating the side effects of cancer chemotherapy	Some people can have drowsiness, changes in heart rhythm, and muscle spasms. It could also cause skin rashes, asthma attacks, increased blood pressure, diarrhea, euphoria, and nervousness (Kaur et al. 2013)
Cinnamon	Cinnamon has antioxidant, antidiabetic, antiseptic, anti-inflammatory, warming, soothing, and carminative properties. It contains essential oils such as eugenol which is antiseptic and useful in dental and gum treatment. Active principles in this spice may increase the motility of the intestinal tract and help in the digestion power by increasing gastrointestinal enzyme secretions. An excellent source of minerals, vitamin A, and flavonoid phenolic antioxidants such as carotenes	Excessive use of the cinnamon stick may cause inflammation of taste buds, gum swelling, and mouth ulcers. Large quantities can cause difficulty breathing, dilate blood vessels, sleepiness, and depression (Healthy herbs nutrition fact: www.nutrition-and-you.com)

6 Health Claims

Safety must be the starting point in drug development strategies for herbal medicines. The consumption of herbal medicines is increasing steadily throughout the world as an alternative treatment for alleviating a number of health problems including heart diseases, diabetes, high blood pressure, and even certain types of cancer. In India, the use of herbal drugs is much more because of their easy accessibility (Kaur et al. 2013). Unlike drugs, herbal products are not regulated for purity and potency. There are no studies on their effectiveness or control over the quality and safety of these preparations. As per Food and Drug Administration mandates, only medicines have to be proven to be safe before being released into market. Herbal products do not fall under the category of medicine as long as they are not marketed for the prevention of any disease. Herbal drugs are considered as "food integrators" and readily available in the market without prescription. They are natural, safe, and have fewer side effects than prescription drugs. However, various studies have reported their possible side effects, if taken irregularly, in excessive amounts or in combination with some medicines (Stickel et al. 2005). A common problem with herb use is that people do not take into consideration how they may interact with any prescription drug they are taking, or with each other. Interaction between drugs and herbs can result in unexpected concentration of drugs and also cause undesired effects (Kaur et al. 2013).

7 Herbs and Spices in Ready-to-Eat Foods

"Ready-to-eat" is defined as the status of the food being ready for immediate consumption at the point of sale. It could be raw or cooked, hot or chilled, and can be consumed without further heat treatment including reheating (The Centre for Food Safety, Food and Environmental Hygiene Department 2007). In 2002, under the advice of the Expert Panel on Microbiological Safety of Food, an expert group setup has been developed by the Food and Environmental Hygiene Department to advise the Director of Food and Environmental Hygiene, a set of microbiological guidelines for ready-to-eat food (The Centre for Food Safety, Food and Environmental Hygiene Department 2007). These guidelines stipulate the safety limits of nine major food-borne pathogens such as *Salmonella* species, *Listeria monocytogenes*, *E. coli* O157, and *Vibrio cholerae*, as well as providing a classification of microbiological quality of ready-to-eat food for reflecting the hygienic status of the food concerned. In light of changing needs and latest expert views, the guidelines were revised in 2007. The revision mainly includes textural amendment of the Guidelines and revising the microbiological limits for *Listeria monocytogenes*, making reference to international practices (The Centre for Food Safety, Food and Environmental Hygiene Department 2007).

Use of plants as medicines predates written human history. Many of the herbs and spices used by humans to season food also yield useful medicinal compounds. Use of herbs and spices in cooking and in Ready-to-eat Foods is developed as a response to the threat of food-borne pathogens (The Centre for Food Safety, Food and Environmental Hygiene Department 2007; Mirriam et al. 2012). However, risks of using herbs vary widely depending on the ingredients and how they are used, whether they contain heavy metals, pesticides, pathogens, or other such contaminations, and whether they do cause a direct risk to public health (Bhushan 2005). Studies show that in tropical climates where pathogens are the most abundant, recipes are the most highly spiced. Further, the spices with the most potent antimicrobial activity tend to be selected (Billing and Sherman 1998). In all cultures, vegetables are spiced less than meat, presumably because they are more resistant to spoilage (Sherman and Hash 2001). Many of the common weeds such as nettle, dandelion, and chickweed also have medicinal properties (Stepp 2004).

A more recent study revealed that edible substances which are more environmental friendly, such as herbs and spices, could be efficient as antibiofilm that do not express undesirable effects. In this study, extracts of anise, lemon leaves, curry leaves, and clove were tested against *Vibrio parahaemolyticus* which exist in seafood. The results indicated that cloves have stronger activity against biofilm-producing resistant strains than other extracts (Elexson et al. 2013). Prevention of biofilm development in the food is a key to control and monitor the level of food safety, especially in seafood industries.

8 Safety

Many plants are highly toxic. Herbal medicine probably presents a greater risk of adverse effects and interactions than any other complementary therapy. There are case reports of serious adverse events after administration of herbal products. In most cases, the herbs involved were self-prescribed and bought over the counter or obtained from a source other than a registered practitioner. In the most notorious instance, several women developed rapidly progressive interstitial renal fibrosis after taking herbs (Vickers and Zollman 1999). As well as their direct pharmacological effects, herbal products may be contaminated, adulterated, or misidentified. In general, patients taking herbal preparations regularly should receive careful follow-up and have access to appropriate biochemical monitoring. As with many complementary therapies, information on the prevalence of adverse effects is limited. It would seem wise for pregnant women to avoid the use of herbal agents. Argue may continue regarding the relative safety of ginger versus conventional antinausea drugs, but there seems little doubt that other herbal agents offer no significant benefits and considerable potential risks (Philp 2007). Healthcare providers must take many factors into account when recommending herbs, including the species and variety of the plant, the plant's habitat, how it was stored and processed, and whether or not there are contaminants (including heavy metals and pesticides). Traditional herbal medicines have been registered for their labeled claims on the basis of traditional use for these conditions for at least 30 years. Traditional herbal medicines now on the market have been judged by experts to relatively safe (British Herbal Medicine Association. PO Box 583).

Although approximately 80 % of people today depend upon herbal medication as a component of their primary health care according to the World Health Organization, there is still great concern about the safety and efficacy of herbal use (Herbal Medicine. University of Maryland Medical Center. http://www.umm.edu/altmed/articles/herbal-medicine-000351.htm#ixzz1i9ZzqMkX). While herbal medicine can potentially contribute to the advancement of healthcare, many major challenges must be overcome prior to the successful integration of herbal remedies into mainstream medicine. Indeed, for the incorporation of safe and effective herbs into the medical system to become a reality, more researchers and doctors need to be trained in both modern medicine and herbal compendium that has been accumulated since ancient times.

9 Future of Herbal Medicine

Healthy herbs have long held an important place in our wellness. Prized since ancient times, and today we even more depend on them to purify our body, mind, and soul. The use of herbs to treat disease is almost universal among non-industrialized societies and is often more affordable than purchasing expensive

modern pharmaceuticals. Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become increasingly more in recent years as scientific evidence about the effectiveness of herbal medicine has become more widely available. In some countries in Europe, unlike the United States, herbs are classified as drugs and are regulated. The German Commission E, an expert medical panel, actively researches their safety and effectiveness. However, in India and Asia herbal medicine may still be harming and pose health risk to millions and many hazardous cases are recorded (Kaur et al. 2013). British Herbal Medicine Association was founded in 1964 to advance the science and practice of herbal medicine in the United Kingdom. It promotes the use of herbal medicinal products manufactured to pharmaceutical standards to ensure consistently high quality and effectiveness for the consumer (British Herbal Medicine Association. PO Box 583).

While still not widely accepted, herbal medicine is being taught more in medical schools and pharmacy schools. More healthcare providers are learning about the positive and potentially negative effects of using herbal medicines to help treat health conditions. Some healthcare providers, including doctors and pharmacists, are trained in herbal medicine. They can help people create treatment plans that use herbs, conventional medications, and lifestyle changes to promote health (Sanjoy and Shukla 2003). To gain public trust and to bring herbal product into mainstream of today's healthcare system, clinical trials should be applied by the researchers, manufacturers, and the regulatory agencies to ensure the quality and consistency of the traditional herbal products. Using modern technologies, the quality and consistency of the heterogeneous herbal products can be monitored. A well-designed clinical trial is the method of choice to prove the safety and effectiveness of a therapeutical product (Stephen 2007).

Basic uses of plants in medicine will continue in the future, as a source of therapeutic agents and as raw material base for the extraction of semi-synthetic chemical compounds such as cosmetics, perfumes, and food industries. Popularity of healthcare plant-derived products has been traced to their increasing acceptance and use in the cosmetic industry as well as to increasing public costs in the daily maintenance of personal health and well-being. In the dual role as a source of health care and income, medicinal plants make an important contribution to the larger development process (Sheetal and Singh 2008). It is a major responsibility of the regulatory authorities to ensure that the consumers get the medication, which guarantee with purity, safety, potency, and efficacy. The quality control of crude drugs and herbal formulations is of paramount importance in justifying their acceptability in modern system of medicine. But one of the major problems faced by the herbal drug industry is non-availability of rigid quality control profile for herbal material and their formulations.

10 Conclusion

Herbal medicine is a component of alternative treatment methods and it includes the use of different plants and their extracts due to their large benefits. It is one of the most effective and safe treatment options and is even getting recognition from practitioners of traditional medicine. However, herbal supplements can be beneficial to consumers, but they also can cause serious side effects and potentially dangerous conditions. Under a regulatory regime, the FDA can do little to protect consumers from these health risks. On other hand, food contamination is enormous public health problem, but it could be controlled by the use of natural preservatives such as essential oils obtained from spices. The fact that many essential oils possess antimicrobial activity has been proved by plenty of investigations in the recent years. The type and optimal concentration of essential oil depend on the product used and which species of bacteria or fungi should be used against. But if essential oils are expected to be widely applied as antibacterial and antifungal agents, the sensory impact should be considered because the use of naturally derived preservatives can alter the taste of food or exceed acceptable flavor thresholds. Therefore, research in this area should be focused on the optimization of essential oil combinations and applications to obtain effective antimicrobial activity at sufficiently low concentrations. It could be concluded that adverse effects of herbal medicines as well as their interactions with other prescription drugs should be known to the consumers and physicians. Herbal remedies under conventional therapy are known to show many benefits to humans, which is true but one should be fully familiar with their side effects at normal and large doses.

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Health Benefits of Trace Elements in Human Diseases

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Abstract Microorganisms are found almost everywhere and they are extremely adaptable to harsh conditions and survive wherever they are. Microorganisms are exploited by biotechnologists in traditional fortified foods, dairy foods, beverage preparation, and in modern technologies based on genetic engineering. However, there are many pathogenic microbes which are harmful and can cause death in human, plants, and animals. Some chronic non-communicable diseases such as diarrhea and respiratory diseases are well known to be caused by harmful microbes. Trace elements show a number of biochemical and physiological functions. Fortification of foods with traces of essential elements such as selenium, zinc, chromium, copper, silicon, as well as iron, nickel, and vanadium can prevent many of communicable and non-communicable diseases. Human health has a vital relationship with the balance of essential trace elements for the healthy functioning of human body. Supplementation with trace elements should be carefully controlled. When given in quantities exceeding those required for accomplishing their biological functions, they will cause toxic effects. The dietary reference intakes provided by national regulatory agencies are guides to define intake, supplementation, and toxicity of trace elements for humans.

1 Introduction

Consumption of a well-balanced diet meets mineral requirements and avoids deficiencies or imbalances. The Food and Nutrition Board recommended that supplements or fortified foods be used to obtain desirable amounts of some nutri-

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ents, such as calcium and iron. Individuals who generally consume lower energy diets and who do not consume dairy products can particularly benefit from calcium supplements. Because of many negative consequences of iron deficiency (anemia), iron supplementation is recommended for vulnerable groups, especially in developing countries (Wardlaw 1999). However, extra caution must be taken to avoid intakes greater than the recommended dosage for specific nutrients because of problems related to nutrient excesses, imbalances, or adverse interactions with medical treatments (Whitney and Rolfes 1996). Major minerals are those required in the amounts of 100 mg (milligrams) or more, while trace minerals are required in amounts of part per million a day. The terms *major* and *trace*, however, do not reflect the importance of a mineral in maintaining health, as a deficiency of either can be harmful (Whitney and Rolfes 1996). Minerals are necessary for three main reasons: (a) building strong bones and teeth; (b) controlling body fluids inside and outside cells; and (c) turning the food into energy (http://dining.unt.edu/nutrition/nutrition_brochures/Minerals.pdf).

Biological metals have been considered to play very important roles in the neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and idiopathic ganglia calcification (Isao 2011). Most nutritionists do not consider a trace element essential unless it has a defined biochemical function that would promote the health and well-being of humans. Some of the trace elements not established as essential but with beneficial health effects could be considered as bioactive (Forrest 2011). This includes boron, silicon, and nickel. Fruits, leafy vegetables, nuts, legumes, and pulses are rich in boron; unrefined grains and some vegetables are rich in silicon; and pulses, grains, nuts, and chocolate are rich in nickel. Appropriate intake of these foods would provide amounts of trace element that are beneficial for health and well-being (Forrest 2011). Of all the essential elements, the role of chromium (Cr) is the most controversial. Chromium was first proposed nearly 50 years ago. Recently, its status as an essential element has been challenged. The objective of this chapter is to reveal the importance and human health benefits of some essential major and trace elements for prevention of human diseases.

2 Minerals

Minerals are inorganic elements that originate in the earth and cannot be synthesized in the body. They play important roles in various bodily functions and are necessary to sustain life and maintain optimal health, and thus are essential nutrients. Most of the minerals in the human diet come directly from plants and water or indirectly from animal foods. However, the mineral content of water and plant foods varies geographically because of variations in the mineral content of soil from region to region (Wardlaw 1999). The amount of minerals present in the body, and their metabolic roles, varies considerably. Minerals can be divided into two types: macro-minerals (major) and micro-minerals (minor or trace) (Whitney and Rolfes 1996). Micro-minerals (Trace minerals) comprise more than 12 minerals which are

not needed in as high amounts as macro-minerals, as they are generally used as catalysts in enzyme reactions (The Linus Pauling Institute. Minerals. http://osu.orst.edu/dept/lpi). On the other side, some minerals compete with each other for absorption, and they interact with other nutrients as well, which can affect their bioavailability (United States Department of Agriculture 2002a).

Bioavailability is the degree to which the amount of an ingested nutrient is absorbed and be available to the body. Mineral bioavailability depends on several factors; the ability to absorb nutrients varies by gender, disease state, and physiologic condition (e.g., pregnancy, aging) (Bioavailability. Wikipedia). Excess intake of one mineral can influence the absorption and metabolism of other minerals. For example, the presence of a large amount of zinc in the diet decreases the absorption of iron and copper. On the other hand, the presence of vitamins in a meal enhances the absorption of minerals in the meal. For example, vitamin C improves iron absorption, and vitamin D aids in the absorption of calcium, phosphorous, and magnesium (Whitney and Rolfes 1996; British Nutrition Foundation. Minerals. http://www.nutrition.org.uk).

Toxicity is the degree to which a substance can damage living organisms, the quantity of substance to which the organism is exposed, and the route of exposure skin (absorption), mouth (ingestion), or respiratory tract (inhalation) (Toxicity. Wikipedia). Toxicity is classified usually as (1) acute: harmful effects produced through a single or short-term exposure, (2) chronic: harmful effects produced through repeated or continuous exposure over an extended period, and (3) subchronic: harmful effects produced through repeated or continuous exposure over 12 months or more but less than the normal life span of the organism (Walum 1998).

3 Major Elements

The major minerals present in the body include sodium, potassium, chloride, calcium, magnesium, phosphorus, and sulfur.

3.1 Function

The fluid balance in the body, vital for all life processes, is maintained largely by sodium, potassium, and chloride. Fluid balance is regulated by charged sodium and chloride ions in the extracellular fluid (outside the cell) and potassium in the intracellular fluid (inside the cell) and by some other electrolytes across cell membranes (http://dining.unt.edu/nutrition/nutrition_brochures/Minerals.pdf). Sodium plays an important role in the absorption of other nutrients, such as glucose, amino acids, and water. Potassium and sodium act as cofactors for certain enzymes. Calcium, magnesium, and phosphorus are known for their structural roles and for

maintaining cell membranes and connective tissue (http://dining.unt.edu/nutrition/nutrition_brochures/Minerals.pdf). They are also essential for the development and maintenance of bones and teeth. Phosphorus is important for bones and teeth to be strong and it is found in the bones as a calcium phosphate salt (hydroxyapatite). Several enzymes, hormones, and proteins that regulate energy and fat metabolism require calcium, magnesium, and/or phosphorus to become active. Calcium also aids in blood clotting. Sulfur is a key component of various proteins and vitamins and participates in drug-detoxifying pathways in the body (Lodish et al. 2004). Role, dosage, and sources of major elements are represented in Table 1.

3.2 Prevention of Diseases

Sodium, potassium, and chloride are linked to high blood pressure (hypertension) due to their role in the body's fluid balance. High salt or sodium chloride intake has been linked to cardiovascular disease as well. High potassium intakes, on the other hand, have been associated with a lower risk of stroke, particularly in people with hypertension (Wardlaw 1999). Research also suggests a preventive role for magnesium in hypertension and cardiovascular disease, as well as a beneficial effect in the treatment of diabetes, osteoporosis, and migraine headaches. Osteoporosis is a bone disorder in which bone strength is compromised, leading to an increased risk of fracture. Along with other lifestyle factors, intake of calcium and vitamin D plays an important role in the maintenance of bone health and the prevention and treatment of osteoporosis. Elderly people with suboptimal diets are also at risk of mineral deficiencies because of decreased absorption and increased excretion of minerals in the urine (Whitney and Rolfes 1996).

3.3 Toxicity

Toxicity from excessive dietary intake of major minerals rarely occurs in healthy individuals. Kidneys that are functioning normally can regulate mineral concentrations in the body by excreting the excess amounts in urine (Whitney and Rolfes 1996). Toxicity symptoms from excess intakes are more likely to appear with acute or chronic kidney failure. Sodium and chloride toxicity can develop due to low intake or excess loss of water (Whitney and Rolfes 1996). Accumulation of excess potassium in plasma may result from insufficient aldosterone secretion (a hormone that acts on the kidney to decrease sodium secretion and increase potassium secretion) or tissue damage (e.g., from severe burns). High intake of magnesium, when kidney function is limited, increases the risk of toxicity. The most serious complication of potassium or magnesium toxicity is cardiac arrest.

Table 1 Role, dosage, and good source of major elements

Major elements	Role	Dose/good source	Reference
Calcium	Keeps the bones and teeth strong, regulates the heart beat along with other muscle functions, used for metabolic functions such as nerve transmission and intracellular signaling	Adults need 700 mg of calcium per day/Dairy products, green leafy vegetables (not spinach). Nuts, egg, and wheat	http://www.nhs.uk/Conditions/vitamins-minerals/Pages/Calcium.aspx; http://ods.od.nih.gov/factsheets/Calcium-HealthProfessional
Phosphorus	Keeps the bones and teeth strong. It is the main structural constituent of cell membranes It is also found in genetic material such as DNA and RNA. Supplying the body with energy	Adults need 550 mg of phosphorus per day/Red meat, dairy products and fish all contain high levels of phosphorus	Lodish et al. (2004), http://www.umm.edu/altmed/articles/phosphorus-000319.htm, http://lpi.oregonstate.edu/infocenter/minerals/phosphorus, http://www.nhs.uk/Conditions/vitamins-minerals/Pages/Other-vitamins-minerals.aspx
Magnesium	Used in over 300 biochemical reactions in the body, helps to convert carbohydrates and fats into energy, used in the synthesis of proteins and nucleic acids such as DNA and RNA. Magnesium has a structural role in the teeth and bones and is also used in cell signaling	270 mg per day (women), and 300 mg (men)/Whole grains, nuts, and green leafy vegetables	http://www.nhs.uk/Conditions/vitamins-minerals/Pages/Other-vitamins-minerals.aspx, http://ods.od.nih.gov/factsheets/Magnesium-HealthProfessional, http://www.umm.edu/altmed/articles/magnesium-000313.htm, http://lpi.oregonstate.edu/infocenter/minerals/magnesium
Sodium	Helps to control water balance in cells, blood volume, and blood pressure. It is also involved in nerve impulses	No more than 2.4 g of sodium (relates to 6 g of salt—sodium chloride)	http://www.nhs.uk/Conditions/vitamins-minerals/Pages/Other-vitamins-minerals.aspx, http://extension.usu.edu/files/publications/publication/FN_220.pdf, http://www.ext.colostate.edu/pubs/foodnut/09354.html

(continued)

Table 1 (continued)

Major elements	Role	Dose/good source	Reference
Potassium	Potassium, like sodium, helps to control the water balance of the body. Recent research has also suggested that potassium may help to reduce blood pressure	Necessary for adults to intake 3500 mg of potassium per day/meats, some fish, dairy products, and fruit and vegetables	http://www.nhs.uk/Conditions/vitamins-minerals/Pages/Other-vitamins-minerals.aspx, http://www.ext.colostate.edu/pubs/foodnut/09355.html, http://www.umm.edu/altmed/articles/potassium-000320.htm
Sulfur	Sulfur is needed in the production of cartilage and other tissues	Adequate sulfur requirements can be gained from a healthy diet/meat, fish, garlic, and broccoli	Toxicity. Wikipedia, http://www.nhs.uk/Conditions/vitamins-minerals/Pages/Other-vitamins-minerals.aspx, http://extension.usu.edu/files/publications/publication/FN_220.pdf, http://www.health.harvard.edu/newsweek/Listing_of_vitamins.htm

4 Trace Elements

Trace elements refer to essential elements or nutrients required in minute quantities much smaller than vitamins and minerals. Trace elements are found naturally in the environment, and human exposure derives from a variety of sources, including air, drinking water, and food (Bowen 1966). Trace elements are minerals, i.e., chromium, cobalt, copper, iron, manganese, molybdenum, selenium, zinc, iodine, and other elements that occur in very small amounts (usually less than 1–10 parts per million). Trace elements are found in small amounts in a variety of foods such as meat, fish, cereals, milk and dairy foods, vegetables, and nuts. Other microminerals include beta-carotene, boron, chromium, cobalt, fluoride, as well as molybdenum, nickel, and silicon (Bowen 1966).

4.1 Function

Trace minerals have specific biological functions as they are essential for absorption and utilization of many nutrients and aid enzymes and hormones in activities that are vital to life. Cellular energy production requires many trace minerals,

including iron, copper, and zinc, which act as enzyme cofactors in the synthesis of many proteins, hormones, and genetic material (Whitney and Rolfes 1996). Physiological roles of trace elements include (Whitney and Rolfes 1996): (a) regulatory role: metallo-enzymes and enzyme activators, (b) structural role: membrane integrity and bone structure; and (c) protective role: antioxidant defense (Se, Cu, Fe, Zn, Mn) and immune defense (Zn, Fe, Se). Trace elements play general roles in growth, maturation, cell integrity, and immune defense. On the other hand, trace elements play a central specific role in (a) coronary heart disease: antioxidant defense, lipoprotein metabolism, thrombogenesis, and blood pressure, (b) cancer: antioxidant defense, cell growth, and replication, and (c) osteoporosis: bone structure and connective tissue (Whitney and Rolfes 1996). Trace minerals are required in very small amounts for the body. Little is known about the role of some trace minerals such as barium, bromine, cadmium, gold, silver, and aluminum in human health (Whitney and Rolfes 1996).

Our organs are made up of billions of cells that constantly carry out chemical reactions to ensure that the organs function properly. All these reactions depend for their success on enzymes which trigger biochemical reactions (Wardlaw 1999). On the other hand, an enzyme needs cofactors, i.e., the trace elements which essentially trigger many biological mechanisms in the digestive, muscular, circulatory, and cerebral systems (Wardlaw 1999; Whitney and Rolfes 1996). Although present in only minute quantities, they are essential in the body to maintain a healthy balance. Trace elements have a number of biochemical and physiological functions: (1) trace elements have important roles as essential components of metallo-enzymes; (2) reduction of risk of non-communicable diseases; (3) protection against damage to the genome by reactive oxygen species thereby reducing the risk of cancer; (4) exhibit a central role in the antioxidative defense; in relation to coronary heart disease, trace elements prevent oxidative damage of lipoproteins; and (5) trace elements are central in the regulation of cholesterol synthesis, blood clotting factors, platelet function, and thrombogenesis (Bowen 1966). Role, dosage, and sources of major elements are represented in Table 2.

4.2 Types of Trace Elements

4.2.1 Non-Essential Trace Elements

Non-essential trace elements are not found in the human organs. Examples are aluminum, silver, lithium, and gold. They have no natural physiological effect but they do have pharmacological properties (Bowen 1966).

4.2.2 Essential Trace Elements

Over 80 elements have been identified in man, but a mere 15 of them appear to be indispensable for proper functioning of the human organs. They are classified as

Table 2 Role, dosage, and good source of trace elements

Trace elements	Role	Dose/Good source	Reference
Iron	Transports oxygen in red blood cells; helps to form hemoglobin molecules in the blood and myoglobin molecules in the muscles	Men should consume 8.7 mg of iron per day; , whereas women should intake 14.8 mg/liver, meat, and dark green leafy vegetables	http://www.nhs.uk/Conditions/vitamins-minerals/Pages/Iron.aspx, http://www.mckinley.illinois.edu/handouts/dietary_sources_iron.html
Copper	Copper is important for iron metabolism; it oxidases iron to the form that is necessary for red blood cell formation. Anti-inflammatory, anti-infectious, inflammatory rheumatic conditions, and fights off free-radicals	It is necessary for adults to consume 1.2 mg of copper per day	http://www.nhs.uk/Conditions/vitamins-minerals/Pages/Other-vitamins-minerals.aspx, http://lpi.oregonstate.edu/infocenter/minerals/copper
Zinc	Cofactor for various functions, including growth, immunity and skin repair. Important for wound healing. It is also involved in many metabolic processes as it forms part of many enzymes	Adult requirement depends on their sex. Men require between 5.5 and 9.5 mg of zinc per day; whereas, women require 4–7 mg per day/red meat and poultry, cereals fortified with zinc, liver, and egg yolk	http://www.nhs.uk/Conditions/vitamins-minerals/Pages/Other-vitamins-minerals.aspx, http://ohioline.osu.edu/hyg-fact/5000/5560.html
Manganese	Forms part of an enzyme present in the mitochondria of cells. This enzyme, known as manganese superoxide dismutase, is responsible for fighting free radicals. Necessary for the production of healthy bones and also collagen which is used in wound healing	Men over the age of 18 should intake 2.3 mg of manganese per day; whereas women (over 18) should consume 1.8 mg per day/tea, nuts, vegetables, soya, eggs, coffee, and cereals	Toxicity. Wikipedia, http://www.nhs.uk/Conditions/vitamins-minerals/Pages/Other-vitamins-minerals.aspx, Bowen (1966), http://www.nhs.uk/Conditions/vitamins-minerals/Pages/Iron.aspx, http://www.csua.berkeley.edu/wuhsi/elements.html, http://lpi.oregonstate.edu/infocenter/minerals/manganese
Iodine	Used in the production of thyroid hormones, such as tri-iodothyronine and thyroxine. Thyroid hormones affect many systems in the body, including the brain, skeleton and organs	Adults should consume 0.14 mg of iodine per day/sea foods, garlic, onion, turnip, and some grains	http://lpi.oregonstate.edu/infocenter/minerals/iodine, http://www.nhs.uk/Conditions/vitamins-minerals/Pages/Iodine.aspx, http://e.hormone.tulane.edu/learning/thyroid.html

(continued)

Table 2 (continued)

Trace elements	Role	Dose/Good source	Reference
Selenium	Destroys free radicals. Helps reinforce the body's anti-aging defenses. Forms at least 25 different seleno-proteins in the body, which aid with many different functions defending against oxidative stress and regulating cell growth	Men need 0.075 mg per day; whereas women need only 0.06 mg per day/meat, fish, whole cereals, poultry, and nuts	http://www.nhs.uk/Conditions/vitamins-minerals/Pages/Other-vitamins-minerals.aspx, http://lpi.oregonstate.edu/infocenter/minerals/selenium, http://www.nap.edu/openbook.php?record_id=9810&page=285

"essential" because they act as catalysts for enzymes and other biological functions in the body (Bowen 1966). These trace elements are minerals which our body can't produce by itself and, thus, their intake should be through diet. A deficiency of any one can lead to malfunctioning.

4.3 Prevention of Diseases

In addition to clinical deficiency diseases such as anemia and goiter, research indicates that trace elements play a role in the development, prevention, and treatment of chronic diseases. Iron, zinc, copper, and selenium have been associated with immune response conditions. Copper, chromium, and selenium have been linked to the prevention of cardiovascular disease. Excess iron in the body, on the other hand, can increase the risk of cardiovascular disease, liver diseases, colorectal cancer, and neurodegenerative diseases such as Alzheimer's disease (The American Dietetic Association 2002). Chromium supplementation has been found to be beneficial in many studies of impaired glucose tolerance, a metabolic state between normal glucose regulation and diabetes (United States Department of Agriculture 2002b). Fluoride has been known to prevent dental caries due to its antimicrobial action, while potassium iodide supplements taken immediately before or after exposure to radiation can decrease the risk of radiation-induced thyroid cancer.

Classifying deaths and diseases (Fig. 1, Leon 2008)

1. Communicable diseases (Group I). Include those where death is directly due to the action of a communicable agent.
2. Non-communicable diseases. Non-communicable Diseases (Group II): cancer, diseases of various organ systems (e.g., respiratory, cardiovascular, etc.), diabetes, mental health, etc.
3. External causes (Group III): injuries, poisonings, and violence.

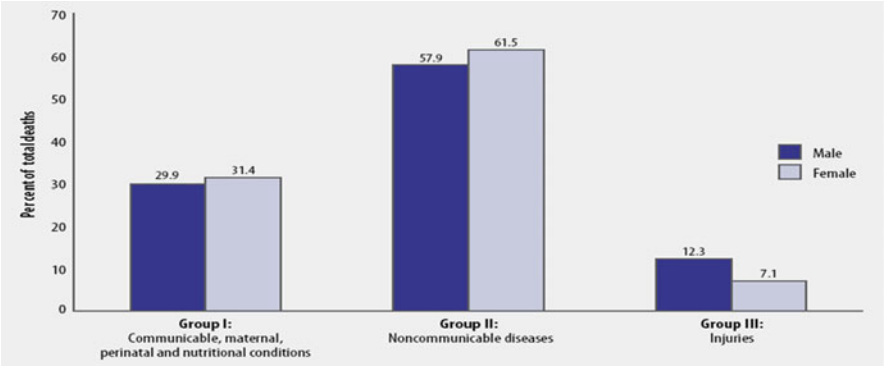

Fig. 1 Distribution of deaths in the world (Leon 2008)

4.3.1 Communicable Disease

A communicable disease is an illness that is transmitted through direct contact with an infected individual or indirectly through a vector. Communicable diseases are caused by microorganisms, and some diseases are passed on by direct or indirect contact with infected persons or with their excretions. Most diseases are spread through contact or close proximity because the causative bacteria or viruses are airborne (http://www.thefreedictionary.com/organisms). Communicable diseases include flu, tuberculosis, measles, pertussis, AIDS, etc. Some infectious diseases can be spread only indirectly, usually through contaminated food or water, e.g., typhoid, cholera, dysentery. Other infections are introduced into the body by animal or insect carriers, e.g., rabies, malaria, encephalitis (Hinman 1998). Control of disease is the reduction of disease incidence, prevalence, morbidity, or mortality to a locally acceptable level as a result of deliberate efforts. It is well documented that appropriate intake levels of certain chemical elements have been demonstrated to be required to maintain optimal health due to activation of the immune system against diseases.

4.3.2 Non-Communicable Diseases

A non-communicable disease (NCD) is noninfectious and non-transmissible among people. NCDs may be chronic diseases of long duration and slow progression, or they may result in more rapid death. NCDs include cardiovascular diseases, diabetes, cancer, and respiratory diseases (Fig. 2). NCDs are caused by behavioral risk factors: (a) tobacco use, (b) physical inactivity, (c) unhealthy diet, and (d) harmful use of alcohol. Risk factors also include high blood pressure, obesity, raised cholesterol, raised blood glucose, as well as decreased vegetable and fruit intake (World Health Organization (WHO) 2010; Nielsen 1993). NCDs already affect low- and middle-income countries where nearly 80 % of NCD deaths—29

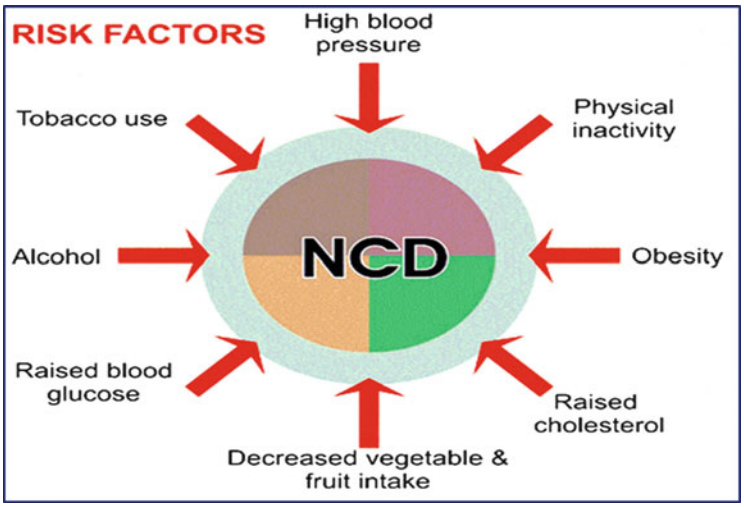

Fig. 2 Risk factors for non-communicable diseases (World Health Organization (WHO) 2010)

million—occur (WHO 2009). On the other hand, level of trace elements is altered in the body during certain diseases like cancer, diabetes, hypertension, and coronary heart disease. There is strong evidence showing that chronic disease can be prevented and controlled through comprehensive and integrated actions. These include policy actions, laws and regulations, tax and price interventions, improving the built environment, and community-based, school-based, workplace screening and clinical interventions at health facility levels. World Health Organization has estimated cancer and cardiovascular diseases to be the most causal of NCDs (Fig. 3). However, tobacco accounts for almost 6 million deaths every year (including over 600,000 deaths from exposure to second-hand smoke), and is projected to increase to 8 million by 2030 (Lim et al. 2012). Approximately 1.7 million deaths are attributable to low fruit and vegetable consumption.

5 Dietary Element

Dietary elements (commonly known as dietary minerals or mineral nutrients) are the chemical elements required by living organisms, other than the four elements carbon, hydrogen, nitrogen, and oxygen present in common organic molecules (Whitney and Rolfes 1996). Chemical elements include the seven major dietary elements calcium, phosphorus, potassium, sulfur, sodium, chlorine, and magnesium. Important trace or minor dietary elements, necessary for life, include iron, cobalt, copper, zinc, molybdenum, iodine, and selenium. Mineral nutrient are metabolized for growth, development, and vitality of living organisms (Skinner 2005; Adame 2002). The elements may be naturally present in the food (e.g.,

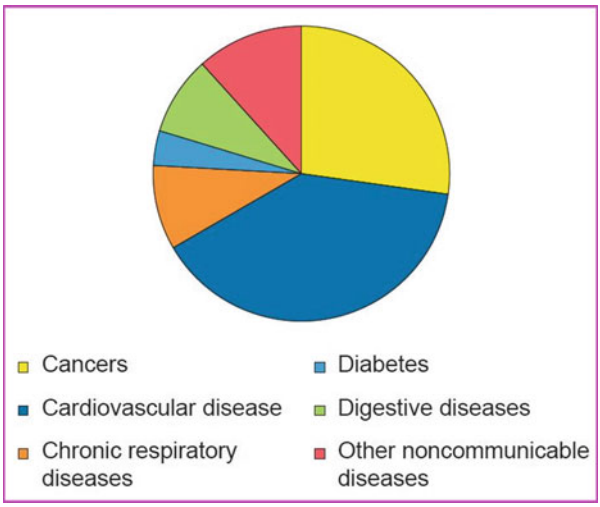

Fig. 3 Proportion of global non-communicable diseases death, under the age of 70, by cause of death (World Health Organization (WHO) 2010)

calcium in dairy milk) or added to the food (e.g., orange juice fortified with calcium; iodized salt, salt fortified with iodine). Dietary supplements can be formulated to contain several different chemical elements (as compounds), a combination of vitamins and/or other chemical compounds, or a single element (as a compound or mixture of compounds) such as calcium (as carbonate, citrate, etc.) or magnesium (as oxide, etc.). The dietary minerals focus on chemical elements required for supporting the biochemical reactions of metabolism (Lippard and Berg 1994). Dietary element overdoses lead to harmful effects. Mineral nutrients in our diet prevent deficiency but in large amounts, however, they trigger toxic side effects.

5.1 Major Elements Overdoses

Major elements overdoses include (a) some symptoms of calcium excesses such as constipation, bloating, nausea, and intestinal gas are common side effects among healthy people taking supplements equal to 1500–4000 mg of calcium a day (Whitney and Rolfes 1996; United States Department of Agriculture 2002a). Doses higher than 4000 mg a day may be linked to kidney damage. Megadoses of calcium can bind with iron and zinc, making it harder for your body to absorb these two essential trace elements; (b) too much phosphorus can lower your body stores of calcium; (c) megadoses of magnesium appear safe for healthy people, but in case of kidney disease, the magnesium overload can cause weak muscles, breathing difficulty, irregular heartbeat, and/or cardiac arrest (Whitney and Rolfes 1996; United States Department of Agriculture 2002a).

5.2 Trace Elements Overdoses

Overdosing of iron supplements can be deadly, especially for young children. The lethal dose for a young child may be as low as 3 g (3000 mg) of elemental iron at one time. This is the amount in 60 tablets with 50 mg elemental iron each (http://www.nhs.uk/Conditions/vitamins-minerals/Pages/Iron.aspx; http://www.mckinley.illinois.edu/handouts/dietary_sources_iron.html). For adults, the lethal dose is estimated to be 200–250 mg elemental iron per kilogram (2.2 pounds) of body weight. Moderately high doses of zinc (up to 25 mg a day) may slow absorption of copper in the body (Fesller 2005). Overdoses (2000 mg) of zinc cause symptoms of zinc poisoning: vomiting, gastric upset, and irritation of the stomach lining. Doses of molybdenum 2–7 times of the adequate intake (45 μg) may increase the amount of copper excretion in urine (Fesller 2005). Doses as high as 5 mg of selenium a day may lead to thickened but fragile nails, hair loss, and perspiration with a garlicky odor (Fesller 2005). Deficiency and toxicity symptoms of some trace element in adults are represented in Table 3.

6 Trace Elements in Dairy Products

Mineral elements occur in milk and dairy products as inorganic ions and salts, as well as part of organic molecules, such as proteins, fats, carbohydrates, and nucleic acids. The chemical form of mineral elements is important because it determines their absorption in the intestine and their biological utilization (Zamberlin et al. 2012). The mineral composition of milk is not constant because it depends on lactation phase, nutritional status of the animal, and environmental and genetic factors (Zamberlin et al. 2012). Unlike the major elements, trace elements are present in the human body in the concentrations lower than 0.01 % of the total body mass. Of the 20 essential minerals, 14 are trace elements and include iron, copper, zinc, manganese, selenium, iodine, chromium, cobalt, molybdenum, and others. Many of the trace elements are toxic, their concentrations in milk are, however, too low to pose a threat to human health (Zamberlin et al. 2012). The concentration of trace elements in milk is not constant. It depends on the lactation stage, nutritional status of the animal, and environmental and genetic factors (Cashman 2002). The content of trace elements in goat and other milk types also depends on the species, its individual characteristics, feeding method, lactation stage, and health condition of udder (Park and Chukwu 1989).

Table 3 Trace element deficiency and toxicity symptoms in adults (Fesller 2005; Baumgartner 1993; Food and Nutrition Board, Institute of Medicine—National Academy of Sciences 2001; Food and Nutrition Board, Institute of Medicine-National Academy of Sciences 2000; Shike et al. 1981; Jensen and Binkley 2002)

Trace element	Deficiency	Toxicity
Iron	Hypochromic microcytic anemia, pallor, fatigue, decreased work performance	Hemosiderosis, hemochromatosis, accumulation in liver and heart, some endocrine tissues; iron toxicity can be fatal
Copper	Hypochromic, microcytic anemia, leukopenia, neutropenia, skeletal abnormalities, and rarely, thrombocytopenia	Accumulation in liver, hepatocellular damage
Zinc	Dermatitis, alopecia, anorexia, reduced taste sensitivity, impaired immune function, impaired wound healing, glucose intolerance	Anemia, hyperamylasemia, fever, central nervous system dysfunction in renal patients; deficiency of Cu (enteral Zn interferes with Cu absorption)
Manganese	Impaired metabolism of carbohydrate and lipid, dermatitis, impaired protein synthesis, weight loss	Extrapyramidal neurologic symptoms: headache, tremor, facial nerve deficit, gait disturbance. Hyperintensity of signals on brain magnetic resonance images in basal ganglia
Selenium	Cardiomyopathy, skeletal myopathy, myalgias, myositis, impaired cellular immunity, discoloration of nails	Alopecia, brittle hair and nails, skin rash, GI disturbance, “garlic” breath odor, nervous system abnormalities
Molybdenum	Tachycardia, tachypnea, headache, night blindness, lethargy	Limited toxicity data for humans. Possible gout (high incidence in areas where soil is high in Mo) and possible excessive urinary copper excretion
Iodine	Hypothyroidism—weakness, cold intolerance, weight gain, thinning hair, goiter (thyroid enlargement)	Thyroiditis, goiter, hypo- or hyperthyroidism, thyroid papillary cancer, dermatoses (iodermia)

7 Trace Elements in Water

Characteristics of water as determinants of disease risk are not a new idea. Organic contaminants, such as chlorinated hydrocarbons, insecticides, or toxic heavy metal imbalances, have adverse health consequences (Leslie and Gerald 2005). The effect depends upon the susceptibility of the individual consuming water explained by individual metabolic characteristics (Leslie and Gerald 2005). These include nutrient mineral status, metabolic utilization, absorption capabilities, and retention rates, in relation to the mineral content of the water being consumed (David 1986).

7.1 Hard Water

Hard water is usually characterized as having a pH greater than 7.0, with total hardness in the range of 250 mg/L or greater. Since most adverse health effects appear to be contributed to by soft water, it would seem logical that hard water is ideal for everyone (Leslie and Gerald 2005). However, hard water can also contribute to mineral deficiencies or imbalances. As an example, excess calcium can antagonize the absorption of other minerals, such as iron, zinc, and potassium (Leslie and Gerald 2005).

7.2 Soft Water

Soft water is characterized as being acidic (pH less than 7.0), with total hardness in the range of less than 180 mg/L. The relationship of soft water influencing the incidence of cardiovascular disease death rates was first documented in 1957 (Sharrett and Feinleib 1975). Since then, other studies have confirmed that death rates from cardiovascular disease were significantly higher in areas with soft water, as compared to hard water regions. Sodium may be a main contributor to various health disturbances found in soft water regions. A high sodium level or high sodium in relation to other specific minerals is frequently found occurring naturally in soft water (Leslie and Gerald 2005). Treated or softened water usually does not raise sodium content above acceptable ranges. Sodium concentration, however, can be over one-thousand times higher in relation to calcium and magnesium levels (Leslie and Gerald 2005). Susceptible individuals, or those who have a tendency to retain sodium, would be adversely affected by consuming water with high concentrations of sodium. Generally, individuals showing low calcium and magnesium levels, in relation to high sodium and potassium, should avoid soft water or water that has been artificially softened, especially if a patient is subject to hypertension. Hard water would be considered therapeutic or protective for these individuals (David 1986).

7.2.1 Minerals in Water Supply

Calcium and magnesium are the main elements that give water its hardness. The minerals calcium and magnesium are known to prevent increased sodium accumulation within the body. Intake of water containing excess sodium however, can contribute to calcium and/or magnesium deficiencies systemically (Leslie and Gerald 2005; Mildvan 1970). This is especially true if dietary calcium or magnesium intake is marginal or inadequate. Calcium produces a stimulatory or hyper excitable effect upon muscle tissue, while magnesium produces a sedative effect. Magnesium has been generally accepted as being the protective factor in preventing

cardiovascular disturbances, due to the beneficial effects of magnesium when administered during the treatment of cardiovascular disorders (Leslie and Gerald 2005).

High zinc-to-copper ratio or a relative copper deficiency may contribute to ischemic heart disease (Klevay 1975). Excess tissue copper accumulation is known to have a suppressing effect upon the thyroid gland. Excess copper is frequently found in soft water, due to the corrosive effect of soft water acidity upon copper pipes (David 1986). Chlorine content of water may also be a factor to consider in heart disease. Drinking chlorinated water with a low calcium diet may result in increasing cholesterol levels which was found to be prevented by calcium intake (David 1986). Chlorine is used as a disinfectant in drinking water and may range from 1 to 3 mg/L. Patients suffering from ischemic heart disease should avoid chlorinated water.

7.2.2 Metabolic Disorders

Many organizations have established drinking water standards for nutrient mineral and toxic metal content. However, detrimental and unfavorable effects could be occurred with overdoses of mineral levels rather than acceptable ranges: (a) *osteoporosis*: prolonged intake of soft water can contribute to osteoporosis (David 1986). Water containing inadequate amounts of magnesium relative to calcium has been shown to be related to a greater incidence of bone fractures with decreased healing time of bones in the elderly (David 1986). Magnesium-deficient soils and water have also been related to osteoporosis and dental caries, even while the calcium content is adequate or high (David 1986). It is recognized that a high calcium to magnesium ratio results in decreased bone matrix formation. (b) *Hypertension and arthritis*: drinking water that contains high amounts of iron can lead to excess tissue deposition (David 1986). The primary deposition sites are in the liver, joints, and muscle. Excess accumulation is associated with arthritis, cirrhosis, and hypertension. (c) *Hyperactivity*: elevated lead accumulation has been well documented in contributing to hyperactivity in children, as well as other intellectual deficit disorders (David 1986). Calcium intake in adequate amounts is necessary to protect from the highly detrimental effects of lead and its absorption (David 1986). (d) *Kidney stones*: soft water areas of the United States have also been correlated with an increased incidence of renal calculus formation. Again, this condition is related to magnesium deficiency, relative to calcium (David 1986). The increased consumption of soft water during the summer months and increased vitamin D synthesis due to exposure to sunlight increase the amount of calcium absorption resulting in a relative magnesium deficiency. This may explain the seasonal occurrence of kidney stone formation during the summer months.

8 Trace Elements in Plant Nutrition

Plants absorb dissolved elements in soils, which are subsequently picked up to the food chain. Bacteria play an essential role in the weathering of primary elements that result in the release of nutrients for their own nutrition and for the nutrition of others in the ecological food chain. Like humans, plants require certain elements to grow well and to remain healthy. Sixteen chemical elements are known to be important to a plant's growth and survival. The sixteen chemical elements are divided into two main groups: non-mineral and mineral nutrients. The non-mineral nutrients are hydrogen, oxygen, and carbon which are found in the air and water (FloridaGardener.com. Nutrients for plant: 16 elements necessary for plants). The 13 mineral nutrients, which come from the soil, are dissolved in water and absorbed through a plant's roots. Not always enough amounts of these nutrients are abundant in the soil for a plant to grow healthy. So, such nutrients are added to the soil as fertilizers. Mineral nutrients are divided into two groups: (a) macronutrients; and (b) micronutrients.

Macronutrients include two groups: primary and secondary nutrients. The primary nutrients are nitrogen, phosphorus, and potassium. These major nutrients usually are lacking from the soil because plants use large amounts for their growth and survival (FloridaGardener.com. Nutrients for plant: 16 elements necessary for plants). The secondary nutrients are calcium, magnesium, and sulfur. These nutrients are usually enough in the soil. Sulfur is usually found in sufficient amounts from the slow decomposition of soil organic matter. Micronutrients or trace elements essential for plant growth are boron, copper, iron, chloride, manganese, molybdenum, and zinc. Recycling organic matter such as grass clippings and tree leaves is an excellent way of providing micronutrients (as well as macronutrients) to growing plants. Iron is essential for almost all life processes such as respiration and DNA synthesis (FloridaGardener.com. Nutrients for plant: 16 elements necessary for plants). Care needs to be taken with trace element applications because the margins between sufficiency levels and toxic levels of a trace element can be very narrow.

Despite being one of the most abundant elements in the Earth's crust, the bioavailability of iron in many environments such as the soil or sea is limited by the very low solubility of the Fe^{3+} ion (Neilands 1995). Siderophores are small, high-affinity iron-chelating compounds secreted by microorganisms such as bacteria, fungi, and grasses. Although there is sufficient iron in most soils for plant growth, plant iron deficiency is a problem in calcareous soil, due to the low solubility of iron (III) hydroxide (Neilands 1995). Calcareous soil accounts for 30 % of the world's farmland. Under such conditions, graminaceous plants (grasses, cereals and rice) secrete phytosiderophores into the soil (Sugiura and Nomoto 1984), a typical example being deoxymugineic acid. Phytosiderophores have a different structure to those of fungal and bacterial siderophores having two α-aminocarboxylate binding centers, together with a single α-hydroxycarboxylate unit. This latter bidentate function provides phytosiderophores with a high

selectivity for iron (III). When grown in an iron-deficient soil, roots of graminaceous plants secrete siderophores into the rhizosphere (Sugiura and Nomoto 1984). The iron (III) complex is then reduced to iron (II) and the iron is transferred to nicotianamine, which although very similar to the phytosiderophores is selective for iron (II) and is not secreted by the roots (Mori et al. 1998; Walker and Connolly 2008).

9 Trace Elements in Microbiology and Microbial Diseases

Under iron restricted condition, many bacteria produce the iron-chelating molecule; siderophore, which chelate iron and supply to bacterial cell by outer membrane receptors. Siderophore structure produced by bacteria is varied greatly. Its production can be obtained from bacteria under iron restrict condition on succinate media (Syed and Nidhale 2013). Siderophore and their derivative have large application in agriculture as to increase soil fertility and biocontrol for fungal pathogen. In medicine, the most important application is selective drug delivery, a new strategy to inhibit drug-resistant bacteria. Siderophores are also used to reduce the level of metal contamination in environment specifically in soil and water.

Siderophores are important for some pathogenic bacteria for their acquisition of iron and are amongst the strongest binders to soluble Fe^{3+} (Wiren et al. 1999; Hider and Kong 2010). For human health, due to this property, siderophores have attracted interest in metal chelation therapy for treatments of iron poisoning in medical science (Zhou et al. 2012). Siderophores have applications in medicine for iron and aluminum overload therapy and antibiotics for improved targeting (Hider and Kong 2010). Understanding the mechanistic pathways of siderophores has led to opportunities for designing small-molecule inhibitors that block siderophore biosynthesis in pathogenic bacteria to inhibit the growth and virulence in iron-limiting environments (Julian et al. 2005). Siderophores are useful as drugs in facilitating iron mobilization in humans, especially in the treatment of iron diseases, due to their high affinity for iron. These drugs are lethal to the microbes when it assimilates the siderophore conjugate (Miller 2008). Through the addition of the iron-binding functional groups of siderophores into antibiotics, their potency has been greatly increased. This is due to the siderophore-mediated iron uptake system of the bacteria. Siderophores can chelate metals other than iron such as aluminum (Neilands 1995), chromium (Neilands 1995), copper (Hider and Hall 1991), zinc (Hider and Hall 1991), manganese (Olmo et al. 2003), cadmium (Olmo et al. 2003), plutonium (John et al. 2001), and uranium (John et al. 2001).

9.1 *Inhibition of Pathogenic Bacteria*

A communicable disease is defined as an illness that arises from transmission of an infectious agent or its toxic product from an infected person, animal, or reservoir to a susceptible host. The most effective means for reducing a disease burden is through preventive strategies to inhibit the pathogens (The Johns Hopkins and the International Federation of Red Cross and Red Crescent Societies). Some strategies for microbial-disease reduction may include (a) water chlorination to ensure water safety; (b) increasing resistance to infectious by promoting better nutrition, immunization, and others means of self-protection; (c) practicing good sanitation, protecting food stores, markets, restaurants, and implementing vector control; (d) health education, food safety, and immunization of children aged 6 months to 15 years with vaccines against diseases (The Johns Hopkins and the International Federation of Red Cross and Red Crescent Societies).

For all microorganisms, acquisition of metal ions is essential for survival in their environment or in the infected host. Metal ions are required in many biological processes as components of metalloproteins and serve as cofactors or structural elements for enzymes (Porcheron et al. 2013). Indeed, host defense strategies against infection consist of metal deprivation or toxicity by highly concentrated release of metals (Porcheron et al. 2013). To overcome these host strategies, bacteria employ a variety of metal uptake and export systems and finely regulate metal-homeostasis by numerous transcriptional regulators, allowing them to adapt to changing environmental conditions. As a consequence, iron, zinc, manganese, and copper uptake systems significantly contribute to the virulence of many pathogenic bacteria. Many studies have reported the inhibition of pathogenic bacteria by metal ions and iron-chelating deprivation. Iron acquisition poses a significant challenge to bacteria in many environments, including pathogenic settings. This raises the possibility of targeting siderophore production as a novel strategy to reduce the fitness of pathogens (Gulick 2014). Inhibitory effect of trace elements upon some bacterial pathogens is discussed below.

9.1.1 *Brucella abortus*

Brucella is a genus of Gram-negative bacteria, non-motile, non-encapsulated coccobacilli, which function as facultative intracellular parasite. *Brucella* is the causative agent of a zoonotic disease, bovine brucellosis (Lopez-Goni and O'Callaghan 2012). It is transmitted by ingesting contaminated food such as unpasteurized milk or milk products. *Brucella abortus* infects the placenta and fetus of gestating cows and can be transmitted to humans by drinking infected unpasteurized milk or from contact with discharges from cattle or goats that abort their fetus. When humans are infected by this organism, they develop a severe fever. *Brucella abortus* has been shown to produce two siderophores: 2,3-dihydroxybenzoic acid (2,3-DHBA) and brucebactin (Jain et al. 2011). Studies

on *Brucella* have shown that 2,3-DHBA is associated with erythritol utilization and virulence in pregnant ruminants. The biosynthetic pathway and role of brucebactin are not known and the only gene shown to be involved so far is entF. An entF mutant was created in wild-type *B. abortus* 2308. Compared with the wild-type strain, the entF strain showed significant growth inhibition in iron minimal media that increased in the presence of an iron chelator (Jain et al. 2011). Addition of $FeCl_3$ restored the growth of the entF strain, suggesting its significant role in iron acquisition. Thus, the possibility of targeting siderophore production is a novel strategy to reduce the fitness of this pathogens (Gulick 2014).

9.1.2 *Klebsiella pneumonia*

Klebsiella pneumoniae has become the predominant pathogen causing primary pyogenic liver abscess. A strain of *K. pneumoniae* was found to possess multiple iron transport systems (Hsieh et al. 2008). By use of siderophore uptake assays, these systems were confirmed to be siderophore-dependent iron acquisition systems. Although there are multiple iron transport systems, it is reported that mutation was found to be necessary to decrease virulence in *K. pneumoniae*. The *tonB* mutant could be a potential vaccine candidate because it can induce a significant protective immune response against challenge with a wild-type strain (Hsieh et al. 2008).

9.1.3 *Treponema denticola*

Treponema denticola is considered as one of the primary pathogens responsible for periodontitis, a chronic inflammatory disease that is the major cause of adult tooth loss (Jackson-Rosario and William 2009). The impact of stannous salts on the growth of *T. denticola* was established. The mechanism of action revealed that, stannous salts impair selenium metabolism in this organism. The biological use of selenium as a catalyst, incorporated into proteins as selenocysteine, is broad. It plays an essential role in energy metabolism, redox balance, and reproduction in a variety of organisms, from bacterial pathogens to eukaryotic parasites to humans. The results of several epidemiological studies indicate that higher levels of selenium in the mammalian diet can have a negative effect on dental health. Given that selenium is required for the synthesis of glycine reductase and, consequently, acetyl phosphate for ATP synthesis, it is proposed that selenium is the root cause for growth inhibition of *T. denticola* (Jackson-Rosario and William 2009). Stannous fluoride is widely used in toothpastes and other oral treatments. Understanding the implications of these results requires a further understanding of the role of selenium metabolism and amino acid fermentation in the oral bacterial community.

9.1.4 *Escherichia coli*

Iron is essential to virtually all organisms but it can be toxic in excess. High concentration of iron and other trace elements could restrict bacterial growth and modify their metabolic pattern as well. Influence of iron, chromium, cadmium, and synergism or antagonism between these elements on growth of a Gram-negative bacterium was examined (Kalantari and Ghaffari 2008). In the series of experiments, *E. coli* has been cultured in nutrient broth supplemented with Fe^{2+}, Fe^{3+}, Cr^{3+}, Cd^{2+} alone or in combination, at 37 °C for 5 h. Bacterial growth was measured every half an hour using spectrophotometer. Chromium shows inhibitory effects on growth of the bacteria and cadmium is very toxic. Cr^{3+} and Cd^{+} exhibit inhibitory effect with iron on the growth of bacteria (Kalantari and Ghaffari 2008). It was also reported that chromium salts act as an iron-chelating agent which could precipitate it and thus removed the excess amounts of these elements. Hence, trace element could have toxic effects against pathogenic bacteria and might have some effect in complex with antibacterial activity of various antibiotics (Kalantari and Ghaffari 2008).

In some other reports, growth profile of *E. coli* was studied in the presence of silver nanoparticles modified substrates. Nomcebo et al. (2014) reported that complete growth inhibition was observed when resin was coated with silver nanoparticles, which were further suggested to be used for inactivation of bacteria and for employment in water treatment. The antimicrobial property of silver is known as an oligodynamic effect, a process in which metal ions interfere with the growth and function of bacteria (Carlos et al. 2014; Vasilev et al. 2009). Several in vitro studies have confirmed the effectiveness of silver at preventing infection, both in coating form and as nanoparticles dispersed in a polymer matrix. However, application of silver in the in vivo system is associated with warnings due to the toxic effect of silver on human tissue.

9.1.5 *Yersinia* spp.

Two species of Yersinia: *Yersinia enterocolitica*, a causative of gastroenteritic water- and foodborne pathogen, and *Yersinia pestis*, the causative agent of plague, were examined for developing small molecule inhibitors of siderophore biosynthesis (Tan 2007). Chelating of iron by bacterial siderophores is a critical process for the pathogen virulence and growth. In the two bacterial species above, the required first steps in siderophore biosynthesis are catalyzed by salicylate adenylation enzymes. Thus, inhibitors of these enzymes should block siderophore biosynthesis and, hence, bacterial iron uptake, growth, and virulence. Development of such inhibitor (considered a new antibiotic strategy), called salicyl-AMS, which has potent activity against salicylate adenylation enzymes in biochemical assays are reported (Tan 2007). Salicyl-AMS also inhibits bacterial siderophore biosynthesis and growth under iron-limiting conditions.

In view of its potent biochemical activity, it is hypothesized that the cellular activity of salicyl-AMS is being obliged by one or more pharmacological factors: permeability, efflux, stability, and/or specificity (Tan 2007). A multidisciplinary approach is used to evaluate this hypothesis and to develop analogs with increased cellular activity through three integrated specific aims: (1) synthesize salicyl-AMS analogs designed to provide improved permeability, efflux resistance, and stability; (2) synthesize analogs designed to provide improved specificity based on an inhibitor binding model; and (3) test salicyl-AMS and its analogs in a panel of assays to evaluate the pharmacological factors that affect cellular activity and to identify optimized inhibitors (Tan 2007).

9.2 Inhibition of Bacterial Biofilm Formation

Different systems have been documented as making contribution to a pathogen survival. Native bacterial communities populate human mucous membranes and epithelial surfaces such as the gastrointestinal tract, oral cavity, and skin. These bacterial communities play key role in the immune systems and protection of the mucosal surfaces and also provide vital functions against exogenous pathogens (Høiby et al. 2010). The relationship between the host and its microbial communities is carefully balanced, but under certain conditions it can break down and result in infectious diseases. According to a recent public announcement from the National Institutes of Health, more than 60 % of all microbial infections are caused by bacterial biofilms. Biofilms consist of communities of microorganisms typically embedded in an organic polymer matrix. Presence of biofilm confirms an important role for bacterial chronic infection associated with antibiotic resistance.

Biofilms develop on all surfaces immersed in natural aqueous environments, including both biological (aquatic plants and animals) and abiological (concrete, metal, plastics, stones). In nature, biofilms constitute a protected growth modality that allows the bacteria to survive in hostile environments (Høiby et al. 2010). Bacterial biofilms colonize any humid surface (plaque on the teeth, slippery slime on river stones, gel-like film on the inside of a vase, and infected tissue). Microorganisms within the biofilms are protected from the normal defense mechanisms in the body. Moreover, biofilms are pervasive and problematic in medical, industrial, and environmental settings because these communities express biofilm-specific properties such as increased resistance to antibiotics, UV light, and chemical biocides, increased rates of genetic exchange, altered biodegradability, and increased secondary metabolite production. Thus, the biofilm provides a source of infection by bacteria and when they infect the body, it is difficult to eradicate them with therapeutic agents (Prakash et al. 2003). Biofilm-growing bacteria cause chronic infections, including foreign-body infections that are characterized by persistent inflammation and tissue damage despite antibiotic therapy (Nomcebo et al. 2014). Some general features of biofilm infections in human include: (1) aggregates of bacteria embedded in a self-produced polymer matrix, (2) tolerant to both innate

and adaptive immune responses, (3) tolerant to clinical dosing of antibiotics despite susceptibility of planktonic cells, and (4) chronic infections.

Understanding the mechanisms of bacterial persistence will suggest novel ways to control infectious diseases. Strategies to plan against bacterial biofilm must be achieved by prevention of biofilm formation rather than dispersal of the formed biofilm (Maria et al. 2014). Strategies for prevention of biofilm formation include both "chemical" and "mechanical" methods (Abdel-Aziz and Aeron 2014). One important strategy against bacterial biofilm is the use of chelating agents. Metal cations, such as calcium, magnesium, and iron, have been implicated in maintaining matrix integrity. Consistent with this observation, chelating agents have been shown to destabilize biofilm architecture besides interfering with bacterial membrane stability. For example, chelation of iron, calcium, and magnesium could prevent biofilm formation. Sodium citrate inhibited biofilm formation by several *Staphylococci* species in vitro (Shanks et al. 2006). In addition, tetrasodium-EDTA eradicated biofilms in an in vitro biofilm model and on explanted hemodialysis catheters, whereas disodium-EDTA, in combination with tigecycline or gentamicin, reduced biofilm formation by *Staphylococcus* species and *P. aeruginosa* (Maria et al. 2014).

10 Prevention and Control of Non-Communicable Diseases

An important way to reduce NCDs is to focus on lessening the risk factors associated with these diseases. Low-cost solutions exist to reduce the common modifiable risk factors (mainly tobacco use, unhealthy diet and physical inactivity, and the harmful use of alcohol) and map the epidemic of NCDs and their risk factors (Lim et al. 2012). To lessen the impact of NCDs on individuals and society, a comprehensive approach is needed that requires all sectors, including health, finance, foreign affairs, education, agriculture, planning, and others, to work together to reduce the risks associated with NCDs to prevent and control them (Lim et al. 2012).

Early detection and timely treatment to reduce NCDs are high impact because, if applied to patients early, it can reduce the need for more expensive treatment (WHO 2009). The greatest impact can be achieved by creating healthy public policies that promote NCD prevention and control and reorienting health systems to address the needs of people with such diseases. Low-income countries generally have lower capacity for the prevention and control of non-communicable diseases (Lim et al. 2012). High-income countries are nearly four times more likely to have NCD services covered by health insurance than low-income countries. Countries with inadequate health insurance coverage are unlikely to provide universal access to essential NCD interventions.

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Fortified Foods and Medicinal Plants as Immunomodulators

Shadia M. Abdel-Aziz, Abhinav Aeron, and Neelam Garg

Abstract There is a strong consensus that nutrition plays an important role in modulating the immune system which needs adequate supply of nutrients to function properly. The complexity of the immune system supports this idea because its optimal functioning involves a variety of biological activities including energy metabolism, production of proteins, cell division, and proliferation. Important micronutrients to the immune function include vitamins A, C, E, and B6, folate, iron, zinc, and selenium. Other nutrients mentioned as playing a role in immune function include beta-carotene (a precursor to vitamin A), vitamin B12, and vitamin D. On the other hand, overdoses for activation of the immune system can lead to detrimental effects such as chronic inflammation or autoimmune diseases. In some individuals with allergies, a normally harmless material can be mistaken as an antigen. Immune functions are responsible to protect the body against attack by pathogens or cancer cells and thus play a vital role for health and well-being. However, the immune functions are disturbed by malnutrition, aging, physical and mental stress, or undesirable lifestyle. Therefore, ingestion of foods with immune-modulating activities is considered an efficient way to reduce the risk of infections or cancer and to prevent the immune function from declining.

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1 Introduction

Immune system is a remarkable defense system within vertebrates to provide protection against invading agents. It is able to generate varieties of cells and molecules capable of recognizing and eliminating foreign and undesirable particles. The immune system is composed of many interdependent cell types that collectively protect the body from bacterial, parasitic, fungal, and viral infections and from the growth of tumor cells. Many of these cell types have specialized functions. The cells of the immune system can engulf bacteria, kill parasites or tumor cells, or kill viral-infected cells. Often, these cells depend on the T helper subset for activation signals in the form of secretions formally known as cytokines, lymphokines, or more specifically interleukins (Saroj et al. 2012).

There is a strong consensus that nutrition plays an important role in modulating the immune system to function properly. The complexity of the immune system supports this idea because its optimal functioning involves a variety of biological activities including cell division and proliferation, energy metabolism, and production of proteins (Yehia et al. 2011). Interest in micronutrient malnutrition has increased greatly over the last few years. One of the main reasons for the increased interest is the realization that micronutrient malnutrition contributes to the global burden of disease. The *World Health Organization Report* identified iodine, iron, vitamin A, and zinc deficiencies as being among the world's most serious health risk factors (World Health Organization and Food and Agriculture Organization of the United Nations 2011). Micronutrients that are most important to immune function include vitamins A, C, E, and B6, folate, iron, zinc, and selenium. Other nutrients mentioned as playing a role in immune function include beta-carotene (a precursor to vitamin A), vitamin B12, and vitamin D. On the other hand, overactivation of the immune system can lead to detrimental effects such as chronic inflammation or autoimmune diseases. In some individuals with allergies, a normally harmless material can be mistaken as an antigen (Yehia et al. 2011).

Micronutrient malnutrition is a public health problem, potentially huge, and significant especially when designing strategies for prevention and control of diseases such as HIV/AIDS, malaria and tuberculosis, and diet-related chronic diseases (World Health Organization and Food and Agriculture Organization of the United Nations 2011). Another reason for the increased attention to the problem of micronutrient malnutrition is that, while micronutrient deficiencies are certainly more frequent and severe among disadvantaged populations, they do represent a public health problem in some industrialized countries. This is particularly true of iodine deficiency in Europe, where it was generally assumed to have been eradicated, and of iron deficiency, which is currently the most prevalent micronutrient deficiency in the world (World Health Organization and Food and Agriculture Organization of the United Nations 2011). In addition, the increased consumption in industrialized countries of highly processed, energy-dense but micronutrient-poor foods is likely to adversely affect micronutrient intake and status (World Health Organization and Food and Agriculture Organization of the United Nations

2011). Micronutrient malnutrition can affect all age groups, but young children and women of reproductive age tend to be among those most at risk of developing micronutrient deficiencies. Micronutrient malnutrition has many adverse effects on human health. This chapter deals with the modulation of immune functions by a variety of food components, mechanisms and interactions between the immune system and some foods. Efficiency of probiotics, herbs, and medicinal plants as healthy foods are also discussed.

2 History

Immunology is a science that examines the structure and function of the immune system. It originates from medicine and early studies on the causes of immunity to disease. The earliest known mention of immunity was during the plague of Athens in 430 BC. Thucydides (an Athenian historian) noted that people who had recovered from a previous bout of the disease could nurse the sick without contracting the illness a second time (Retief and Cilliers 1998). In the eighteenth century, Pierre-Louis Moreau de Maupertuis made experiments with scorpion venom and observed that certain dogs and mice were immune to this venom (Ostoya 1954). These observations of acquired immunity were later exploited by Louis Pasteur in his development of vaccination and his proposed germ theory of disease (Plotkin 2005). It was not until Robert Koch's 1891 proofs that microorganisms were confirmed as the cause of infectious disease, for which he was awarded a Nobel Prize (1905). Viruses were confirmed as human pathogens in 1901, with the discovery of the yellow fever virus by Walter Reed (Walter 2007). Immunology made a great advance towards the end of the nineteenth century, through rapid developments, in the study of humoral immunity (relating to the body fluids, especially with regard to immune responses involving antibodies in the body fluids) and cellular immunity (Metchnikoff 1905). Particularly important was the work of Paul Ehrlich, who proposed the side-chain theory to explain the specificity of the antigen–antibody reaction; his contributions to the understanding of humoral immunity were recognized by the award of a Nobel Prize in 1908, which was jointly awarded to the founder of cellular immunology, Metchnikoff (1905).

3 Immune System

The immune system is composed of many interdependent types of cell that can collectively protect the body from bacterial, parasitic, fungal, and viral infections and from the growth of tumor cells. Many of these cell types have specialized functions (Alberts et al. 2002). The cells of the immune system can engulf bacteria, kill parasites or tumor cells, or kill viral-infected cells (Fig. 1) (Brinkmann 2005). Pathogens can rapidly evolve and adapt and thereby avoid detection and

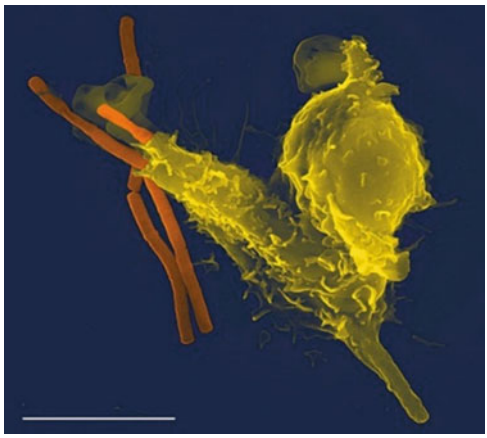

Fig. 1 Scanning electron microscope image of a single neutrophil (*yellow*) engulfing anthrax bacteria (*orange*). Neutrophils are the most abundant white blood cell and the first line of defense against invading microbes (Brinkmann 2005)

neutralization by the immune system; however, multiple defense mechanisms have also evolved to recognize and neutralize pathogens. Even simple unicellular organisms such as bacteria possess a rudimentary immune system in the form of enzymes that protect against bacteriophage infections (Beck and Gail 1996). The immune system is, therefore, crucial to our survival. It can be classified into two subsystems: as the innate immune (non-specific) system and the adaptive or acquired immune (specific) system. In immunology, an antigen, or antibody generator, is any substance which provokes an adaptive immune response. An antigen is often foreign or toxic to the body which, once in the body, attracts and is bound to a respective and specific antibody (Fig. 2) (Wikipedia: antigen, lactic acid bacteria). An antibody is a protein produced by the body's immune system when it detects harmful substances, called antigens.

3.1 Innate Immune System

The innate immune system is a subsystem of the overall immune system that comprises the cells and mechanisms that defend the host from infection by other organisms in a non-specific manner. It is known as non-specific immune system and first line of defense (Charles et al. 2001). This means that the cells of the innate system recognize and respond to pathogens in a generic way, but, unlike the adaptive immune system, it does not confer long-lasting or protective immunity to the host (Charles et al. 2001).

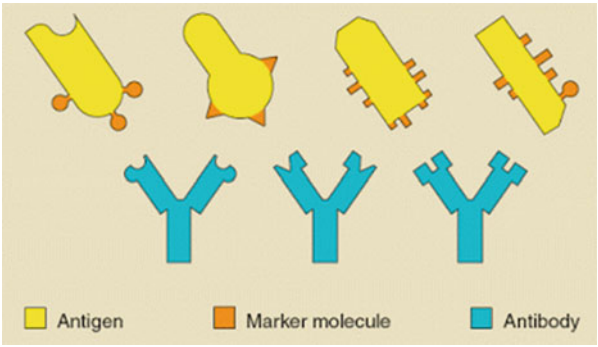

Fig. 2 Types of antigens with marker molecule receptors of antibodies (Wikipedia: Antigen, Lactic acid Bacteria)

3.2 Adaptive Immune System

Adaptive or acquired immunity differs from the innate response as it is specific, has an element of memory, and is unique to vertebrates. Adaptive immune allows for a stronger immune response as well as immunological memory, where each pathogen is remembered by a signature antigen (Pancer and Cooper 2006). The adaptive immune response is antigen specific and requires the recognition of specific "non-self" antigens during a process called antigen presentation. Antigen specificity allows for the generation of responses that are tailored to a specific pathogen.

4 Important Cells of the Immune System

Cells of the immune system and their important biological functions are reported in Table 1 (www.mhhe.com.biosci.genbio.raven6b.graphics.raven06_57; Naga and Rajeshwari 2014). Lymphocyte cells are divided into two major subsets that are functionally and phenotypically different: (a) T helper cells function to potentiate the immune response by the secretion of specialized factors that activate the white blood cells to fight off infection, and (b) natural killer cells are important in directly killing certain tumor cells, viral-infected cells, and sometimes parasites (Saroj et al. 2012). The major function of B lymphocyte cells, on the other hand, is the production of antibodies in response to foreign proteins of bacteria, viruses, and tumor cells. Antibody production and binding to a foreign substance or antigen are often critical as a means of signaling other cells to engulf, kill, or remove that foreign substance from the body. Macrophage cells are important in the regulation of immune responses. They are often referred to as scavengers or antigen-presenting cells because they pick up and ingest foreign materials and present these antigens to other cells of the immune system such as T cells and B cells (Saroj et al. 2012). This is one of the important first steps in the initiation of an immune response. Stimulated macrophages exhibit increased levels of phagocytosis (Saroj et al. 2012).

Table 1 Cells of the immune system (www.mhhe.com.biosci.genbio.raven6b.graphics.raven06_57; Naga and Rajeshwari 2014)

Cell type	Function
Helper T cell	Commander of the immune response; detects infection and sounds the alarm, initiating both T-cell and B-cell responses
Inducer T cell	Not involved in the immediate response to infection; mediates the maturation of other T cells in the thymus
Cytotoxic T cell	Detects and kills infected body cells; recruited by helper T cells
Suppressor T cell	Dampens the activity of T and B cells, scaling back the defense after the infection has been checked
B cell	Precursor of plasma cell; production of antibodies in response to foreign proteins of bacteria, viruses, and tumor cells
Plasma cell	Biochemical factory devoted to the production of antibodies directed against specific foreign antigens
Monocyte	Precursor of macrophage
Macrophage	The body's first cellular line of defense; also serves as antigen-presenting cell to B and T cells and engulfs antibody covered cells. Important in the regulation of immune response
Natural killer cell	Functions as effector cell. Recognizes and kills infected body cells; natural killer cell detects and kills cells infected by a broad range of invaders; killer cell attacks only antibody-coated cells
Dendritic cell	It originates in the bone marrow, function as antigen-presenting cells. These cells are found in the structural compartment of the lymphoid organs
Leukocytes	Leukocytes comprise both monocytes and macrophages. These cells are predominantly important in the removal of bacteria and parasites from the body. They engulf any foreign bodies and degrade them using their powerful enzymes
Mast cell	Initiator of the inflammatory response which aids the arrival of leukocytes at a site of infection; secretes histamine and is important in allergic responses

5 Disorders of the Human Immunity

The immune system is a remarkably effective structure that incorporates specificity, inducibility, and adaptation. Failures of host defense do occur, however, and fall into three broad categories: immunodeficiencies, autoimmunity, and hypersensitivities (Chetan et al. 2014).

5.1 Immunodeficiency

Immunodeficiencies occur when one or more of the components of the immune system are inactive. Malnutrition is, however, the most common cause of immunodeficiency in developing countries. Diets lacking sufficient protein are associated with impaired cell-mediated immunity, complement activity, phagocyte function, IgA antibody concentrations, and cytokine production. Deficiency of single

nutrients such as zinc, selenium, iron, copper, vitamins A, C, E, and B6, and folic acid (vitamin B9) also reduces immune responses. Disorders of the immune system can result in autoimmune diseases, inflammatory diseases, and cancer (Lisa and Werb 2001; O'Byrne and Dalgleish 2001). Immunodeficiency occurs when the immune system is less active than normal, resulting in recurring and life-threatening infections.

The ability of the immune system to respond to pathogens is diminished in both the young and the elderly, with immune responses beginning to decline at around 50 years of age due to immunosenescence (Aw and Silva 2007). In developed countries, obesity, alcoholism, and drug use are common causes of poor immune function. However, malnutrition is the most common cause of immunodeficiency in developing countries (Aw and Silva 2007). Deficiency of single nutrients such as iron, copper, zinc, selenium, vitamins A, C, E, and B6, and folic acid (vitamin B9) reduces immune responses (Saroj et al. 2012). In addition, the loss of the thymus at an early age through genetic mutation or surgical removal results in severe immunodeficiency and a high susceptibility to infection (Miller 2002). AIDS and some types of cancer cause acquired immunodeficiency where phagocytes have a reduced ability to destroy pathogens (Copeland and Heeney 1996).

5.2 Autoimmunity

Autoimmunity, in contrast to immunodeficiency, arises when the body mounts an immune response against itself due to failure to distinguish self-tissues and cells from foreign antigens (Chetan et al. 2014). Common autoimmune diseases include chronic lymphocytic thyroiditis, rheumatoid arthritis, diabetes mellitus type 1, and systemic lupus erythematosus. Overactive immune responses comprise the other end of immune dysfunction, particularly the autoimmune disorders. Here, the immune system fails to properly distinguish between self and non-self and attacks part of the body. Under normal circumstances, many T cells and antibodies react with "self" peptides (Miller 1993). One of the functions of specialized cells is to present young lymphocytes with self-antigens produced throughout the body and to eliminate those cells that recognize self-antigens, preventing autoimmunity (Sprou et al. 2000).

5.3 Hypersensitivity

Hypersensitivity is an immune response that damages the body's own tissues and is mainly due to genetic causes. As clinical condition and immune parameters change in allergic patients, it is possible to observe the effects of foods by measuring the immune parameters associated with allergic reactions (Kaminogawa and Nanno

2004). Food-derived materials could prevent allergy by counteracting at least one step of allergic reactions.

6 Immunomodulation

Immune modulation via dietary strategies may hold promise as well for maintaining immune homeostasis in the healthy population (Yehia et al. 2011). There is a decline in the functional capacity to elicit specific immune responses with increasing age, and regulatory cells show a decrease in production and response to regulatory signals. Overall, these developments result in the impairment with age of innate and adaptive immune responses, increased self-antigen reactivity, and increased incidence of infection, leading to increased risk of mortality (Burns and Goodwin 2004; Harry 2009). On the other hand, immunomodulators are substances that have been shown to modify the immune system response and potentiate the immune system against any threat. When the immune system is highly strengthening, it will attack, destroy, and/or weaken the invading microorganisms (Vetvicka and Vetvickova 2014; Shruti et al. 2014). Many proteins, amino acids, and natural compounds have shown a significant ability to regulate immune responses including both adaptive and innate immune systems. Clinically, immunomodulators can be classified into following three categories (Naga and Rajeshwari 2014; Arya and Gupta 2011).

6.1 Immune Adjuvants

An adjuvant is a pharmacological and/or immunological agent that modifies the effect of other agents. Adjuvants may be added to vaccine to modify the immune response by supporting it to give a higher amount of antibodies and a longer lasting protection, thus minimizing the amount of injected foreign material (Definition of immunological adjuvant—NCI Dictionary of cancer terms. www.cancer.gov).

6.2 Immune Stimulants

Immunostimulants, also known as immunostimulators, are substances (drugs and nutrients) that stimulate the immune system by inducing activation or by increasing the activity of any of its components. One notable example is the macrophage colony-stimulating factor (Susan and Sally 2009).

6.3 Immune Suppressants

Immunosuppression is performed to prevent the body from rejecting an organ transplant or for the treatment of autoimmune diseases such as rheumatoid arthritis or Crohn's disease. An immunosuppressant is any agent that causes immunosuppression, including immunosuppressive drugs and some environmental toxins. A person who is undergoing immunosuppression or whose immune system is weak for any reasons (e.g., chemotherapy) is known to be immunocompromised (Susan and Sally 2009).

7 Immune Functions

Immune function is the target for the development of functional foods (Chetan et al. 2014; Harry 2009). In particular, vitamins, such as A, C, D, and E, and minerals, such as zinc, chromium, and selenium, are focal points to reinforce the immune system. Modulating the immune response may well serve as the basis for the development of functional foods. Immunonutrition products are typically meant for clinical use to fortify hampered immune function. Probiotics such as lactic acid bacteria and some vitamins enhance phagocytic activity and natural killer cell activity (innate immunity), while vitamins, minerals, amino acids, fatty acids, and oligosaccharides augment T-cell responses and antibody production (acquired immunity). A balance of innate and acquired immunity is desirable for good health (Kaminogawa and Nanno 2004). The immune system plays a vital role in the symptoms of many chronic disorders, such as psoriasis, diabetes type I, asthma, allergic disorders, or Crohn's disease (a type of inflammatory bowel disease that may affect any part of the gastrointestinal tract from mouth to anus). Modification and balancing such immune responses offer, therefore, a huge potential for the development of health-promoting food ingredients for specific consumer groups with an immature or defective immune system, such as children or elderly, or those affected by specific immune-related disorders (World Health Organization and Food and Agriculture Organization of the United Nations 2011).

7.1 Immunomodulation by Foods

Several reports have shown that improvement of immune functions by foods reduced the severity of infectious diseases due to immune system activation. Moreover, proliferation and metastasis of cancer cells are decreased when the immune functions are strengthened by immunomodulators. An immunomodulator may be defined as a substance, natural or synthetic, which can stimulate, suppress, or modulate any of the components of the immune system including both innate and

adaptive arms of the immune response (Yamamoto 1996). The primary function of food in the human diet is to provide nutrients and energy. Nutrients include macronutrients (protein, fat, and carbohydrates) and micronutrients (minerals, vitamins, and trace elements). Food also has the secondary function of giving sensory satisfaction by its flavor, taste, and color. Recently identified is a third function: the capacity of food to modulate physiological systems (immune, endocrine, nervous, circulatory, and digestive) beyond the accepted nutritional effects. Food components having these functions are called "functional foods" (Gibson and Roberfroid 1995).

Immunonutrition by functional foods for ill patients with problems in the immune functions is of paramount importance. Such foods contain specific amino acids such as arginine and glutamine, minerals such as zinc and selinium, various compounds with antioxidative properties, vitamins, and specific polyunsaturated fatty acids such as *Omega-3* (Harry 2009). Immunonutrition is specific for the immune-function modulation and used in relatively well-described clinical cases. Its application should be, however, building up on scientific basis. The concept of immunomodulation relates to nonspecific activation of the function and efficiency of macrophages, granulocytes, natural killer cells, and lymphocytes as well as to the production of various effector molecules generated by activated cells (Harry 2009). It is expected that these nonspecific effects give protection against different pathogens including bacteria, viruses, fungi, etc., and constitute an alternative to conventional chemotherapy (Sumit et al. 2014). Some dietary components can affect the immune function. Protein malnutrition, for instance, affects all aspects of immune function, with these effects being more pronounced in children and elderly. The functioning of T-cell and B-cell subsets and functions of innate immunity are strongly related to protein nutritional status. Immune responses can be restored by relieving this protein shortage (Harry 2009). Modulating the immune response and beneficial effects of functional foods differs according to ages, different requirements for immune mitigation, and size of dose.

7.1.1 Traditional Foods

Traditional means proven usage on the domestic market for a period that allows transmission between generations; this period is to be at least 30 years (EuroFIR 2013). *Traditional food* is a food of a specific feature or features, which distinguish it clearly from other similar products of the same category, in terms of the use of traditional ingredients (raw materials or primary products), traditional composition, or traditional type of production and/or processing (Trichopoulou et al. 2007). Many microorganisms play an important role as protective cultures against some pathogenic and spoilage bacteria such as *Listeria monocytogenes*, *Salmonella enteritidis*, *Pseudomonas* spp., and *Enterobacteria*, especially for application in traditional meat products. Beneficial bacteria in the food chain can have a protective role to reduce the growth of pathogenic and/or spoilage bacteria in foods, i.e., *Biopreservation*, which refers to extended shelf life and enhanced safety of foods

using microorganisms and/or their metabolites (Ross et al. 2002). These biopreservatives can also have a probiotic role, i.e., conferring a beneficial effect upon the host either on a farm animal through feeds or on humans through food products (Diana 2011). Medicinal plants are extensively reported as a potent source of bioactive compounds such as flavonoids, alkaloids, terpenoids, saponins, tannins, and glycosides. Such compounds help to (a) activate the immune system, (b) protect the body from free radicals, (c) kill pathogenic germs, and (d) decrease the risk of cancer (Diana 2011). Interaction between beneficial bacteria, especially gut microbiota, and functional compounds may lead to the activation of immune system, a good health, and well-being.

7.1.2 Probiotics and Prebiotics

Probiotics are a big and rapidly growing business, with annual global sales of products expected to rise to $42 billion by 2016 (Berkeley 2014). The term probiotic refers to dietary supplements (tablets, capsules, powders, lozenges, and gums) and foods (such as yogurt and other fermented products) that contain "beneficial" or "friendly" bacteria referred to as *Probiotics* which mean "for life," as opposed to *Antibiotics*. Probiotics confer health benefits primarily by rebalancing the normal microflora in the large intestine (Berkeley 2014). Probiotics contain strains of living bacteria that are similar to the healthy bacteria normally found in the digestive system. The purpose of taking probiotics is to increase the levels of those healthy bacteria. Products with probiotic may contain a single or a number of strains in a daily dose and can range from 1 billion to more than 250 billion. There is a close connection between bacteria in the colon and the immune system, and that probiotics have been linked to enhanced immune responses. Well-established probiotic effects are as follows: (1) Prevention and/or reduction of duration of antibiotic-associated diarrhea and lactose intolerance. (2) Reduction in concentrations of cancer-promoting enzymes and/or putrefactive bacterial metabolites in the gut. (3) Beneficial effects concerning prevention of inflammatory diseases of the gastrointestinal tract, *Helicobacter pylori* infection, or bacterial overgrowth. (4) Adjustment and normalization of stool block in patients who are suffering from constipation or an irritable colon. (5) Prevention of respiratory tract infections (common cold, influenza) and other infectious diseases as well as treatment of urogenital infections. (6) Prevention of cancer colon, hypocholesterolemic effect, and prevention or therapy of ischemic heart diseases or amelioration of autoimmune diseases (e.g., arthritis). (7) Improvement of the mouth flora and prevention of caries (De Vrese and Schrezenmeir 2008).

Probiotic bacteria are nonpathogenic microorganisms that when given in adequate numbers exert positive health effects on the host, whereas *Prebiotics* are a category of functional food defined as "nondigestible food ingredients that beneficially affect the host by stimulating growth and/or activity of a number of bacteria in the colon, and thus improve host health" (Gibson and Roberfroid 1995). Lactic acid bacteria and bifidobacteria are the best candidates for use as protective and

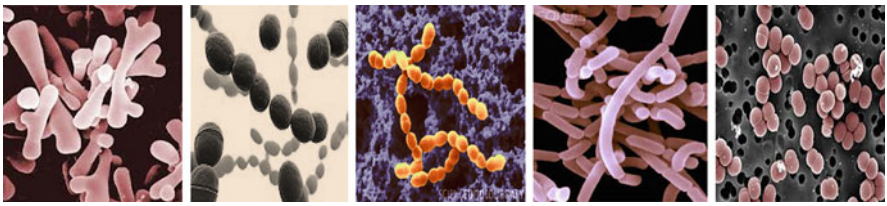

Fig. 3 Some genera of probiotic bacteria: *Bifidobacterium*, *Streptococcus*, *Lactococcus*, *Lactobacillus*, and *Pediococcus* (Wikipedia: Antigen, Lactic acid Bacteria)

probiotic cultures because they have a long history of consumption as starter cultures and for safe use. Lactic acid bacteria (LAB) are part of the natural microbiota of farm animals and human and are present in almost all fermented foods, meat products, and dairy products. LAB is generally employed because they significantly contribute to the flavor, texture, and, in many cases, to the nutritional value of the food products and has a GRAS status (generally recognized as safe). LAB exert antimicrobial effect as a result of different metabolic processes such as lactose metabolism, proteolytic enzymes, citrate uptake, bacteriophage resistance, bacteriocin production, polysaccharide biosynthesis, metal-ion resistance, and antibiotic resistance (Gibson and Roberfroid 1995; Zotta et al. 2009). Some species of lactic acid bacteria are represented in Fig. 3 (Wikipedia: antigen, lactic acid bacteria). Strains most commonly used as probiotics are lactobacilli and bifidobacteria which are also normal inhabitants of the gut. These beneficial species induce a balance with coliforms, *Staphylococcus aureus*, and gut microbiota and were supposed to be appropriate for proper activation of intestinal immunity. Probiotics species include lactobacilli (*L. acidophilus, L. gasseri, L. casei, L. rhamnosus, L. bulgaricus, L. plantarum, L. lactis, L. johnsonii, L. reuteri),* bifidobacteria (*B. longum*, *B. bifidum*, *B. breve*, *B. lactis*, *B. infantis*, *B. adolescentis*), yeasts such as *Saccharomyces boulardii* and *Saccharomyces cerevisiae*, and bacteria such as *Streptococcus thermophilus* and *Enterococcus faecalis* (Garssen et al. 2003). Probiotics may, however, influence the immune system and may have deleterious effects on health. Unfortunately, little information is available on the deleterious effects of probiotic use. Reported studies revealed that infections, endocartitis, dental caries, rheumatic vascular disease, and septicemia were described due to the ingestion of lactobacilli. Thus, one issue that clearly needs more attention is safety of probiotics (Garssen et al. 2003). Most common side effects of probiotics include diarrhea, gas, bloating, cramps, and rashes. Assuming that the adverse effect is relatively minor and does not persist for more than 14 days, these side effects should be declared, especially for young children, elderly, and people who have immune system problems or intestinal damage.

A synergistic combination of probiotics and prebiotics is called synbiotic. Prebiotics (particularly inulin, its hydrolysis product oligofructose, and galactooligosaccharides) fulfill all the criteria for prebiotic classification. They are dietary fibers with a well-established positive impact on the intestinal microflora such as (1) prevention of diarrhea or constipation and modulation of the metabolism of the

intestinal flora, (2) immunomodulatory properties, (3) positive effects on lipid metabolism and stimulation of mineral adsorption, and (4) cancer prevention (De Vrese and Schrezenmeir 2008). Other health effects of prebiotics are indirect, i.e., mediated by the intestinal microflora, and therefore less well proven. In the last years, successful attempts have been reported to make infant formula more breast milk-like by the addition of fructo- and (primarily) galactooligosaccharides (De Vrese and Schrezenmeir 2008).

7.1.3 Fortified Foods

Food fortification or enrichment is the process of adding micronutrients (essential trace elements and vitamins) to food. In 1831, the French physician "Boussingault" urged adding iodine to salt to prevent goiter. However, it was between the First and Second World Wars (1924–1944) that supplementation was established as a measure either to correct or prevent nutritional deficiencies in populations or to restore nutrients lost during food processing. Thus, during this period the adding of iodine to salt, vitamins A and D to margarine, vitamin D to milk, and vitamins B1, B2, niacin, and iron to flours and bread was established (Borenstein 1971). While fortification and enrichment refer to the addition of nutrients to food, the true definitions do, however, slightly vary. *Enrichment* is performed to restore nutrients lost during food processing. In this case, the amount of nutrients added is approximately equal to the natural content in the food before processing. *Fortification* is the process of adding micronutrients that may not be present naturally in foods. Fortification also standardizes the contents of nutrients that show variable concentrations (Borenstein 1971). A typical example is the addition of vitamin C to orange juice to standardize vitamin C concentration and improve any changes due to seasonal and processing variations. Fortification of foods helps to support the body's immune defenses and boost immune function for protection of human health.

Enhancement of immune functions by foods may comprise ingesting both macronutrition (basic nutrition) and micronutrition. However, limits should be set on what nutrients can be added to food, how much of an individual vitamin or mineral can be added, and which foods cannot be fortified. Food fortification was identified as a strategy by the WHO and FAO to begin decreasing the incidence of nutrient deficiencies at the global level (World Health Organization and Food and Agriculture Organization of the United Nations 2011). As outlined by the FAO, the most common fortified foods are as follows: (a) cereals and cereal-based products, (b) milk and milk products, (c) fats and oils, (d) tea and other beverages, and (e) infant formulas (Liyanage and Hettiarachchi 2011). Types of food fortification include microbial fortification (i.e., addition of probiotic bacteria to foods), commercial and industrial fortification of foods, and biofortification (i.e., breeding crops to increase their nutritional value, which can include both conventional selective breeding and modern genetic modification). However, care must be

taken concerning processed and packaged foods because when these types of foods are routinely eaten, nutritional deficiencies and imbalances within the body will occur. This processed food diet can result in a lot of health symptoms: allergies, headaches, blood sugar imbalances, digestion problems, fatigue, joint pain, sleep problems, and stress.

7.1.4 Cereal β: Glucans

β-(1,3)-(1,6)-glucans are non-starch polysaccharides having glucose polymers with the property of enhancing innate immunity by binding with the macrophages and natural killer cells. β-glucans can be found in bacteria, yeasts, mushrooms, seaweed, and cereals. Barley, oats, and corn bran are considered to be major sources of cereal β-glucans which are a major component of natural water-soluble dietary fibers. Significant positive health effects have been attributed to oat β-glucans including total cholesterol control, modulation of glucose and insulin responses, weight management, and improved gastrointestinal function (Harry 2009; Bonfilius 2014). The efficacy of oat and barley β-glucans in reducing the risk of coronary heart disease has been recognized by the U.S. Food and Drug Administration. β-glucans significantly enhanced the immune response to bacterial infection through the stimulation of neutrophils. β-glucans appear also to exert their immunomodulatory effects via activation of innate immune pathways, e.g., in macrophages (Harry 2009). Oral ingestion of β-(1,3)-glucan in individuals with allergic tropism could reduce the spontaneous increase in both allergen-specific and total IgE titers, and the clinical responses to treatment were well correlated with the capacity of monocytes to bind to β-(1,3)-glucan (Yamada et al. 2007).

7.1.5 Fungal Immunomodulatory Proteins

Fungal immunomodulatory proteins are 15 kDa proteins of fungal origin and have been found in *Flammulina velutipes* (golden needle mushroom), *Volvariella volvacea* (paddy straw mushroom), *Ganoderma lucidum*, and *G. tsugae* (Japanese lacquer mushroom). Fungal proteins are stable in food processing conditions such as freezing, thawing, dehydration, and acid/alkali conditions (Harry 2009). They enhance and/or activate macrophage immune responses leading to immunomodulation. The biological relevance of fungal proteins for allergy mitigation lies in the observation that they were able to inhibit food allergic and respiratory-allergic reactions in mouse models when applied orally or nasally (Hsieh et al. 2003).

7.2 *Immunomodulation by Herbs and Medicinal Plants*

Herbal and medicinal plants have been used since ancient times for the treatment of various diseases and disorders. Phytochemicals (low-molecular-weight compounds like alkaloids, terpenes, phenols, flavonoids, saponins, etc.) and extracts (high-molecular-weight compounds like lectins, polysaccharides) can be applied as immunomodulators for stimulation and alteration in the immune system. Many literatures gave the reference of many plants for prevention of different diseases and the capacity of the body to resist diseases (Jane et al. 2010; Nagarathna et al. 2013; Roshan and Savitri 2013). However, isolation of the active principals involved did not gain achievement till the nineteenth century (Phillipson 2001). A study in 1990 showed that 64 % of the world's population use botanic drugs to combat health problems (Retief and Cilliers 1998). Plants synthesize chemicals as part of their defense against pathogens. Many such compounds occur in nature as antifeedant and anti-infectant chemicals and are also effective against microbes (Chetan et al. 2014). Flavonoids and hydroxylated phenols are naturally synthesized by plants in response to infection. Flavones and flavanones, being bitter, also have natural antifeedant effects. Alkaloids are the most common plant metabolites. An alkaloid derivative, nicotine, has been shown to have insecticidal activities. Quinine, another alkaloid isolated from the bark of the *Cinchona* tree, was the first effective antimalarial drug (Chetan et al. 2014). Immunomodulation by herbs and medicinal plants has extensively been studied regarding their effects and side effect (Chetan et al. 2014; Sumit et al. 2014; Benny and Vanitha 2004; Amitava and Catherine 2011; Shivaprasad et al. 2011). Compared to synthetic drugs, herbal drugs are frequently considered to be less toxic with fewer side effects. Therefore, the search for more effective and safer agents exerting immunomodulatory activity is becoming a field of major interest all over the world. Some medicinal herbs with immunomodulatory properties are represented in Table 2.

Certain medicinal plants promote positive health and maintain organic resistance against infection by reestablishing body equilibrium. Many polysaccharides isolated from higher plants are considered to be biological response modifier and enhance various immune responses, like complement activation, proliferation of lymphocytes, and stimulation of macrophages (Chetan et al. 2014). Plant flavonoids are used as immunostimulator which is important for growth, development, and immunity. Recently, some phytoconstituents were found to be useful to combat breast cancer cells (Wang et al. 2012; Renuka et al. 2014). In clinical practice, however, use of phytochemicals to support the immune system or to fight infections should be based on cautions because most in vitro or in vivo models by immunomodulatory agents may be inadequate to ensure its use as drugs and since high doses tend to be immunosuppressive. Some plants and the parts used, fruits, leaves, seeds, flowers, roots, and bark, with biological immunomodulator activity are reported in Table 3.

Table 2 Effects of some medicinal herbs on immune cells and microbes (Benny and Vanitha 2004)

Species	Plant part	Chemical identity	Biological activity
Aloe vera	Leaves	Polysaccharide	Selectively stimulates cytokines and activates lymphocytes (Josias 2008)
Angelica gigas	Roots	Polysaccharide, decursin	Modulates cytokines, anticancer and anti-inflammatory activities (Zhang et al. 2012)
Astragalus membranaceus	Roots	Polysaccharide	Increases macrophage count (Shao et al. 2004)
Panax ginseng	Roots	Saponins	Stimulates lymphocytes and cytokines (Jong et al. 2005)
Panax ginseng	Leaves	Saponins	Enhances macrophage actions (Wang et al. 2009)
Panax notoginseng	Roots	Peptide	Toxic to *Coprinus comatus*, *Physalospora piricola*, *Botrytis cinerea*, and *Fusarium oxysporum* (Benny and Vanitha 2004)
Scutellaria albida	Roots	Flavonoid	Toxic to *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa* (Skalta et al. 2000)
Zingiber officinale	Rhizome	Sesquiterpene	Toxic to *Rhizoctonia solani* (Agarwal 2001)
Licorice	Roots	Saponins, minerals flavonoids, pectins, polysaccharides, amino acids	Exhibits steroid like anti-inflammatory activity, similar to the action of hydrocortisone, treatment for chronic hepatitis, promotes healing of ulcers, protects against aspirin-induced damage to the gastric mucosa, antiviral effect (Sumit et al. 2014; Wagner 1997)

8 Conclusion

The immune system is complex and includes highly specialized cells. The immune system is a network protective barrier against infection. Innate and adaptive immunity depends on the activity of white blood cells. Innate immunity largely depends upon leukocyte and macrophages, while adaptive immune response depends upon lymphocytes which provide long-term immunity. Immunodeficiency of an immune system occurs when one or more of the components of the immune system are inactive. Immunomodulators are becoming very popular in the worldwide natural health industry as people start to realize the importance of a health immune system in the maintenance of health and the prevention of diseases. Herbs and medicinal plants are established to contain bioactive compounds. Some compounds appear to act synergistically to produce the desired effects on host immunity. Identification and evaluation of new bioactive compounds from herbs and medicinal plants can

Table 3 Plants investigated pharmacologically for immunomodulatory activity (Naga and Rajeshwari 2014)

Botanical source	Chemical identity	Biological activity
Couroupita guianensis (flowers)	Steroids, phenolics, flavonoids, glycosides, carbohydrates, and proteins	Immunostimulant activity on both specific and non-specific immune mechanisms (Pradhan et al. 2009)
Azadirachta indica (flowers)	Steroids, phenolics, flavonoids, and glycosides	Stimulates both specific and non-specific immune responses. Potent immunostimulant against cytotoxic drug (Haggag et al. 2007)
Ocimum sanctum (leaves)	Ascorbic acid and flavonoids	These phytochemicals possess potent immunostimulant activity. In combination they showed synergistic activity which might be due to antioxidant property (Nawale and Poojari 2013)
Cassia auriculata (leaves)	Steroids, alkaloids, flavonoids, tannins, and phenolics	Significant immunostimulant effect on cell-mediated immunity and no effect on humoral immunity (Chakraborthy 2009)
Tridax procumbens (leaves)	Flavones, glycosides, polysaccharide, and monosaccharide	Stimulatory effect on humoral immunity and stimulated phagocytosis and offered protection against *P. aeruginosa* infection (Oladunmoye 2006)
Boerhavia diffusa (roots)	Alkaloids, carbohydrates, glycosides, triterpenoids, steroids, phenols, and tannins	Roots possess antistress, adaptogenic, and immunopotentiating activity (Meera and Mustafa 2007)
Premna integrifolia (roots)	Premnine, ganikarine, premnazole, flavonoids, luteolin, sterols, and terpenes	Immunomodulator (higher response to specific immunity as compared to nonspecific immunity) (Gokhani et al. 2007)
Trapa bispinosa (fruits)	Alkaloids, carbohydrates, starch, tannins, phenolic compounds, saponin, and glycosides	Immunostimulatory activity (Nawale and Poojari 2013)
Terminalia belerica (fruits)	Gallic acid, ellagic acid, ethyl gallate, chebulic acid, β-sitosterol, and 3-lignans	Shows immunosuppressant effect at low concentration while stimulatory activity at high concentration (Aurasorn et al. 2008)
Alstonia boonei (bark)	Alkaloids—indole, terpenes, lactones, steroids, and triterpenes	Antimicrobial and antimalarial action (Dibua et al. 2013)
Acacia catechu (bark)	Tannins catechin, quercetin, and catechuic acid	Anti-inflammatory effect (Rupa et al. 2012)
Bauhinia racemosa (bark)	Tannins, glycosides, saponins, and flavonoids	Immunostimulant activity on both specific and non-specific immune system, analgesic (Rupa et al. 2012)

help in the development of novel, less harmful, and clinically useful drugs to support the immune system as well as to inhibit or kill pathogenic microorganisms.

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Health Benefits of Probiotic Consumption

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Abstract In recent years, a novel term—functional food—was introduced which refers to preventional and/or curing effects of food beyond its nutritional value. There is a wide range of functional foods that were developed recently, and many of them are being produced in all over the world including probiotic, prebiotic, and synbiotic foods as well as foods enriched with antioxidants, isoflavones, phytosterols, anthocyanins, and fat-reduced, sugar-reduced, or salt-reduced foods. Among these foods, probiotic functional food has exerted positive effects on the overall health. Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" by the FAO/WHO. This chapter presents an overview of probiotic food consumption on the prevention and/or treatment of gastrointestinal tract diseases, immune-related diseases, coronary heart disease, bacterial vaginosis, diabetes management, oral health, and dentistry.

1 Functional Foods

The main role of food is to provide enough nutrients to meet metabolic requirements in human body, while giving the consumer a satisfaction feeling and well-being. Beyond meeting nutrition needs, food may have different physiological functions and may play beneficial or detrimental roles in some diseases (Koletzko

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et al. 1998). Functional foods were developed in order to promote a well-being state, improve health, and reduce the disease risk. "Functional food" means special foods which have preventional and/or curing effects beyond its nutritional value. There is a wide range of functional foods that were developed recently, and many of them are being produced in all over the world including probiotic, prebiotic, and synbiotic foods as well as foods enriched with antioxidants, isoflavones, phytosterols, anthocyanins, and fat-reduced, sugar-reduced, or salt-reduced foods. Among these foods, probiotic functional foods are the first choice to exert positive effects on the human health. Some of dairy probiotic foods including probiotic ice cream, frozen fermented dairy desserts, probiotic cheese, bio-yogurt, drinking yogurt, kefir, freeze-dried yogurt, and spray-dried milk powder have been employed as possible delivery vehicles for probiotic bacteria (Kailasapathy and Rybka 1997; Ravula and Shah 1998; Stanton et al. 2001; Haynes and Playne 2002; Homayouni et al. 2008b).

The number of microbial cells in the human gut is ten times more than the cells of an adult body (Mountzouris and Gibson 2003). So, the change of microbial balance in human intestine can impress the host health. The ratio between the beneficial microbes (probiotics) and harmful microbes would have an important effect on host health. One way of keeping probiotic cells in the gut is to introduce probiotics into the intestine through the regular consumption of foods containing these bacteria. Dairy products have an important role in human health and form the main part of the food pyramid. The therapeutical and health-care characteristic of fermented dairy products has been used over long years. Another way to keeping up the probiotic cells in the gut is to entering prebiotics into the intestine through the regular consumption of food containing these components. It is clear that versus probiotics, the amounts of prebiotics do not change during the passage from upper intestinal tract (Homayouni et al. 2008a).

2 Probiotic Foods

Consumption of probiotic bacteria via dairy food products is an ideal way to reestablish the intestinal microbiota balance. It must conform to certain requirements for a dairy food product to be considered as a valuable alternative for delivery of probiotic bacteria in one hand and for variety of probiotic cultures to use as a dietary adjunct and to exert a positive influence in the other hand. The culture must be native of the human gastrointestinal tract, have the ability to ferment prebiotics, survive passage through the stomach and small bowel in adequate numbers, be capable of colonizing in site of action, and have beneficial effects on human health. In order to survive, the strain must be resistant to acidic conditions (gastric pH 1–4), alkaline conditions (bile salts present in the small bowel), enzymes present in the intestine (such as lysozyme), and toxic metabolites produced during digestion. For example, in traditional yogurt production, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were used as starter culture.

These bacteria do not belong to the indigenous intestinal microbiota, are not bile acid resistant, and do not survive passage through the gut. So, the traditional yogurt culture is not to be considered as probiotic. In the case of dairy food product to be considered as a valuable alternative for delivery of probiotics, it must match definite necessities such as neutral pH, high enough total solid level, absence of oxygen, and near to ambient temperatures (Homayouni et al. 2008b, c). A number of dairy food bio-products have been employed and developed as delivery vehicles of probiotic bacteria. Around 80 bifid-containing products are estimated to be on the world markets. Most of these products are from dairy origin including fresh milk, fermented milk, dairy beverages, ice cream, dairy desserts, cheese, cottage cheese, and powdered milk (Tamime et al. 1995).

2.1 Definition of Probiotics

Probiotics are distinct as live microorganisms which when administered in sufficient amounts present a health benefit on the host (Homayouni 2009). In recent years probiotic bacteria have increasingly been incorporated into dairy foods as dietary adjuncts. *Lactobacillus* and *Bifidobacterium* are the most common probiotic bacteria cells that were used in the production of fermented and non-fermented dairy products.

Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO 2002; Champagne et al. 2011). This adequate amount varies from country to country; in Japan a product should contain a minimum of 107 colony-forming unit (CFU)/g of probiotic bacteria to be considered a probiotic one, while the USA has developed a standard which requires at least 108 CFU/g of the product to be labeled as probiotic (Vuyst 2000). But generally a probiotic product should contain >106–108 CFU/g or >108–1010 CFU/day of viable cells which has shown to be efficacious. Some of the species used in probiotic products are *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Bacillus*, *Propionibacterium*, *Saccharomyces*, and spore-forming and nonflagellated rod or *Coccobacilli* (Saraf et al. 2010). A microorganism can be called probiotic if fulfilling the criteria below:

1. The culture can be produced in industrial scale.
2. Can survive during production and storage.
3. Can tolerate the gut environment of the host.
4. Exerts health effects when consumed (Homayouni 2008).

2.2 Delivery Vehicles

Traditionally yogurt was the first food to present consumers with probiotics. Recently development of novel probiotic foods has attracted a great attention, and manufacturers are coming out with new products including ice cream, cheese, chocolate, beverages, cereals, and vegetable products (Randheera et al. 2010). Different forms of probiotic supplements are also available in the market including pills, capsules, tablets, gelcaps, liquids, and powders. Clinical trials investigating the health benefits of probiotics have used these bacteria either in foods or as supplements.

2.3 Prebiotics Versus Probiotics

In recent years there has been an increasing interest in the field of prebiotics and probiotics. As discussed before probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. This adequate amount varies from country to country but all in all should not be less than 108–109 CFU/g of the probiotic carrier (Vuyst 2000; Champagne et al. 2011). Some of the mostly documented health benefits for probiotics include effectiveness against diarrhea, improvement of lactose metabolism, immunomodulation, as well as anticarcinogenic, antidiabetic, hypocholesterolemic, and hypotensive characteristics (Shah 2007; Mai and Draganov 2009).

On the other hand, prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improve host health (Gibson et al. 1995). The stimulated bacteria should be of a beneficial nature, namely, *Bifidobacteria* and *Lactobacilli* (Gibson et al. 2004). Some health effects associated with prebiotic consumption are reduction in acute gastroenteritis, decrease in cancer risk, increase in mineral absorption, and lipid regulation (Gibson and Rastall 2006).

Despite the significance of these two concepts, there has been little discussion on which of them plays a more important role in public health promotion, so far. This to answer, one should first give a reply to which precedes the other (Homayoni et al. 2012).

Fetuses are in a germ-free environment before birth. After delivery intestinal microbiota colonizes the composition of which depends on factors including environmental ones like human milk, weaning, infant environment, maternal microbiota, and especially diet (Weng and Walker 2006); prebiotic foods play a major role in the context of the latter one (Cummings et al. 2001). Regarding this, we seem to have encountered prebiotics prior to probiotics. This alone surely can't justify the predominance of prebiotics. But still there are other issues to take into

account when it comes to deciding which, for promotion of the public health, is potentially a more important field to invest in.

Colonic microbial ecosystem of each man, since dependant on a great number of factors including his nutritional pattern, is identical to him; this ecosystem differences have also been reported to vary for different regions which is not surprising at all. Therefore it may better amplify the endogenous probiotics of each man by feeding these bacteria with their desired substances, prebiotics, instead of introducing unfamiliar strains to the gut in foods or supplements (Gibson and Roberfroid 1995; Cummings et al. 2001; Gibson et al. 2004).

Probiotics are susceptible to environmental stresses. Manufacturing processes like heating, metabolites produced by them during storage including acids, and gastrointestinal condition such as gastric acid and bile salts may affect the viability of these microorganisms (Gibson and Roberfroid 1995). Prebiotics are devoid of these downcomings and are stable to a great extent during storage and in the gut before they reach colon where they are fermented and consumed by the probiotics. Thus less of prebiotics will be wasted in the market or the gut; this is favorable economically.

Prebiotics are cheaper sources and affordable for the majority of the societies. They have been consumed by people as a part of their foods from the beginning of human life in the earth. Besides, people still have their doubts about consuming live bacteria and don't trust probiotics as much as prebiotics. This is not weird as they remember eating prebiotics since their very young age, while they have always been frightened of microorganisms. It is also worth mentioning that probiotic foods have been claimed to be more effective in the promotion of public health compared with probiotic supplements; the reasons include the availability of nutrients and energy sources, with prebiotics being the main source for the bacteria in the food matrix and colon. Because prebiotics could enter the colon within any change or metabolization (Homayouni et al. 2012). In other words, probiotic bacteria, when administered with the purpose of delivering health benefits, cannot function as they are supposed to nor as efficiently as desired, in the absence of prebiotics. These all put together drive us to think that the advantages of prebiotics overweigh those of probiotics, from different points of view. So, although a great attention is concentrated in the field of probiotics during the last few decades, prebiotics may be substantially a more important field to conduct studies on, which does not necessarily overlook the value of investigations seeking the effects of probiotics on human health.

The efficacy of probiotic bacteria is mainly based on two factors: viability (being live in food products and supplements) and survivability (sustaining their life through harsh conditions) as well as activity (Vrese and Schrezenmeir 2008). Several parameters including probiotic strain, pH of the matrix, nutritional component of the carrier, and heat treatment can affect viability and survivability (Kailasapathy and Chin 2000; Kolida et al. 2000; Homayouni et al. 2008a, b, c; Sleator and Hill 2008; Bazrafshan and Homayouni 2010; Randheera et al. 2010). Some techniques have been developed for increasing bacterial viability and survivability including preexposing to sublethal stresses such as salt, heat, bile and low

pH, immobilized cell technology, microencapsulation, genetic modification, combining different synergistic strains, and incorporation of nutrients and prebiotics to the matrix. Selection of the proper method depends on the type of product to be added to the probiotic bacteria (Farnworth 2008; Homayouni et al. 2008a, b, c; Sleator and Hill 2008). Factors affecting bacterial activity are water activity of the carrier, access to essential nutrients for probiotic growth, pH of the matrix, and growth promoters (Vuyst 2000).

Returning to the hypothesis posted at the beginning of this section, it is now possible to state that foods are better carriers for probiotics than supplements. This chapter has given an account of widespread use of food products for treatment of several diseases in human. The reason for this widespread application of probiotic foods may be due to the buffering properties of foods for probiotics during passage through the gut, provision of essential nutrients for maintaining the activity, and efficacy of the probiotic bacteria, synergistic effects of food ingredients on probiotic growth, and consumer attitude toward probiotic foods vs. supplementation with tablets, capsules, and other drug forms (Vrese and Schrezenmeir 2008; Randheera et al. 2010; Del Piano et al. 2011).

3 Health Benefits of Probiotic Consumption

3.1 Gastrointestinal Tract Diseases

3.1.1 Diarrhea

The major disorders related to gastrointestinal tract are diarrhea and lactose intolerance. Several evidences have shown that probiotics can help relieve the symptoms of both disorders. Alleviation of lactose intolerance symptoms by probiotic bacteria is attributed to their intracellular β-galactosidase content.

3.1.2 Metabolic Syndrome

The metabolic syndrome, a concurrence of disturbed glucose and insulin metabolism, overweight and abdominal fat distribution, mild dyslipidemia, and hypertension, is most important, because of its association with subsequent development of type 2 diabetes mellitus and cardiovascular disease. Probiotics have been showed to be connected with almost all the elements of metabolic syndrome and have revealed considerable results. It has been shown that the intestinal microbiota composition of obese subjects differs from that of lean ones. But not many clinical trials have used probiotics to modify the gut microbiota in order to combat obesity. Inflammation has a key role in development of insulin resistance. Probiotics through their

immunomodulatory effects have been shown to decrease inflammation and thus increase insulin sensitivity (Lye et al. 2009). Release of angiotensin converting enzyme (ACE) inhibitory peptides from the parent protein through proteolytic action explains how probiotics can exert antihypertensive effects (Lye et al. 2009).

3.1.3 Cancer

Cancer involves the abnormal division and reproduction of cells that can spread throughout the body (Barbara and Kathryn 2012). Eating behaviors play a very important role in health promotion and disease prevention (Kashfi 2009). One of the important dietary compounds that have a significant role in cancer treatment and/or control is probiotic. Reports on the benefits of oral administration of probiotic cultured milks and lactic acid bacteria on tumors have been connected with changes related to tumor induction and promotion (Gorbach et al. 2004). The colonic microbiota has been suggested to have a critical role in setting the tone for a healthy bowel including the risk for developing cancer (O'Keefe 2008). Key physiological functions that might be related to cancer risk include control of epithelial cell proliferation and differentiation, production of essential nutrients and/or bioactive food components, prevention of overgrowth of pathogenic organisms, and stimulation of intestinal immunity (Tappenden and Deutsch 2007). A number of studies have focused on the effect of probiotics on intestinal microecology and cancer. *Lactobacillus acidophilus*, *Lactobacillus casei* Shirota strain, and *Lactobacillus* GG have been shown to have inhibitory properties on chemically induced tumors in animals (De Roos and Katan 2000; McFarland 2000).

3.2 Immune-Related Diseases

The physiological role of immune responses is protection against infectious microbes; however even non-harmful foreign substances can stimulate immune system (Abbas and Lichtman 2006–2007). Host defense system consists of a complex array of cells and molecules, which include innate and acquired mechanisms. The innate immune system mediates the initial protection against infections that are nonspecific to a given pathogen; the main mechanism of native immunity is phagocytosis. In contrast to innate immunity, the adaptive immune system shows a high level of specificity and memory. There are two key components of adaptive immunity: humoral and cellular immunity (Gill et al. 2009). All diseases may affect the immune system. Two extremities of the variety immune-related diseases are immune deficiency and immune hypersensitivity that have been extensively studied in connection with probiotics.

Disarrays caused by imperfection immunity are called immunodeficiency diseases. When consequence from genetic defects in one or more mechanisms of the

immune system occurs, these are called primary immunodeficiency. Other imperfection in the immune response may result from pollutions, dietary, or microbes which called acquired immunodeficiency (Abbas and Lichtman 2006–2007). Among the acquired diseases, the acquired immunodeficiency syndrome (AIDS). AIDS is a human immunity disorder caused by the human immunodeficiency virus (HIV) (Sepkowitz 2001). At the beginning of infection with HIV, several million viruses may exist per milliliter of blood (Piatak et al. 1993). These responses reduce numbers of circulating CD4+ T-cells, consequently infections with a variety of opportunistic microbes extend. This acute viremia is linked in approximately all patients with the activation of CD8+ T-cells, which kill HIV-infected cells and therefore produce antibody or seroconversion. The symptoms of AIDS patients include weight loss, respiratory tract infections, prostatitis, and skin rashes. Diarrhea is a common complication in patients with HIV infection (Pantaleo et al. 1997).

The adaptive immune reaction provides particular resistance against infection with microorganisms, but some immune responses have increase to an excessive and unsuitable effect; this is usually called hypersensitivity. There are four most important types of hypersensitivity: type (I), allergic reaction occurs when a person's immune system answers to normally benign substances in the environment replies; they produced through release of particular mediators. Type (II) hypersensitive reactions are mediated by antibodies binding to specific cells or tissues. In type (III), immune complements are placed in the tissue causing local tissue damage and swelling, those due to autoimmune disorders such as inflammatory bowel disease, rheumatoid arthritis, and type (I) diabetes mellitus. In type (IV) hypersensitive answers, T-cell effectors secrete lymphokines when encountering the same antigen for the second time which ends in inflammatory responses (Abbas and Lichtman 2006–2007; Brostoff et al. 2006). In the next session, the beneficial effects of probiotics on autoimmune diseases, allergies, and AIDS, as well as enhancing the quality of patients live, will be discussed.

3.2.1 Effect of Probiotic on Immune-Related Disease

Several in vivo and in vitro investigations have shown that particular strains of probiotics are able to modulate the performance of the immune system, stimulate the immune function to defend against infectious disorders and cancers, and regulate overexpressed immune responses connected with immuno-inflammatory diseases such as allergy and IBD (Gill and Guarner 2004).

3.2.2 Probiotic and AIDS

HIV/AIDS is a prominent problem in the human race which revolutionizes the human landscape in the world. Comparatively the minority patients obtain antiretroviral therapy, and acute diarrhea influence on their quality of life. Utilization of

probiotics improves immune response and feature of AIDS patients' life (Suttajit 2007). *L. plantarum* 299v supplementation in the HIV-positive child could shorten acute diarrhea (Cunningham-Rundles et al. 2000). 2.5×10^{10} CFU of the *B. bifidum* and *S. thermophilus* in formula showed that an increase in the CD4+ count in children with HIV infections (Trois et al. 2007). Anukam et al. (2008) demonstrated rising in the mean CD4+ count in the AIDS female by *L. rhamnosus* and *L. reuteri* at dose of 2.5×1012 CFU/day. It was shown that consuming yogurt supplemented with 1010 CFU/day of the probiotic strain *L. rhamnosus* may successfully improve gastrointestinal symptoms and alleviate tolerance to antiretroviral treatment (Irvine et al. 2011).

3.2.3 Probiotic and Autoimmune Diseases

There are two significant categories of autoimmune diseases: immune system attack to particular organs such as stomach, pancreas, adrenal, and thyroid; the other one is the non-organ-specific diseases that contain the rheumatological disorders. Regulation of enteric microbiota combination by probiotics may alleviate the symptoms of some autoimmune disorders. Superlative studies in this regard are inflammatory bowel disease (IBD), rheumatoid arthritis, and type (I) diabetes mellitus (Brostoff et al. 2006). Inflammatory bowel disease (IBD) comprises two discrete disorders: Crohn's disease (CD) and ulcerative colitis (UC). Pouchitis is another disease that consequences from complex ileal pouch-anal anastomosis (IPAA) surgery for UC. Some studies showed the potential of probiotic supplementation in the prohibition and/or treatment of various inflammatory bowel disorders (Matsumoto et al. 2005; Gill et al. 2009). Same strains of *Lactobacilli*, *Bifidobacteria*, and *Streptococcus salivarius* ssp. thermophilus (VSL #3) deferred the first onset of acute pouchitis and improved life quality of people with ileal pouch-anal anastomosis (Gionchetti et al. 2000, 2003). Supplementation with $1–2 \times 1010$ CFU *L. rhamnosus* GG in capsule during 3 months changed the intestinal microbiota, but endoscopic or clinical response was not improved (Kuisma et al. 2003). *L. rhamnosus* GG at dose of 1.4×1010 CFU in the fermented food product delayed episodes of pouchitis (Gosselink et al. 2004). Dose of 6×1011 CFU/day of (VSL #3) maintained antibiotic introduced remission (Mimura et al. 2004).

The roles of beneficial microbes in administration active UC have also been reported in literature. The probiotic *E. coli* Nissle at dose of $5–50 \times 109$ CFU demonstrated to have a clinical effect similar to that obtained by the Mesalazine (Kruis et al. 2004). 1.8×1010 CFU of *L. rhamnosus* GG in tablet seems to be beneficial and harmless for maintaining remission in patients suffering from UC (Zocco et al. 2006). In patient with moderate UC, using VSL#3 supplementation was safe and effective in achieving clinical responses and remissions (Sood et al. 2009; Tursi et al. 2010). In a double-blind clinical study, the efficiency of rectal *E. coli* Nissle at dose of $1–4 \times 109$ CFU/day consumption was significant in per protocol analysis in moderate distal UC (Matthes et al. 2010). In pediatric patients with active distal UC, rectal infusion of *L. reuteri* in enema solution with

1010 CFU/day was effective in altering mucosal expression levels of some cytokines which improved mucosal inflammation (Oliva et al. 2012).

Decline in disease activity have been attained in children with CD by probiotic intervention (Gupta et al. 2000). The yeast *Saccharomyces boulardii*, when administered 1 gr tree times a day with Mesalamine in capsule, remained in the remission in CD patients (Guslandi et al., 2000). *L. rhamnosus* GG supplementation was unable to reduce the risk of postoperative recurrence in CD (Prantera et al. 2002; Bousvaros et al. 2005).

Evidences have revealed that there is a correlation between the gastrointestinal microbiota and the development of rheumatoid arthritis. Using capsules containing 1010 CFU of *L. rhamnosus* GG did not show considerable difference in the activity of rheumatoid arthritis, although reported improvement in subjective well-being (Hatakka et al. 2003). Two-month supplementation of *Bacillus coagulans* with 2×109 CFU appeared to be safe and effective for patients with rheumatoid arthritis (Mandel et al. 2010). After 3 months consuming capsules containing 4×109 CFU of *Lactobacillus rhamnosus* and *Lactobacillus reuteri* with rheumatoid arthritis, patients did not show clinical improvement, but functional improvement was seen (Pineda et al. 2011).

3.2.4 Probiotic and Allergy

The incidence of allergic diseases is growing dramatically in both developing and developed countries; this increase is particularly problematic in children. Atopic dermatitis (AD), asthma, and allergic rhinitis represent the most common chronic disease (Pawankar et al. 2011–2012). Alteration of the intestinal microbiota or lack of beneficial microbes during childhood is factor in the increased prevalence of allergic diseases (Ghadimi et al. 2008). Beneficial bacteria showed competently downregulate inflammation related with hypersensitivity responses in patients with atopic eczema and food allergy (Majamaa et al. 1995; Dugas et al. 1999). The application of 5×109 CFU/day of the probiotic strains *L. rhamnosus* and *L. reuteri* significantly altered the production of the cytokines IL-2, IL-4, IL-10, or IFN-γ; thereupon they improved clinical symptoms in children with AD (Rosenfeldt et al. 2003). In a clinical study in children with cow's milk allergy and AD, it was illustrated that the utilization of *L. rhamnosus* GG at dose of 5×109 CFU/day for 4 weeks increased production of IL-10 and IFN-γ (Pohjavuori et al. 2004). *L. gasseri* and *L. coryniformis* at a dose of 2×108 CFU/day for each strain in yogurt decreased IgE in plasma and increased regulatory T-cells (CD4+/CD25+) in pediatric suffering from allergic (Martinez-Canavate et al. 2009).

3.3 Coronary Heart Diseases

Coronary heart disease (CHD) is one of the major causes of death in adults in the developed and developing countries which are referred to the condition in which the main coronary arteries supplying the heart are no longer able to supply sufficient blood and oxygen to the heart muscle (myocardium). The main cause of the reduced flow is an accumulation of plaques, mainly in the intima of arteries, a disease known as atherosclerosis (Akbarzadeh and Toufan 2008). A number of risk factors known to affect an individual to CHD have been categorized such as hyperlipidemia (high levels of lipids in the blood), hypertension (high blood pressure), obesity, cigarette smoking, and lack of exercise. Probiotics as a live microbial food supplement may beneficially affect the host by improving its intestinal microbial balance and are generally consumed as fermented milk products containing *Bifidobacteria* and/or *Lactobacilli*. The supposed health benefits of probiotics include improved resistance to gastrointestinal infections, reduction in total cholesterol and TAG levels, and stimulation of the immune system. A number of mechanisms have been proposed to explain their putative lipid-lowering capacity and these include a "milk factor," which has been thought to inhibit HMG-CoA reductase and the assimilation of cholesterol by certain bacteria. The mechanism of action of probiotics on cholesterol reduction includes physiological actions of the end products of fermentation SCFAs, cholesterol assimilation, deconjugation of bile acids, and cholesterol binding to bacterial cell walls. It has been well documented that microbial bile acid metabolism is a peculiar probiotic effect involved in the therapeutic role of some bacteria. The deconjugation reaction is catalyzed by conjugated bile acid hydrolase enzyme, which is produced exclusively by bacteria. The mechanism of cholesterol binding to bacterial cell walls has also been suggested as a possible explanation for hypocholesterolemic effects of probiotics. Probiotics have received attention for their beneficial effects on the gut microbiota and links to their systemic effects on the lowering of lipids known to be risk factors for CHD, notably cholesterol and TAG (Ranjbar et al. 2007).

Investigations have revealed the possible hypocholesterolemic properties of milk products, especially in a fermented form. 18 % fall in plasma cholesterol after feeding 4–5 l of fermented milk per day for 3 weeks (Mann and Spoerry 1974). The mechanisms of action of probiotics on cholesterol reduction are physiological actions of the end products of fermentation SCFAs, cholesterol assimilation, deconjugation of bile acids, and cholesterol binding to bacterial cell walls. The SCFAs that are produced by the bacterial anaerobic breakdown of carbohydrate are acetic, propionic, and butyric. It has been well documented that microbial bile acid metabolism is an irregular probiotic effect involved in the therapeutic role of some bacteria. The deconjugation reaction is catalyzed by conjugated bile acid hydrolase enzyme, which is produced exclusively by bacteria. Deconjugation ability is widely found in many intestinal bacteria including genera *Enterococcus*, *Peptostreptococcus*, *Bifidobacterium*, *Fusobacterium*, *Clostridium*, *Bacteroides*, and *Lactobacillus* (Hylemond 1985). This reaction releases the amino acid moiety and the

deconjugated bile acid, thereby reducing cholesterol reabsorption, by increasing fecal excretion of the deconjugated bile acids. Many in vitro studies have investigated the ability of various bacteria to deconjugate a variety of different bile acids. Bifidobacterium longum is the most efficient bacterium when tested against six different bile salts (Grill et al. 1995). Also *Lactobacillus* species had varying abilities to deconjugate glycocholate and taurocholate (Gilliland et al. 1985). Oral administration of certain bacterial species led to an increased excretion of free and secondary bile salts (Marteau et al. 1995; De Smet et al. 1998). Certain bacteria can assimilate cholesterol *L. acidophilus* and *B. bifidum* that had the ability to assimilate cholesterol in in vitro studies, but only in the presence of bile and under anaerobic conditions (Rasic et al. 1992; Gilliland et al. 1985). However, despite these reports there is uncertainty whether the bacteria are assimilating cholesterol or whether the cholesterol is coprecipitating with the bile salts. Klaver and Van Der Meer (1993) concluded that the removal of cholesterol from the growth medium in which *L. acidophilus* and a *Bifidobacterium* sp. were growing was not due to assimilation, but due to bacterial bile salt deconjugase activity. The same question was addressed by Tahri et al. (1995) with conflicting results, and they concluded that part of the removed cholesterol was found in the cell extracts and that cholesterol assimilation and bile acid deconjugase activity could occur simultaneously. The mechanism of cholesterol binding to bacterial cell walls has also been suggested as a possible explanation for hypocholesterolemic effects of probiotics. Hosono and Tono-Oka (1995) reported *Lactococcus lactis* subsp. biovar had the highest binding capacity for cholesterol of bacteria tested in the study. It was speculated that the binding differences were due to chemical and structural properties of the cell walls, and that even killed cells may have the ability to bind cholesterol in the intestine. The mechanism of action of probiotics on cholesterol reduction could be one or all of the above mechanisms with the ability of different bacterial species to have varying effects on cholesterol lowering.

It has been demonstrated that microbial bile acid metabolism is a main effect in the therapeutic role of probiotic bacteria. The deconjugation reaction is catalyzed by conjugated bile acid hydrolase enzyme, which is produced by *Bifidobacterium* and *Lactobacillus*. This reaction releases the amino acid and deconjugated bile acid, which is reducing cholesterol reabsorption, by increasing fecal elimination of the deconjugated bile acids.

3.4 Bacterial Vaginosis

Bacterial vaginosis (BV) is the most common urogenital disease in women, affecting about 19–24 % of them in generative ages (Parent et al. 1996). BV is supposed to occur as a result of an imbalance in the normal vaginal microbiota (Eriksson et al. 2005). BV develops when the normal *Lactobacillus* bacteria in the vagina are disrupted and afterwards replaced by predominantly anaerobic bacteria including *Gardnerella vaginalis*, *Mycoplasma hominis*, *Prevotella*, and *Peptostreptococcus*

(Ya et al. 2010). Other bacteria such as *E. coli* from the rectum have also been shown to cause this disease. *Lactobacilli*, by producing a natural antibacterial agent such as hydrogen peroxide, keep the healthy normal balance of vaginal microorganisms (Mastromarino et al. 2009). BV is mostly followed by irritating symptoms mainly foul, fish-like, or musty odor which is sometimes stronger after a woman had sex, watery or foamy and white (milky) or gray vaginal secretions, itching on the outside of the vagina, and burning or discomfort during urination (Razzak et al. 2011). The most common oral treatment for BV in both pregnant and nonpregnant women is metronidazole and clindamycin (Joesoef et al. 1999). As many as 30 % of women relapse within one month of treatment, with unprompted relapse occurring more commonly among women treated with topical compared with systemic antibiotics (Sobel et al. 2001). Since antimicrobial treatment of urogenital infections is not constantly effectual and problems remain due to bacterial and yeast resistance, recurrent infections, as well as side effects, it is no wonder why alternative medicines are sought for by patients and their caregivers (Reid and Bruce 2003; Cribby et al. 2009).

Probiotic may promote or support a beneficial balance of the microbial population of the gastrointestinal tract (Holzapfel et al. 2001; Homayouni et al. 2008a, b, c). When ingested, some of these probiotic microorganisms are able to resist the physicochemical conditions prevailing in the digestive tract (Salminen et al. 1998). A beneficial effect of "lactic acid-producing" microorganisms on vaginal microbiota has been suggested more than 100 years ago (Döderlein 1892). The basis for use of probiotics in BV treatment emerged in 1973, when healthy women with no history of urinary tract infections (UTI) were reported to have *Lactobacilli* in their vagina (Reid and Bruce 2003). *Lactobacillus* microorganisms that predominate in the vagina of healthy women spread from their rectum and perineum and form a barrier to the entry of uropathogens from vagina into the bladder (Reid et al. 2001). Probiotics are believed to protect the host against infections by means of several mechanisms including (1) occupation of specific adhesion sites at the epithelial surface of the urinary tract; (2) maintenance of a low pH and production of antimicrobial substances including organic acids, hydrogen peroxide, and bacteriocins; (3) degradation of polyamines; and (4) the production of surfactants with antiadhesive properties (Anukam et al. 2006; Goldin and Gorbach 2008). Probiotics have been administered both orally and vaginally in BV treatment; however it is still not clear as to which route is more efficient. Foods and supplements have been used as carriers when oral administration was aimed (Reid et al. 2001; Homayoui et al. 2014).

3.5 Diabetes

Type 2 diabetes mellitus (T2DM) has rapidly increased in the world during the past few decades. Experimental and clinical evidence has suggested that oxidative stress plays a major role in the pathogenesis and progression of diabetes and its

complications (Ceriello and Motz 2004; Stephens et al. 2009). Diabetes is usually accompanied by an increased production of free radicals and impaired antioxidant defenses (Lipinski 2001; Maritim et al. 2003). These conditions can lead to cellular organelle damage, the dysfunction of enzymes, an impairment of the binding of paraoxonase-1 to high-density lipoprotein and protection against lipid peroxidation, and the development of insulin resistance and may explain the presence of inflammation in T2DM (Lipinski 2001; Maritim et al. 2003; Ceriello and Motz 2004; Zozulinska and Wierusz-Wysocka 2006). Studies have shown that special strains of lactic acid bacteria have antioxidant properties (Lin and Yen 1999; Uskova and Kravchenko 2009). The antioxidative mechanisms of probiotics could be assigned to reactive oxygen species scavenging, metal ion chelation, enzyme inhibition, and the reduction activity and inhibition of ascorbate autoxidation (Lin and Yen 1999). In healthy persons, the consumption of goat milk fermented with *Lactobacillus fermentum* ME-3 has been shown to increase total antioxidative status (TAS) and decrease markers of oxidative stress (Kullisaar et al. 2003; Songisepp et al. 2005). The antioxidative properties of other probiotic strains have also been reported in healthy persons (Naruszewicz et al. 2002; Chamari et al. 2008). Studies using animal models of diabetes have also shown that *Lactobacillus acidophilus* and *Lactobacillus casei* attenuate oxidative stress and have antidiabetic effects (Yadav et al. 2007; Harisa et al. 2009). Alterations in gut microbiota composition have recently been documented in patients with T2DM, providing a target for probiotic intervention (Larsen et al. 2010). Modification of gut microbiota by probiotics may be seen as a novel means of regulating glucose metabolism and improving oxidative stress in T2DM.

3.6 Oral Health and Dentistry

The mouth or oral cavity is the first part of the gastrointestinal tract. Mouth temperature is optimal for bacteria to grow; there is adequate moisture, frequent serving of nutrients, and a variety of different surfaces for microbial attachment. These include mucosal surfaces of keratinized and nonkeratinized epithelium, dental hard tissues (enamel, dentin, and dental root cement), and often man-made appliances such as dental prostheses and dental restorations. Hence, more than 700 microbial species have been identified in the mouth, and it is estimated that each individual may carry 200–300 species at the same time. Microorganisms are found free in saliva, covering mucosal surfaces, and organized in supra- and subgingival dental plaque. In plaque the microorganisms form highly structured biofilms where bacteria appear to communicate with each other. 1 mg of plaque may contain up to 1011 bacteria. The viridans group *Streptococci* constitutes the majority of the indigenous oral microbiota, but practically all human pathogenic bacteria known occasionally may also harbor in the mouth. In addition, yeasts are

prevalent in the oral microbiota and in particular in elderly individuals. *Candida albicans* is the predominant species of yeast in the oral cavity.

Based on the experience from using probiotic preparations mainly in connection with gastrointestinal diseases, partly the same mechanisms of function can be anticipated to affect also the oral cavity after probiotic administration. Several bacterial strains have been investigated experimentally and in clinical trials for their anticipated probiotic effects on oral and dental diseases.

L. rhamnosus strain GG is one of the most extensively studied probiotic bacteria, and it is of particular interest for oral biology since the bacterium does not readily ferment sucrose and might hence be more "safe for teeth" than other lactic acid-producing bacteria. Later clinical studies have indeed shown its efficacy in caries prevention in children and in controlling oral *Candida* in the elderly. The probiotic milk fermented by *L. rhamnosus* was found to reduce the risk of caries significantly (OR = 0.51, $p = 0.004$). The effect was particularly clear in the 3- to 4-year-olds. The probiotic cheese contained *Lactococcus lactis* and *L. helveticus* starter cultures with added *L. rhamnosus* GG, *L. rhamnosus* LC705, and *Propionibacterium freudenreichii* subsp. Shermanii JS, while the control cheese only contained the starter culture of *Lactococcus lactis* with no probiotic strains (Hatakka et al. 2003). 32 % decrease in the prevalence of high yeast count in the probiotic group increase in the control group from 28 to 34 %. Probiotic intervention reduced the risk of high yeast counts by 75 %. Also, other *Lactobacillus* strains have been investigated with respect to their effect on oral microorganisms, in particular with dental caries prevention in mind. For example, *L. reuteri*, *L. casei*, and *L. acidophilus* have been found to inhibit cariogenic *Streptococci* both in experimental and clinical studies.

Adhesion capacity to oral surfaces and antimicrobial effect of the putative probiotic species are key characteristics needed for successful effect on oral microbiota. Hence, systematic investigations are needed in this regard when screening potential probiotic bacteria. Research data accumulated so far have shown huge differences in the attachment of putative probiotic strains onto surfaces in test models that mimic oral tissues (Haukioja et al. 2006). The *Lactobacillus* strains that are effective against oral *Streptococci* and periodontal microorganisms do not necessarily inhibit *Candida*. *L. salivarius* and *L. gasseri* might be good candidates as potential probiotic strains against dental infections but not for oral yeast infections (Strahinic et al. 2007).

According to the current knowledge, different probiotic strains are needed for different indications. Hence, no single bacterial strain is expected to be suitable for all the oral and dental problems, whether symptoms or disease states, where probiotic therapy is being considered. Because dental diseases in particular pose such a big socioeconomic burden to mankind, not to mention about individual suffering, there is great potential in the future for oral probiotics.

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Application of Active Edible Film as Food Packaging for Food Preservation and Extending Shelf Life

Pimonpan Kaewprachu and Saroat Rawdkuen

Abstract The shelf life of food is defined as the duration of time from its date of manufacture to the date it is no longer acceptable for a consumer. Factors that affect food quality vary: processing, storage, delivery, chemical reactions, and physical and biological changes that occur naturally in the food. In general, the major factor that determines shelf life is the rate of chemical change. The factors that contribute to food shelf life are normally classified as intrinsic and extrinsic. Packaging is the most important extrinsic factor for extending shelf life. When the properties of the packaging material are changed, the shelf life is directly altered and may be undesirable. Packaging functions as a barrier to food spoilage and thereby extending shelf life and maintaining the desirable appearance of the product. Moreover, packaging plays a role in storage and delivery of the food products to retailers and consumers. It is not only important to simply package food. It is becoming equally important for packaging to serve more active functions such as being antimicrobial and antioxidant, being intelligent to limit moisture transfer or having air absorption, and also displaying the expiry date and recommendations for consumption. Films have been used for a long time for packing food, and now they are undergoing advanced development. Film or coating is a thin layer of material used for wrapping or coating layers between the foods. Materials currently used for film manufacturing are bio-based or naturally biodegradable and are environmentally friendly. These newer film materials have replaced conventional plastic films because of their harm to the environment. Active biodegradable packaging, edible packaging, and other alternatives have become increasingly interesting and widely researched and applied. Not only are they interesting for researchers but also for governments and industrial sectors. According to the benefits of these alternative packagings, much research is published every year focusing on its application to both meat and vegetable products. The objective of this review is to summarize and present the work currently in progress about active edible films as well as natural biodegradable films that are normally applied in food products in order to prevent their spoilage and extend their shelf life.

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1 Introduction

Food products are highly prone to microbial deterioration since they are rich in essential nutrients and perishable in nature. This is further accelerated by some intrinsic factors including pH and water activity of food materials. In general, most fresh food, especially fresh meat, has a water activity value higher than 0.85, and its pH value falls within the favorable pH range for spoilage microorganism (Dave and Ghaly 2011). A significant level of spoilage of food products takes place at different levels of the production steps. Not only chemical reaction but enzymatic spoilage and microbial growth also play a significant role in food deterioration.

To produce the safest and highest-quality products possible, several thermal and nonthermal food preservation techniques have been used, alone or in combination, to prevent or minimize the growth of spoilage, pathogenic microorganisms, and lipid oxidation such as conventional heating, chilling, drying, freezing, etc. Unfortunately, some of these techniques cannot be fully applied to some food products because of the nutrition loss of the final product if the severe processing techniques are applied. Therefore, the use of packaging films or coating containing antimicrobial or antioxidant agents is an alternative way to solve this limitation that acts as active packaging.

Active packaging is a system in which the product, the package, and the environment interact in a positive way to extend shelf life or improve microbial safety or sensory properties while maintaining the quality of food products. This active packaging could be more efficient to control the microbial growth, enzymatic deterioration, and oxidation of lipid in the products. Antimicrobial substances incorporated into packaging materials can control microbial contamination by reducing the growth rate and maximum growth population and/or extending the lag phase of the target microorganism or by inactivating microorganisms by contact. Nowadays, a biomaterial resource has interest to use as biodegradable-based films due to being environmentally friendly such as polysaccharide, protein, and lipid. These biodegradable-based films could be incorporate active agents and applied with many types of foods to delay or prevent the food quality deterioration caused by microbial growth and chemical reactions. So, the food shelf life can be extended.

2 Shelf Life and Factors

The quality of foods is decrease with time, it follows that there will be a finite length of time before the product becomes unacceptable. This time from production to unacceptable is referred to as “shelf life.” In addition, “shelf life” refers to the time on the retailer’s shelf as well as the consumer’s shelf. Inadequate shelf life will often lead to consumer dissatisfaction and complaints, and it can lead to loss in nutrition or cause illness. Shelf life may be defined in different ways depending on the

specific purpose. The Institute of Food Science and Technology (IFST) in the United Kingdom has defined the "shelf life" as "the period of time during which the food product will remain safe; be certain to retain desired sensory, chemical, physical, microbiological and functional characteristics; and comply which any label declaration of nutritional data when stored under the recommended conditions" (IFST 1993). Another definition is that "the period of time under defined conditions of storage, after manufacture or packing, for which a food product will remain safe and be fit for use" (Man 2002) or "the period of time from the production and packaging of a product to the point at which the product first becomes unacceptable under defined environment conditions" (Lee et al. 2008).

There are many factors that can influence the shelf life and can be categorized into two factors: the nature of the food and its surrounding. These factors are referred to as intrinsic and extrinsic factors. They include the following:

2.1 Intrinsic Factors

Intrinsic factors are an inherent part of the food and include water activity (a_w), pH, oxidation-reduction potential (E_h), oxygen content, and nutrients.

Water Activity (a_w) The parameter a_w is defined as the ratio of the water vapor pressure of a food to the vapor pressure of pure water at the same temperature. Water activity values have been widely used to indicate the stability of foods with respect to the potential for microbial growth, chemical, and biochemical changes and physical transfer such as moisture migration (Labuza and Hyman 1998). Humectants such as invert sugar, glycerol, dextrose, and various glucose syrups are used in food formulation to influence this potential (Man 2002).

Oxidation-Reduction Potential (E_h) The oxidation-reduction potential is a physicochemical parameter that determines the oxidizing or reducing properties of the medium, and it depends on the composition of food, pH, temperature, and a concentration of dissolved O_2. E_h play an important role in the cellular physiology of microorganisms such as growth capacity, enzyme expression, and thermal resistance.

Product Composition and Formulation The composition of a food product can be the single most important shelf life-determining factor in many food products. Fried foods contain a significant percentage of oil, the quality of which is of parameter important to determining their shelf life due to the fact that it can cause lipid oxidation, resulting in developing of off-odor and flavor (rancidity) and short shelf life. Research has demonstrated that besides an adequate heat process and effective temperature control during storage and distribution, product formulation is a critical factor in determining the shelf life of foods (Gould 1999).

pH Value and Acidity The pH value of a food product varies according to its composition and formulation, and it needs to be controlled where acidity has a

major influence on the shelf life and safety of the product. The pH of a system is related to the concentration of hydrogen ions which, in the case of food, come from "acid" ingredient that dissociate in water, releasing them in the process. The pH of food product may vary during its shelf life, as a result of changes taking place in the food, e.g., mold growth.

2.2 Extrinsic Factors

Extrinsic factors that control the rates of reaction include temperature, relative humidity (RH), gas atmosphere, light, and packaging that can influence on the rate of reactions, depending on the specific packaging material.

Temperature A key factor in determining the rates of deteriorative reactions and in certain situations the packaging material can affect the temperature of foods.

Relative Humidity The RH of the ambient environment is important and can influence a_w of the food.

Gas Atmosphere The presence and concentration of gases in the environment surrounding the food have a considerable influence on the growth of microorganisms.

Light Many deteriorative changes in the nutritional quality of foods are initiated or accelerated by light. The intensity of light and the length of exposure are significant factors in the production of discoloration and flavor defects in foods.

Packaging Important for protecting food products which are fragile or sensitive to oxygen, moisture, or light and prevents contamination during transport, storage, and distribution. Suitable packaging materials also offer a barrier against light, gaseous exchange, and/or moisture vapor transfer, protecting the food from many of the deteriorative changes that can be limiting the shelf life of foods.

3 Food Packaging and Functions

Food packaging is simply the enclosure of food product in a plastic pouch, a metal can, or a glass bottle. It is a coordinated system designed for the efficient delivery of high-quality, safe food product throughout every phase of the supply chain, from raw material production to food manufacture, packing, retail, wholesale, consumer use, disposal and recycling, or other means of resource recovery (Lee et al. 2008).

The Packaging Institute International defines packaging as the enclosure of products and items, packaged in a wrapped pouch, bag, box, cup, tray, can tube, bottle, or other container form, to perform one or more of the following functions: containment, protection, preservation, communication, utility, and performance

(Anonymous 1988). Other definitions of packaging include a coordinated system of preparing goods for transport, distribution, storage, retailing and end use, a means of ensuring safe delivery to the ultimate consumer in sound condition at optimum cost, and a techno-commercial function aimed at optimizing the costs of delivery while maximizing sales (and hence profits) (Coles et al. 2003).

Packaging performs a series of disparate tasks: it protects contents from contamination and spoilage, makes it easier to transport and store goods, and provides uniform measure of contents. (Hine 1995). A package must do three things: it must protect the contents, promote the product, and inform the consumer. A fourth function, convenience, is closely related to promotion since convenient packages promote sales (Doyle 1996). Four primary functions of packaging have been identified: containment, protection, convenience, and communication. These four functions are interconnected and all must be assessed and considered in the package development process (Robertson 2006).

Containment The containment function of packaging makes a huge contribution to protecting the environment from the myriad of products which are moved from one place to another on numerous occasions each day in any modern society.

Protection To protect the food from physical damage, physicochemical deterioration, microbial spoilage, and product tempering is probably the most important function of packaging. Good packaging is required to protect against loss of hermetic condition and microbial penetration (Lee et al. 2008).

Convenience This is an important function to satisfy the busy consumer lifestyle. Examples of convenient food packages are ready-to-eat meals, heat-and-eat meals, and shelf-heating packages or easy opening, resealability, and microwavability (Lee et al. 2008).

Communication The package communicates with the consumer through written texts, brand logo, and graphics. In many countries, nutritional facts such as calories, fat, cholesterol, and carbohydrates are required on all food packaging (Lee et al. 2008).

4 Active Packaging

In the recent years, new food packaging technologies are developing as a response to consumer demands or industrial production trends toward mildly preserved, fresh, tasty, and convenient food products with prolonged shelf life and controlled quality. Active packaging is the main area in which most of recent ideas have been applied to satisfy these needs and redefining the function of food packaging (Lee et al. 2008). In addition, changes in retailing practices or consumers' way of life present major challenges to the food packaging industry and act as driving forces for the development of new and improved packaging concepts that extend shelf life while maintaining and monitoring food safety and quality.

Active packaging is a system in which the product, the package, and the environment interact in a positive way to extend shelf life or improve microbial safety or sensory properties while maintaining the quality of food products (Han 2000; Quintavalla and Vicini 2002). A multinational European study, Acti Pack, has also defined it as a packaging system that actively changes the condition of the packaged food to extend shelf life or to improve food safety or sensory properties while maintaining the quality of the packaged food (Ahvenainen 2003).

All active packaging technologies involve some physical, chemical, or biological action for altering the interactions between the package, the product, and the package headspace to achieve certain desired outcome (Lee et al. 2008). They can be divided into three categories of absorber, releasing system, and other systems. *Absorbing system* is a group of technologies that use packaging films or sachets to remove undesired gases and substances (such as oxygen, carbon dioxide, moisture, ethylene, and taints) from the package so that a favorable internal package environment and food condition are achieved (Rooney 1995; Vermeiren et al. 1999). *Releasing system* is a group of technologies that actively add or emit desired or active compounds (such as carbon dioxide, ethanol, antimicrobials, antioxidants, and nutraceuticals) to protect and enhance food quality (Lee et al. 2008). Most attention of controlled release concept has been focused on antimicrobial packaging and antioxidant packaging.

4.1 Antimicrobial Film Packaging

Antimicrobial packaging is a system that can kill or inhibit the growth of microorganisms and thus extend the shelf life of perishable products and enhance the safety of packaged food products (Han 2000). Most food packaging systems consist of the food products, the headspace atmosphere, and the packaging materials. Any one of these three components of food packaging systems could possess an antimicrobial element to increase antimicrobial efficiency. Antimicrobial packaging can take several forms including:

1. Addition of sachets/pads containing volatile antimicrobial agents into packages—oxygen absorbers, moisture absorbers, and ethanol vapor generators are the main types of sachets (Appendini and Hotchkiss 2002).
2. Incorporation of volatile and nonvolatile antimicrobial agents directly into polymers—one way is the addition of antimicrobial agents into the melt form of polymer, and the other is the addition into the wet polymer solution. Packaging materials should be in contact with the surface of the food for the diffusion of the nonvolatile agent to the surface of the food (Ouattara et al. 2000). For the volatile antimicrobial agents, packaging materials do not need to be in contact with the surface of the food (Appendini and Hotchkiss 2002; Suppakul et al. 2003).
3. Coating or absorbing antimicrobials onto polymer surface—this technique is generally used for the antimicrobials which are sensitive to high temperature,

such as enzymes, and cannot be used in polymer processing, so they are often coated onto the material or added to cast films (Guillard et al. 2009).
4. Immobilization of antimicrobials to polymers by ion or covalent linkages—if both antimicrobial agent and the polymer have functional groups, immobilization of the antimicrobial agents to polymers by ionic or covalent bonding occurs.
5. Use of polymers that are inherently antimicrobial—cationic polymers such as chitosan and poly-L-lysine are inherently antimicrobial and have been used in films and coatings. These polymers interact with negative charges on the cell membrane and cause the leakage of their intracellular components (Appendini and Hotchkiss 2002).

4.2 Antioxidant Film Packaging

Antioxidant can be incorporated into or coated onto food packaging material to control the oxidation of fatty components and pigments and thus can contribute to the quality preservation of foods (Vermeiren et al. 1999). The antioxidants incorporated into plastic packaging materials may have the dual role of protecting the polymer as well as the packaged food from oxidation (Waite 2003). The forms of antioxidant packaging also use the same process with antimicrobial packaging as mentioned above. Antioxidative packaging can retard the oxidative reactions of fatty ingredients in any packaged foods.

Antimicrobial and antioxidant agents can incorporate into edible films. They can release onto the surface of food to control or inhibit microbial growth and control the oxidation of fatty components and pigments. Edible films have become popular in the food industry because they produce less waste, are cost effective, and offer protection after the package has been opened. Components of edible films can be divided into three categories: hydrocolloids, lipids, and composites (Cha and Chinnan 2004). Hydrocolloids include proteins (such as soy protein, whey protein, collagen and gelatin, and corn zein) and polysaccharides (such as starch, alginate, cellulose derivatives, chitosan, and agar). Lipids include waxes, acylglycerols, and fatty acids. Composites contain both hydrocolloid components and lipids. The choice of materials for a film or coating is largely dependent on its desired function.

5 Active Compounds Incorporated in the Film Packaging

Growth of microorganisms on the surface of packaged food products and oxidation of fatty components and pigments are the predominant cause of spoilage, which may be counteracted using antimicrobial and antioxidant compounds. Loss of color, desired flavor, or texture and generation of off-flavors are among the physiological and chemical deterioration processes that lower product quality. Edible films are conducive to the use of antimicrobials and antioxidants. Incorporated active

compound into edible films to act as active packaging allows improvement of nutritional and quality aspects without destroying the integrity of the food product.

5.1 Antimicrobial Agents

Antimicrobial agent can be described as a chemical or natural compound that either destroys or inhibits the growth of microscopic and submicroscopic organisms. Various antimicrobial agents could be incorporated into conventional food packaging and material to create new antimicrobial packaging systems. Table 1 shows potential antimicrobial agents and food-grade preservatives. A range of agents has been selected with the aim of prolonging shelf life and ensuring food safety. Antimicrobial agents can be divided into two categories according to their sources as chemical antimicrobial agents and natural antimicrobial agents (Appendini and Hotchkiss 2002). Table 2 shows that significant progress has been made by effectively integrating antimicrobial agents into various biodegradable polymers, particularly polysaccharides and protein-based films. Such antimicrobial films have demonstrated inhibitory activity against the growth of various microorganisms. Understandably, the physico-mechanical properties of the films are other important aspects to be considered when designing the film for food packaging applications.

Table 1 Examples of antimicrobial agents for potential use in food packaging materials

Classification	Antimicrobial agents
Bacteriocin	Nisin, pediocin, lacticin
Enzyme	Glucose oxidase, lysozyme, lactoperoxidase
Organic acids	Malic acid, sorbic acid, acetic acid, propionic acid, lactic acid, benzoic acid, citric acid
Acid salts	Potassium sorbate, sodium benzoate
Acid anhydrides	Sorbic anhydride, benzoic anhydride
Para-benzoic acid	Propylparaben, methylparaben, ethylparaben
Polysaccharide	Chitosan
Plant/spice extracts	Grapefruit seed extract, green tea extract, cinnamon oil, clove, rosemary, lemongrass, oregano
Fatty acids	Lauric acid, palmitoleic acid
Antibiotic	Natamycin
Fungicides	Benomyl, imazalil, sulfur dioxide
Phenolics	Catechin, cresol, hydroquinone
Plant volatile	Allyl isothiocyanate, cinnamaldehyde, eugenol, thymol, carvacrol, pinene
Metals	Silver, copper, zirconium
Probiotics	Lactic acid bacteria

Source: Han (2005)

Table 2 Antimicrobial agents in biodegradable packaging materials

Packaging material	Antimicrobial agent	Loading	Microorganism(s)	References
Polysaccharides films				
Cellulose	Nisin		*L. innocua* and *S. aureus*	Coma et al. (2001)
	Olive leaf extract	0.5 to 3 % (w/v)	*S. aureus*	Ayana and Turhan (2009)
Chitosan	Tea tree oil	1 % (p/p)	*L. monocytogenes*, *E. coli*, and *S. aureus*	Sánchez-González et al. (2011)
	Mexican oregano	0 to 4 % (v/v)	*A. niger* and *P. digitatum*	Avila-Sosa et al. (2012)
	Chitosan	1 % (w/w)	*E. coli* and *L. plantarum*	Leceta et al. (2013)
Starch	Cinnamon essential oil	0.4–0.8 g	*P. commune* and *E. amstelodami*	Souza et al. (2013)
Starch-alginate	Lemongrass oil	0.1 to 0.4 % (w/v)	*E. coli* O157:H7	Maizura et al. (2007)
K-carrageenan	*Zataria multiflora* Boiss and *Mentha pulegium*	1, 2, and 3 % (v/v)	*S. aureus*, *E. coli*, *B. cereus*, *S.* Typhimurium, and *P. aeruginosa*	Shojaee-Aliabadi et al. (2014)
Quince seed mucilage	Oregano essential oil	1, 1.5, and 2 % (v/v)	*S.* Typhimurium, *P. aeruginosa*, *E. coli*, and *Y. enterocolitica*	Jouki et al. (2014)
Protein films				
Soy protein isolate	Grape seed extract + EDTA	1 % (w/w)	*E. coli* O157:H7, *L. monocytogenes* and *S.* Typhimurium	Sivarooban et al. (2008)
Whey protein	Nisin	50 IU/mL	*P. aeruginosa*, *L. monocytogenes*, *Y. lipolytica*, *P. roqueforti*, and *P. commune*	Pintado et al. (2010)
	Lactic acid	10 % (w/w)	*E. coli*, *S. aureus*, and *Y. lipolytica*	Ramos et al. (2013)
Corn zein	Lysozyme	479 to 958 μg/cm^2	*E. coli* and *B. subtilis*	Güçbilmez et al. (2007)
	Lysozyme	11.7 mg/g	*L. innocua*	Arcan and Yemenicioğlu (2013)
	Lysozyme	11.7 mg/g	*L. monocytogenes*	Ünalan et al. (2013)
Wheat gluten	Potassium sorbate	0 to 15 wt%	*A. niger* and *F. incarnatum*	Türe et al. (2012)
Gelatin	Catechin-lysozyme	0.5 wt%	*E. coli*, *S. aureus*, *S. cerevisiae*, and *L. innocua*	Rawdkuen et al. (2012)

Table 3 Examples of antioxidant agents for potential use in food packaging materials

Classification	Antioxidant agents	References
Synthetic	Ethylenediaminetetraacetic acid (EDTA)	Güçbilmez et al. (2007))
	Butylated hydroxytoluene (BHT)	Jongjareonrak et al. (2008)
	Butylated hydroxyanisole (BHA) tert-Butylhydroquinone (TBHQ)	
Natural extract	Thyme oil Rosemary Oregano Borage extract Phenolic compound Ginseng extract α-Tocopherol Mint extract Pomegranate peel extract Grape seed extract Tea tree oil Lemon oil	Gómez-Estaca et al. (2009a) Oussalah et al. (2004) Gómez-Estaca et al. (2009b) Salgado et al. (2013) Jongjareonrak et al. (2008)

5.2 Antioxidant Agents

Antioxidants can be incorporated into edible films in order to enhance food stability, functionality, and safety and also to control the oxidation of fatty components and pigments, contributing to food quality preservation (Lee 2005). Table 3 lists the synthetic and natural antioxidants to be considered for inclusion or adsorption into food packaging materials. Most active compounds from biological origins are sensitive to heat; the thermal fabrication of plastics (such as extrusion and injection) offers limited possibilities for the inclusion of these substances directly in the polymer matrix without loss of their activity (Han 2005). Therefore, solvent compounding and solution coating are preferred and will prevent the loss of these compounds' activity.

The antioxidant agents of packaging films incorporation are shown in Table 4. In recent years, there are many researches that studied these agents to act as active packaging to retard lipid oxidation and extend shelf life of the product.

6 Applications of Active Edible Films

As consumer demand for convenience continues to increase, demand for ready-to-eat foods will also continue to grow. Quality, safety, and shelf life of ready-to-eat foods are often dictated by the type and numbers of pathogenic and spoilage bacteria present on the food surface and development of off-flavor and odor on food. Antimicrobial and antioxidant packaging can be used in several food-related products such as meat, fruits, and vegetable-based products. The first one is to extend the shelf life and promote safety by reducing the rate of the growth of

Table 4 Antioxidant agents in biodegradable packaging materials

Packaging material	Antioxidant agent	Loading	Method for measurement	References
Polysaccharide films				
Chitosan	α-Tocopherol	0.1 and 0.2 % (w/v)	DPPH radical scavenging activity	Martins et al. (2012)
	Resveratrol	1:1:0.01 and 1:1:0.1	DPPH radical scavenging activity	Pastor et al. (2013)
	Tea polyphenols	1:1 (10 %, 20 %, 30 %, and 40 % of the chitosan)	DPPH radical scavenging activity and total phenolic content	Wang et al. (2013)
Methylcellulose	Resveratrol	ratio of 1:1:0.01 and 1:1:0.1	DPPH radical scavenging activity	Pastor et al. (2013)
K-carrageenan	*Satureja hortensis*	1, 2, and 3 % (v/v)	DPPH radical scavenging activity	Shojaee-Aliabadi et al. (2013)
	Zataria multiflora Boiss and *Mentha pulegium*	1, 2, and 3 % (v/v)	DPPH radical scavenging activity	Shojaee-Aliabadi et al. (2014)
Agar + gelatin	Green tea extract	50/50 (v/v) mixture of distilled water and green tea extract	FRAP ferric-reducing ability and ABTS radical scavenging capacity	Giménez et al. (2013)
Quince seed mucilage	Oregano essential oil	1, 1.5 and 2 % (v/v)	DPPH radical scavenging activity and total phenolic content	Jouki et al. (2014)
Protein films				
Gelatin	BHT	200 ppm	DPPH radical scavenging activity	Jongjareonrak et al. (2008)
	Kaffir lime oil Longan seed extract or BHT	50 % (w/w) based on protein 50–100 ppm	DPPH radical scavenging activity, ferric ion-reducing capacity and ABTS radical scavenging ability Lipid oxidation Peroxide value TBARS	Tongnuanchan et al. (2012) Sai-Ut et al. (2014) and Vichasilp et al. (2014)
	Catechin-lysozyme	0.5 wt%	Lipid oxidation Peroxide value TBARS	Rawdkuen et al. (2012)
Sunflower protein	Phenolic compounds	5 % (w/v)	ABTS radical scavenging capacity	Salgado et al. (2013)
Hazelnut meals protein isolates	Fraction of protein extracts (peptide)	10–12.5 % (w/v)	TEAC and ORAC, iron chelating, ACE-inhibitory activity	Ayedemir et al. (2014)

(continued)

Table 4 (continued)

Packaging material	Antioxidant agent	Loading	Method for measurement	References
Sodium caseinate	Cinnamon essential oils	Ratio protein/oil 1:0.100	Quantified by means of an accelerated test of oxidative rancidity	Atarés et al. (2010)
Hake proteins	Essential oils	0.25 ml oil/g protein	DPPH radical scavenging activity and reducing power	Pires et al. (2013)
Chicken feet protein	Marjoram, coriander, and clove bud oil	1 g of essential oil	DPPH and ABTS as radical scavenging activity	Lee et al. (2015)
Zein	Catechin	50 mg/g film-forming solution	Oxygen radical absorbance capacity (ORAC) values	Ünalan et al. (2013)

specific microorganisms and protect against oxidative rancidity and enzymatic browning in fruits and vegetables by direct contact of the package with the surface of solid foods or in the bulk of liquids. Second, antimicrobial packaging materials could be self-sterilizing or self-sanitizing. Overall, the purpose of using active films for meat and fruits and vegetable-based product is to reduce the rate of microbial growth and retard the oxidation of lipid, pigments, and enzymatic browning to extend the shelf life and maintain the quality of the products.

6.1 Muscle-Based Food Products

Meat and meat-based products being a primary cause of foodborne disease are also prone to spoilage during storage as with all proteinous foods and development of off-flavor and odor caused by oxidation of lipid during storage. As in many refrigerated foods, microbial growth is generally responsible for the spoilage in meats and meat-based products together with biochemical and enzymatic deteriorations. One of alternative ways to controlling microbial growth and biochemical and enzymatic change in these products is application of active films incorporation either antimicrobial or antioxidant agents on the surface of the product, thus improving safety and delaying any spoilage. Applications of some edible films as support of active ingredients for improving the quality and extending the shelf life of meat and meat-based products are summarized in Table 5.

Table 5 Selected bioactive agents directly incorporated into the packaging for potential application of meat and meat-based products

Packaging material	Antimicrobial/ antioxidant agent	Amount incorporated	Example of test substrate	Observation	References
Polysaccharide films					
Chitosan	Green tea extract	20 % (w/v)	Pork sausages	Inhibition of lipid oxidation and microbial growth	Siripatrawan and Noipha (2012)
	Sunflower oil	1 wt%	Pork meat hamburgers	Reduction in the MtMb content and microbial count (chitosan-based films)	Vargas et al. (2011)
N,O-carboxymethyl chitosan	Oregano essential oil	1 % (v/v)	Chicken meat fillets	Inhibition of *L. monocytogenes*	Khanjari et al. (2013)
Agar	Green tea extract	50/50 (v/v) green tea extract	Fish	Delayed the growth of microorganisms	López de Lacey et al (2014)
Methylcellulose	Citrus extract	1 % (v/v)	Fish fillets	Reduced the growth of *P. fluorescens* and *A. hydrophila/caviae* and *L. innocua*	Iturriaga et al. (2012)
Sodium alginate	Vitamin C	5 % (w/v)	Refrigerated bream (*Megalobrama amblycephala*)	Inhibited the growth of bacteria, reduced the degree of chemical spoilage, retarded water loss, and enhanced the overall sensory values of bream	Song et al. (2011)
Calcium alginate	Cinnamon	10 μL mL^{-1}	Fresh northern snakehead fish fillets	Inhibiting bacterial growth and reduce the degree of chemical spoilage (lipid oxidation)	Lu et al. (2010)
Protein films					
Whey protein isolate	Oregano oil	0.5, 1, and 1.5 % (w/w)	Fresh beef	Inhibiting the growth of lactic acid bacteria (1.5 % (w/w))	Zinoviadou et al. (2010)
Soy protein isolate	Thyme and oregano essential oils	1, 2, 3, 4, and 5 % (v/v)	Fresh ground beef patties	Reduction in coliform and *Pseudomonas* spp.	Emiroglu et al. (2010))
	Essential oil	0–6 % (w/w)	Chilled pork	Inhibiting the growth of *E. coli*, *S. aureus*, *Pseudomonas*, and yeast	Zhang et al. (2010)

(continued)

Table 5 (continued)

Packaging material	Antimicrobial/ antioxidant agent	Amount incorporated	Example of test substrate	Observation	References
Gelatin	Citrus extract	1 % (v/v)	Fish fillets	Reduced the growth of *P. fluorescens* and *A. hydrophila/caviae* and *L. innocua*	Iturriaga et al. (2012)
	Catechin-lysozyme	0.5 % (w/v)	Minced pork	Microbial growth rates in the sample wrapped with the gelatin film (TPC 4.15 Log CFU/g; yeast and mold 2.99 Log CFU/g) lower than using PVC film	Kaewprachu et al. (2015)
Gelatin-chitosan	Clove essential oil	0.75 mL/g	Fish	Inhibition of natural microbiota of the fish	Gómez-Estaca et al. (2010)
Gelatin-chicken feather protein	Clove oil	0.5, 1.0, and 1.5 g	Smoked salmon	Films containing 1.5 g clove oil decrease in the populations of *E. coli* O157:H7 and *L. monocytogenes* by 1.41 and 1.34 log CFU/g	Song et al. (2014)

Table 6 Application of active films and edible coatings for improving the quality and extending the shelf life of fresh fruits and vegetables

Fruits and vegetables	Coating material	Antimicrobial/antioxidant agent	Amount incorporated	Observation	References
Broccoli	Polycaprolactone/ alginate film	Organic acids + rosemary extract + Asian spice essential oil and Italian spice essential oil	60 g/L organic acids, 13.5 g/L rosemary extract, 6 g/L Asian EO, 6 g/L Italian EO	Inhibition of *S.* Typhimurium and control growth of *L. monocytogenes* and *E. coli*	Takala et al. (2013a)
Baby carrot	Pullulan	Caraway essential oil	0.12 to 10.0 %	Inhibition of *S.* Enteritidis, *S. aureus*, *S. cerevisiae*, or *A. niger*	Gniewosz et al. (2013)
Strawberry	Chitosan	Lemon essential oil	3 % (w/v)	Reduction of *Botrytis cinerea*	Perdones et al. (2012)
Broccoli	Chitosan	Bioactive compounds and essential oils	120 μg/ml bioactive compounds and 15 uL/mL essential oils	Against the native microflora, *E. coli*, and *L. monocytogenes*	Alvarez et al. (2013)
	Polycaprolactone/ methylcellulose film	Organic acids + extract of rosmarinic acid + Asian essential oil and Italian spice essential oil	6 % (w/v) organic acids, 1.35 % rosemary extract, 0.6 % Asian EO or Italian EO	Reduction of *E. coli* and *S.* Typhimurium	Takala et al. (2013b)
Mango	Cellulose film	Nisin	7,500 and 15,000 IU/mL	Against *S. aureus*, *L. monocytogenes*, *A. acidoterrestris*, and *B. cereus*	Barbosa et al. (2013)
Cherry tomato	HPMC-beeswax	Sodium propionate, potassium carbonate, ammonium phosphate, and ammonium carbonate	2 % (w/w)	Reduced the growth of *B. cinerea*	Fagundes et al. (2014)

(continued)

Table 6 (continued)

Fruits and vegetables	Coating material	Antimicrobial/antioxidant agent	Amount incorporated	Observation	References
Organic leafy green (romaine lettuce, iceberg lettuce, mature spinach, and baby spinach)	Pectin + apple, carrot, or hibiscus purees film	Carvacrol	3.0 %	Reduced the *Salmonella* population by 5 $\log_{10}$ CFU/g	Zhu et al. (2014)
Avocados	Candelilla wax film	Ellagic acid	0.01 %	Reduction of *C. gloeosporioides*	Saucedo-Pompa et al. (2009)

6.2 Fruits and Vegetable-Based Products

Fruits and vegetables are more perishable and can be spoiled by biochemical, enzymatic deterioration and microbial growth on the surface. To retard these negative effects is of great interest to use dipping of aqueous solutions containing antimicrobial edible coatings or films with fruits and vegetables. Edible coatings may contribute to extend the shelf life of fruits and vegetables to reduce the risk of pathogen growth on food surfaces (Pranoto et al. 2005). Therefore, the applications of some edible films and coatings as support of active ingredients for improving the quality and extending the shelf life of fruits and vegetables are summarized in Table 6.

7 Conclusions

There is a continued demand of foods that are minimally processed and possess fresh-like quality. Numerous types of food packaging can be used in combination with food preservation techniques in order to extend the effectiveness of food preservation chain. The idea of combining antimicrobials or antioxidants with packaging films to control the growth of microorganisms and delay or prevent oxidation of lipid in food could have a significant impact on shelf life extension and food safety. The application of active films might allow for migration of the antimicrobial or antioxidant agents to the film surface and therefore a continued agents effect at the food surface during extended exposure. The use of active packaging materials in food packaging can minimize the microbial contamination of food product surfaces, enzymatic deterioration, and oxidation of lipid and pigments during storage, transportation, and handling.

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Edible Membranes Containing Antimicrobial Compounds: Current Approach and Future Prospects

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Abstract There is increased consumer demand for a reduction in food additives particularly chemical preservatives, and, as a result, there is currently a huge deal of research being carried out into bio-preservation for food applications. Among these compounds, lactic acid bacteria (LAB) enjoy an advantage as they are considered as GRAS. LAB can inhibit the growth of different microorganisms, including bacteria, yeasts, and fungi, through the production of several metabolites such as organic acids, hydrogen peroxide, enzymes, defective phages, lytic agents, and antimicrobial peptides and bacteriocins. To guarantee food safety, the incorporation of lactic acid bacteria and their metabolites into biopolymer films appears an interesting novel concept. Active packaging is the novel approach for prolong shelf life or to enhance safety and sensory of the food.

1 Introduction

Due to the interruption of cold chain, foods undergo physical, chemical, and microbiological spoilage during storage and transportation. Stability is dependent on a function of changes in their components (proteins, lipids, carbohydrates, and water) mainly due to environmental and processing factors (Cha and Chinnan

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2004). Regular incidence of spoilages leading to occurrence of food-borne outbreaks is the driving force in the search for innovative way to inhibit microbial growth in food. To encourage high quality of the food products and ability to delay the food spoilage is a novel concept i.e. active food packaging incorporated with antimicrobial compound. Maximal incident of food spoilage occurs on fresh and processed food products on their surface. So to enhance the shelf life of the food, it is necessary to control the surface flora causing spoilage of food first. Effective control of food spoilage can be significantly achieved by active food packaging containing antimicrobial substances or using antimicrobial polymers. Antimicrobial packaging materials extend the lag phase and reduce the growth rate of spoilage microorganisms to prolong the shelf life and maintain food quality and safety (Han 2000). The antimicrobial chemicals incorporated into packaging materials contain organic or inorganic acids, metals, alcohols, ammonium compounds, amines, and bacteriocins (Appendini and Hotchkiss 2002; Suppakul et al. 2003; Hugas 1998). Keeping in view of consumers' concerns about chemicals, there is a particular interest in food industry to use natural bio-preservatives such as antimicrobials, enzymes, and bacteriocins for antimicrobial packaging instead of chemical agents (Devlieghere et al. 2004a, b; Suppakul et al. 2003).

Different matrices can be used to incorporate antimicrobial agents, including proteins, lipids, polysaccharides, or composites. The most frequent antimicrobials incorporated in food packaging films are organic acid (e.g., sorbic, benzoic, citric, and propionic acids), enzymes (e.g., lysozyme), bacteriocins (e.g., nisin), polysaccharides (e.g., chitosan), and essential oils (e.g., bergamot, cinnamon, citronella, clove, ginger, oregano, pimento, and rosemary).

Peptides are widely recognized as promising alternatives to use as antimicrobial agents. According to Dangaran et al. (2009), the protein-based materials experienced a boom of interest in the early twentieth century. The inherent properties of proteins make them appropriate starting materials for films and coatings. The distribution of charged, polar, and nonpolar amino acids along the protein chain creates chemical potential. For example, β-lactoglobulin, the major protein in whey, illustrates the domains of polar and nonpolar areas along the protein chain. The resulting interactive forces produce a cohesive protein film matrix. Films are formed and stabilized through electrostatic interactions, hydrogen bonding, van der Waals forces, covalent bonding, and disulfide bridges (Krochta et al. 1994). Protein film-forming capabilities are most significantly demonstrated in emulsified systems in which amphipathic proteins form films at air–water or water–oil interfaces. Proteins have multiple sites for chemical interaction as a function of their diverse amino acid functional groups, which can allow for improvement and tailoring. Cross-linked protein films are often more stable than their polysaccharide-based counterparts and have a longer shelf life (Dangaran et al. 2009; Barone and Schmidt 2006).

In general the protein-based films and coatings are also biodegradable and compostable. As they degrade, they provide a source of nitrogen, which contributes a fertilizer benefit not available from other nonprotein-based films and coatings. Finally, there is emerging evidence that the bioactive peptides produced upon

digestion of proteins (dairy sources in particular) have antihypertensive and radical scavenging health benefits (Aimutis 2004).

Many antimicrobial peptides (AMPs) have significantly potent activity against bacteria, including those that are resistant to conventional antibiotics. Their activity is often relatively specific directed against certain genera or groups of microorganism, which could be advantage on a patient's commensal flora. AMPs generally exhibit high stability to withstand over the wide ranges of pH and temperature, characteristics that may be beneficial for their scale-up production. High specificity, low toxicity and specific mode of action advocates edible food membrane as a alternative packaging solution. The mode of action is also linked to a low propensity for resistance development in target bacteria (Upton et al. 2012). Edible packaging material is rapidly advancing by incorporating antimicrobial edible molecules such as proteins, polysaccharides, lipids and/or resins, and other edible components (Krochta 2002). Considerable interest and advanced research progress in bioactive packaging in the food and packaging industries have been driven by consumer demand for safe, convenient foods with long shelf life. Edible packaging solutions have been regarded as potent alternatives for some applications because of their significant properties. During the last decade, research concerning edible film coatings and antimicrobials has increased. The application of edible films are highly functional and acceptable as a food additive globally (Giannakopoulos and Guilbert 1986) the gradual liberation of the antimicrobial to the food giving origin to what is named technically as "active packaging".

2 Methods of Preparation of Antimicrobial Films

Packaging plays a very crucial role in controlling the quality of foods during storage, transport, and handling. So, it is necessary to protect food products from environmental contaminations and minimize the use of chemical additive packaging waste (Quintavalla and Vicini 2002).

According to Appendini and Hotchkiss (2002), bioactive packaging can be of different types incorporating:

- Sachets or pads containing volatile antimicrobial agents incorporated
- Volatile and nonvolatile antimicrobial agents directly into polymers
- Coating and adsorption of antimicrobials onto polymer surfaces
- Immobilized antimicrobials to polymers by different linkages
- Polymers that are inherently antimicrobial in nature

The incorporation of antimicrobials into a food packaging material can have various approaches like to blend the antimicrobial substance into the film when the film or the co-extruded film is produced (Fig. 1). The limitation of this approach is that the antimicrobial material could not be exposed to the surface firmly the film and hence is not totally available to control the microbial activity. An alternative to

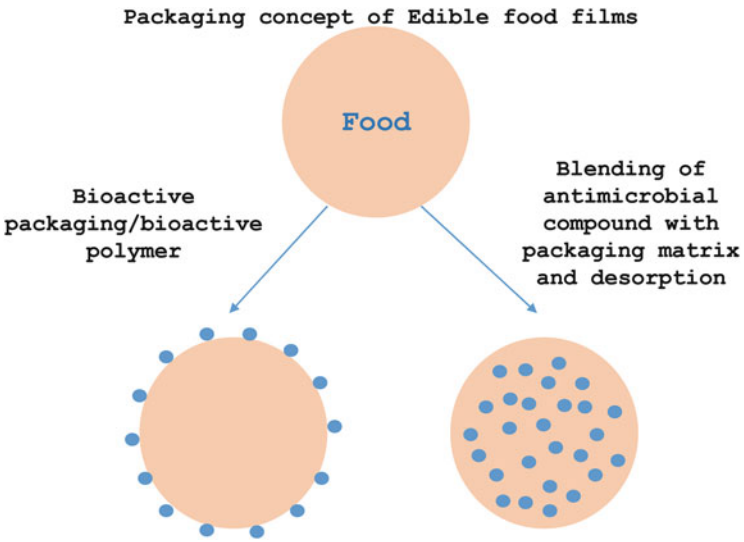

Fig. 1 The incorporation of antimicrobial in food packaging material

this could be to incorporate into the food contact layer. The technique incorporates in controlled spreading of a suspension on a surface to be dried, which can be an alternative for the preparation of films. Current method can be applied to the production of polysaccharides and protein films. The production of composite films (based on cassava starch, glycerol, and cellulose or sisal fibers) was performed by the tape casting technique (Du et al. 2008).

3 Edible Films and Coatings

Edible food films have become very popular in the food industry because of their merit of less leftover, cost-effectiveness, and safety (Cha and Chinnan 2004). They are natural biodegradable material, consumed along with the food packed provide additional health benefits, enhance sensory properties, and may composed of antimicrobials (Ryu et al. 2002). During the fifteenth century, Yuba, the first practice of standing edible film, was developed from soy milk in Japan (Debeaufort et al. 1998). "Larding," coating food with fat, the edible protective coating was applied on meats to prevent shrinkage (Krochta et al. 1994; Debeaufort et al. 1998).

According to Krochta et al. (1994), the main purposes of the films applied on foods:

- Provide protection against humidity and/or oxygen
- Delay microbial spoilage of the surface

- Maintain water activity
- Improve the mechanical properties to facilitate handling on the manufacturing line or during storage and to reduce spoilage and provide structural integrity of a food product
- Improve or modify the color, aroma, or flavor of foodstuffs
- Trap flavor during manufacture and storage

Edible packagings do not provide a significant nutritional value to the coated food. Therefore, we should consider them more like of an additive than an ingredient. It can also be employed to improve the nutritional value of the food and so be regarded as a food ingredient. As food components, edible films and coatings usually have to be as tasteless as during the consumption of the packaged food product. Edible films and coatings have to fulfill some requirements: have good sensory qualities, mechanical efficiencies, physiochemical, microbial stability and safe for human consumption with low cost of production.

3.1 Film Components

Components of edible food packaging and coatings can be classified into three groups: hydrocolloids, lipids, and composites.

a. **Hydrocolloid films**: Hydrocolloid films are mainly used in preventing barrier to oxygen, carbon dioxide, and lipids. Hydrocolloids films can be grouped into two classes as carbohydrates and proteins according to their composition (Krochta et al. 1994).

   1. **Carbohydrate films**: Polysaccharides are nontoxic in nature and widely available. Polysaccharide-based films have a hydrophilic nature. Therefore, they are a poor barrier to water vapor.
   2. **Protein films**: Proteins may be obtained from corn, soybeans, peanut, milk, or gelatin. Protein-based coatings have good barriers to O_2 and CO_2 but not to water. The following are various protein-based edible films:
   3. **Whey protein isolates (WPI)**: Whey proteins account for 20 % of total milk proteins. Liquid whey is a by-product of cheese processing sector and is produced in large quantities, but much of this whey is not utilized significantly. Hence, it leads to serious waste disposal problems. Whey proteins are water soluble and form hydrophilic edible food films which are transparent, flexible, colorless, and odorless films (Krochta 2002). Whey protein-based edible food films at pH 5.2 showed inhibition of *L. monocytogenes* and *E. coli* containing p-aminobenzoic acid (PABA) or sorbic acid (SA) Cagri et al. (2001).
   4. **Collagen casings**: Collagen, traditionally utilized in the meat industry for production of edible sausage casings. To obtain casings, the hide coriums is delcalcified and grinded. Ground collagenous material is mixed with acid to

produce a swollen slurry. The slurry is homogenized and extruded into tubular casings and dried.

b. **Starch**: Starch mainly composed of amylose and amylopectin and is hydrophilic in nature and cost-effective. Starch is primarily obtained from cereal grains, potatoes, etc. Amylose is responsible for the film-forming capacity of starches, in contrast to amylopectin films, which are brittle and noncontinuous. In addition, since the water activity (aw) is critical for microbial, chemical, and enzymatic spoilages, studies have demonstrated that films can resist the migration into the food packed during the storage (Cha and Chinnan 2004).
c. **Alginate**: Alginates are linear copolymers of D-mannuronic acid and L-guluronic acid, extracted from seaweeds, and major structural polysaccharides of brown seaweeds (Yuan-Hui 1991). Gels prepared with alginates rich in L-gulopyranosyluronic acid are stronger and brittle. Alginates have potential film-forming properties making the alginates particularly useful in food applications. Reports demonstrated organic acids used in immobilized calcium alginate gels inhibited *Listeria monocytogenes* on a lean beef tissue. The idea of acid–alginate gels indicated a promising method for preserving raw meat. Na–alginate-based films containing lysozyme, nisin, grapefruit seed extract, and EDTA. The film incorporated with grapefruit seed extract–EDTA showed the strongest inhibitory effect on *Listeria innocua*, *Escherichia coli*, *Salmonella enteritidis*, *Staphylococcus aureus*, and *Micrococcus luteus*.
d. **Lipid films**: Lipidic compounds composed of neutral lipids of glycerides. From this group, acetylated monoglycerides, natural waxes, and surfactants are commonly utilized in edible coatings. Lipids are commonly incorporated to food coatings to impart hydrophobicity (Cha and Chinnan 2004). Wax and glycerides are examples of lipid-based films (Table 1).

4 Bioactive Packaging

Post-packaging problems are the main challenges of food-borne pathogens. The post-packaging antimicrobial packaging shows promise to control the food-borne pathogens (Cagri et al. 2004). Preservation of foods by application of natural technologies can be efficient strategy in order to control many food spoilage issues. Lactic acid bacteria (LAB) or their metabolites were checked for their potential to control the growth of pathogen in various foodstuffs. The choice of an antimicrobial agent mainly depends on their activity against a target pathogen. The incidence of spoilage-causing microorganisms is expectable due to the food intrinsic characteristics such as pH, water activity, composition of food, as well as storage conditions. The direct amendment of additives in food packaging films is a potential practice by which antimicrobial activity can be achieved (Rivero et al. 2013; Gallagher and Corrigan 2000). Nisin is commonly used in the food processing industry as a safe and natural preservative and has been studied for its suitability to be added into

Table 1 Antimicrobial used in films which showed antimicrobial activity against some microorganisms

Matrix film	Antimicrobial agent	Microorganisms	Reference(s)
Whey protein	Potassium sorbate	*E. coli O157:H7*	Perez et al. (2011)
Whey protein	Malic acid, nisin and natamycin	*Penicillium aeruginosa*, *Y. lipolytica*, *L. monocytogenes*, *Penicillium commune*, *and Penicillium chrysogenum*	Pintado et al. (2009)
Hydroxypropyl methylcellulose	Kiam wood (Cotyleobium lanceotatum) extract	*E. coli O175:H7*, *S. aureus*, *and L. monocytogenes*	Chana-Thaworn et al. (2011)
Whey protein	Lactic and propionic acids, chitooligosaccharides, and natamycin	*E. coli*, *S. aureus*, *and Y. lipolytica*	Ramos et al. (2012)
Soy protein	Grape seed extract, nisin, and EDTA	*E. coli O175:H7*, *L. monocytogenes*, *and S.* Typhimurium	Sivarooban et al. (2008)
Wheat gluten	Potassium sorbate	*Aspergillus niger and Fusarium incarnatum*	Ture et al. (2012)
Whey protein	p-Aminobenzoic and sorbic acids	*L. monocytogenes*, *E. coli O157:H7*, *and S.* Typhimurium	Cagri et al. (2001)
Hydroxypropylmethylcellulose, methylcellulose, or sodium caseinate	Bacteriocins	*L. innocua*	Sanchez-Gonzalez et al. (2013)

whey protein, soy protein isolate, chitosan, and others films (Sivarooban et al. 2008; Pintado et al. 2009; Pranoto et al. 2005). Nisin is peptide (bacteriocin) with antimicrobial properties produced by strains of *Lactococcus lactis* subsp. *lactis* that shows antimicrobial activity of microorganism (Pranoto et al. 2005; Malheiros et al. 2010; Taylor et al. 2008). The film incorporated with nisin can be efficient against pathogenic bacteria such *L. monocytogenes*, *S. aureus*, and *B. cereus* (Sivarooban et al. 2008; Pranoto et al. 2005).

One of the most important significant characteristics of LAB is their potential to produce various metabolites with antimicrobial properties (Davidson et al. 1993). LAB are known to produce lactic acid, acetic acid, ethanol, diacetyl, hydrogen peroxide, hydroxy lactic acid, and also small peptides designated bacteriocins. These metabolites can inhibit target microorganisms such as molds, yeasts, vegetative bacteria and bacterial spores. Nisin is a bacteriocin, produced by *Lactococcus*

lactis and recognized as GRAS. Nisin is used to control the *Clostridium* in pasteurized cheese, meat, and salads (Chung et al. 1989; Cutter and Siragusa 1998). Nisin was also incorporated into low-density polyethylene (LDPE) (Siragusa et al. 1999). Palmitoylated alginate polymer can be used to produce hydrophobic and water-vapor-resistant beads and films (Le-Tien et al. 2004). Palmitoylated matrices could be used to entrap enzymes or LAB as a control delivery viability of the bacteria. Entrapment or immobilization of nisin in modified alginate matrices can be used a control delivery system to protect and to increase the antimicrobial activity duration to control the growth of pathogenic bacteria in fresh meat system. The bacteriocins are an attractive option of antimicrobial compounds as they constitute natural preservatives, avoiding the addition of synthetical compounds to food (Cotter et al. 2005). The bacteriocins are antimicrobial proteins or peptides produced by bacteria that inhibit or retard the growth of other bacteria (Cleveland et al. 2001). Some bacteriocins are produced by lactic acid bacteria of food origin, what make possible their application to control some specific bacteria growth in food (Cotter et al. 2005). A polyethylene film entrapped with immobilized bacteriocin reduced viable counts of *Listeria monocytogenes* (Mauriello et al. 2004). Pediocin has been documented as an antilisterial agent in turkey slurries when combined when synergistically combined with sodium acetate (0.3–0.5 %) (Schlyter et al. 1993). Pediocin has also demonstrated antilisterial effect on sliced cooked sausages and frankfurter sausages (Mattila et al. 2003). Antimicrobial and physical properties of soy protein films with various natural antimicrobials have been reported (Ko et al. 2001). The hydrophilic/hydrophobic nature of the amino acid content in the soy protein film matrix can be responsible for retention of incorporated antimicrobials (Dawson et al. 2003).

The objective of food packaging is to preserve the quality and safety of the food from the time of manufacture to the time it is used by the consumer (Dallyn and Shorten 1998). An equally important function of packaging is to protect the product from physical, chemical, or biological damage (Dallyn and Shorten 1998). The most efficient packaging materials that meet these parameters are polyethylene or copolymer. Worldwide production of packaging materials is estimated at more than 180 million tons per year. Edible films, gels, or coatings are considered as biopolymers with biodegradable properties such as polylactic acid (PLA) (Cha and Chinnan 2004) (Fig. 2).

5 Polylactic Acid-Blended Packaging Material

The durability and degradability of packaging films are two different subjects: desirable for packaging stability and shelf life and rapid degradation in the environment (Bohlmann 2005). Biopolymers are produced from natural resources and crude oil. Four categories of biopolymers are generally known (a) extracted directly from natural materials, such as polysaccharides and proteins; (b) chemically synthesized from bio-derived material such as polylactic acid (PLA), also known as

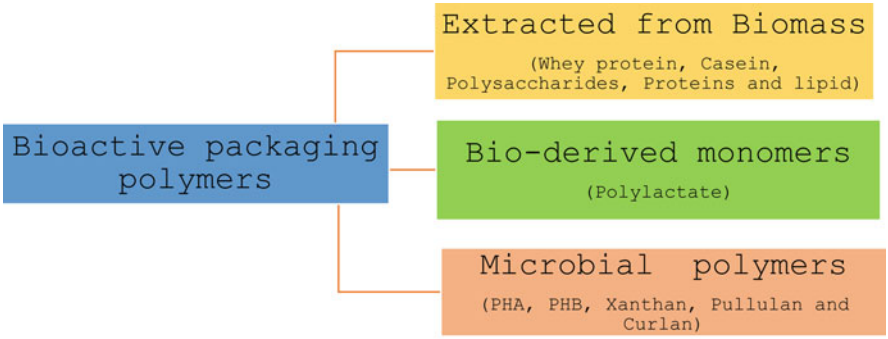

Fig. 2 Components of bioactive packaging system (Adopted and recreated from Weber et al. 2002)

poly(lactic acid); and (c) produced by microorganisms or genetically modified bacteria such as polyhydroxyalkanoates (PHA), polyhydroxybutyrate (PHB), hydroxyl-valerate (PHV), bacterial cellulose, xanthan, and pullan (Mohanty et al. 2000).

In comparison to other biopolymers, PLA has various significances including:

- Production of the lactide monomer by fermentation of a renewable agricultural residues
- The ability to recycle back to lactic acid by hydrolysis or alcoholysis
- Reduction of landfill/composting area

PLA is an agricultural, biological, and chemical sciences and technologies. Further it is classified as generally recognized as safe (GRAS) and is safe for all food packaging applications. The use of biopolymers in food packaging has already attained wide attention (Weber et al. 2002; Frederiksen et al. 2003). An antimicrobial packaging material based on PLA would be superior to other antimicrobial packaging due to its low cost, significant antimicrobial activity, regulatory concerns, and environmental sustainability. Although, the potential for antimicrobial packaging has not been explored yet. Direct incorporation of nisin results in an immediate reduction of bacterial population's cell growth by direct addition if residues of the antimicrobial are rapidly depleted (Yuan et al. 2004). Nisin, at various concentrations incorporated into polyethylene or other edible films, was effective against *L. plantarum*, *L. monocytogenes*, *E. coli*, and *Salmonella* spp. (Cutter et al. 2001; Eswaranandam et al. 2004). A variety of polymer films have been used for delivering nisin. Examples are sodium caseinate films (Kristo et al. 2008), alginate films (Natrajan and Sheldon 2000; Cha et al. 2003; Millette et al. 2007), methylcellulose/hydroxypropyl methylcellulose films (Franklin et al. 2004), linear low-density polyethylene and nylon films (Natrajan and Sheldon 2000), and whey protein, soy protein, egg albumin, and wheat gluten (Ko et al. 2001). Nisin is incorporated into polylactic acid and had antimicrobial

effectiveness against various food-borne pathogens such as *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella enteritidis* (Jin and Zhang 2008). Salmaso et al. (2004) carried nisin into PLA sheet and evaluated the sustained antimicrobial activity of nisin from the nisin/PLA particles. The findings of the various studies suggest that the incorporation of bacteriocins into PLA polymer could provide a possible delivery system for bacteriocins in food applications. Further research will be required to establish the facts for maximal antimicrobial efficiency.

6 Bacteriocin Blended with Other Edible Packaging Materials

Alginate from brown algae is a salt of alginic acid. It is used as an edible coating material because of its colloidal behavior and its ability to form gels or insoluble polymers upon reaction with multivalent metal cations, like calcium (Rhim 2004). Alginate coating containing antimicrobial agents such as nisin had been studied (Jin and Zhang 2008; Lu et al. 2009). Controlled release of such agents from packaging films could be the growth of target microorganisms and thus prolonged the shelf life of packaged products (Quintavalla and Vicini 2002). Among other available packaging materials, cellulose-based material have encouraged increasing interest due to their edibility, biodegradability, and potential as a good carrier (Cagri et al. 2004) (Table 2). Research describing the incorporation of nisin into nonbacterial cellulose-based packaging films to create antimicrobial materials has been documented. The films developed have been used to inhibit *L. monocytogene*s on the surface of frankfurters (Luchansky and Call 2004) and reduce *Listeria innocua* and *Staphylococcus aureus* on cheese and processed ham (Scannell et al. 2000).

Chitosan is a natural carbohydrate polymer obtained by the deacetylation of chitin. The potential of chitosan to act as a food preservative of natural origin has been widely reported on the basis of in vitro trials (Ribeiro et al. 2007). The antimicrobial activity of chitosan depends on several factors such as the deacetylation degree, molecular weight, pH of the medium blended, temperature, and other environmental components (Devlieghere et al. 2004a, b). The increasing demand for fresh seafood has intensified the search for new technologies for better preservation. One of the possibilities is the application of an edible film or coating. Gelatin–chitosan-based edible film together with refrigeration and high pressure decreased microbial growth of cold-smoked sardine in comparison to uncoated samples (Sallam 2007).

Some bacteriocins like nisin, enterocins A and B, sakacin, and pediocin have been demonstrated to control the proliferation of *Listeria monocytogenes* in artificially challenged food products (Aymerich et al. 2000; Cleveland et al. 2001; Garriga et al. 2002; Alves et al. 2006). *L. monocytogenes* is ubiquitous food-borne pathogen which may contaminate foods at pre- and postharvest production

Table 2 Examples of edible film incorporated with the bacteriocins

Bacteriocins	Target organism	Film material	References
Nisin and pediocin	*Listeria monocytogenes*	Packing bags	Ming (1997)
Bacteriocin 32Y by Lact. curvatus	*Listeria monocytogenes*	Polythene films	Mauriello et al. (2004)
Nisin	*M. luteus* ATCC 10240	Plastic film	Mauriello et al. (2005)
Nisin	*Brochothrix thermosphacta*	Polymer films	Cutter et al. (2001)
Nisin	*Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella enteritidis*	PLA film	Jin and Zhang (2008)
Nisin (Nisaplin)	*Listeria monocytogenes*	Polylactic acid film	Liu et al. (2012)
	Staphylococcus aureus and Escherichia coli cells	Nanocomposite films in PLA matrix	Fortunati et al. (2012)
Nisin	*Listeria monocytogenes* CIP 82110 *and Staphylococcus aureus* CIP 4.83	Hydroxylpropyl methylcellulose (HPMC), chitosan (CTS), sodium caseinate (SC), and polylactic acid (PLA) films	Imran et al. (2014)
Nisin	*Listeria monocytogenes*	Lauric acid and nisin-impregnated soy-based films	Dawson et al. (2002)
Nisin	*Brochothrix thermosphacta*	Whey protein edible film	Rossi-Marquez et al. (2009)
Bacteriocin (nisin or lactin NK24)	*Listeria monocytogenes*, *Brochothrix thermosphacta*, *Micrococcus flavus*	Bacteriocin-coated plastic packaging film	Kim et al. (2002)
Lactobacillus sakei (bacteriocin)	*Listeria monocytogenes*	Sodium caseinate film	Gialamas et al. (2010)
Nisin	*Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* Typhimurium	Soy protein edible film	Sivarooban et al. (2008)
Pediocin	*Listeria innocua and Salmonella* sp.	Film incorporated with pediocin	Santiago-Silva et al. (2009)
Nisin	*Staphylococcus aureus*	Alginate film	Millette et al. (2007)
Nisin	*Lactobacillus helveticus and Brochothrix thermosphacta*	Polyethylene based plastic film	Siragusa et al. (1999)
Nisin	*Listeria innocua*	Sodium caseinate film	Cao-Hoang et al. (2010)
Enterocin 416K1 (bacteriocin)	*Listeria monocytogenes*	LDPE film	Iseppi et al. (2008)

(continued)

Table 2 (continued)

Bacteriocins	Target organism	Film material	References
Bacteriocins (nisin and lacticin 3147)	*Lactococcus lactis* subsp. *lactis HP*, *in addition to Listeria innocua* DPC 1770 *and Staphylococcus aureus* MMPR3	Cellulose-based packaging paper (Perganorm Dresden Papier, Heidenau, Germany)	Scannell et al. (2000)
Nisin and a-tocopherol	*Micrococcus flavus*	Paper	Ho Lee et al. (2004)
Cinnamon and nisin	*E. coli* O157:H7 and *Listeria innocua*	Alginate-calcium coating	Lu et al. (2010)
Nisin	*Listeria monocytogenes*	Cellulose film	Nguyen et al. (2008)
Nisin	*Micrococcus luteus ATCC10240*	Methylcellulose (MC), hydroxypropyl methyl cellulose (HPMC), k-carrageenan, and chitosan films	Cha et al. (2003)

(Chen et al. 2003). The ubiquity and the growth at low temperature of *L. monocytogenes* make its control extremely difficult. The researchers encouraged strongly attracted by the incorporation of bacteriocins (Mauriello et al. 2004). Bacteriocins have to be applied to the surface of the packaging materials in a way that allows them to be effective against *L. monocytogenes* (Mauriello et al. 2004).

Modern meat processing relies upon the use of vacuum packaging. The regular means of vacuum-packaging meat is to place the product in a plastic bag with limited oxygen permeability with a mechanical vacuum-package sealing. As much as 90 % of the meat produced is vacuum packaged (American Meat Institute, pers. comm.). The importance of this type of approach for preservation technique is increasing as meat export becomes more prevalent and issues of microbial safety. Bacteriocins are unique food antimicrobials (for review see Nettles and Barefoot 1993). Nisin and pediocin have been demonstrated to be active against *Listeria monocytogenes* on meat surfaces (Holzapfel et al. 1995); additionally, it has been documented that immobilizing antimicrobials by incorporating into edible alginate gels (Siragusa and Dickson 1992) or spray application of bacteriocin to the surface of meats followed by vacuum packaging improves their antimicrobial efficacy (Cutter and Siragusa 1996). Adsorption of bacteriocins to film surfaces and to siliconized surfaces with retention of activity has been reported in prevention of meat spoilage.

7 Current Status and Future Prospective

The prospective of edible packaging has been well documented by many research groups and food industries as an unconventional or synergistic addition to conventional packaging to enhance food protection. Diverse innovative utilizations of

edible films and coatings have been proposed as novel applications as well as alternatives to existing technologies. The dry thermoplastic process is evolving rapidly as a viable commercial edible packaging manufacturing process. Bacteriocin or bioactive packaging is also at the forefront of edible packaging research and development. Nano-biotechnology encouraged scientists to engineer the novel packaging materials to achieve desirable barriers and delivery of antimicrobials entrapped and to better perform their designed functions. However, edible packaging technology still has to overcome several challenges to achieve potent commercial application. Incorporation of bacteriocin directly into a packing material could provide several advantages.

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Irradiation: A Technique for Microbial Decontamination of Medicinal Plants

Neelam Garg and Prakash Chander Gupta

Abstract Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants growing in different parts of the world. These plants are used for cosmetics, spices, herbs, and food supplements and referred to as "medicinal plants" in this chapter. Due to their nutritional, antioxidant, antimicrobial, and medicinal properties, these plants can be exploited for widespread applications. Many medicinal plants are grown in developing countries where sanitation and handling practices may not be satisfactory. Plant materials are exposed to microbial contaminants during their cultivation, harvest, processing, storage, distribution, and sale which exert a significant impact on overall quality and shelf life of the products. Therefore, decontamination of medicinal plants is necessary to eliminate pathogenic and reduce the number of spoilage microorganisms. A number of different techniques including fumigation, steaming, and irradiation have been used for microbial decontamination with varying degrees of success. Among all these techniques, irradiation has become the most important technique. In this article, various techniques of decontamination of medicinal plants and regulatory aspects of irradiation are discussed.

1 Introduction

The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as "medicinal plants." "A medicinal plant is any plant which, in one or more of its parts, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of

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useful drugs" (WHO 1977). Although there are no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important.

Recently, there has been a shift in worldwide trend from synthetic to traditional system of medicine, i.e., "Return to Nature," due to high prices and harmful side effects of synthetic drugs. Medicinal plants have been used in India for thousands of years and are increasingly being used worldwide during the last few decades as evidenced by rapidly growing global and national markets of herbal drugs (Bhowmik et al. 2009). The nutritional, antioxidant, antimicrobial, and medicinal properties of these plants have widespread applications. Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments.

Medicinal plants play an important role in the economies of several exporting and importing countries of the world. Natural color and flavors present in the medicinal plants also have a position in the international market. In the alternative system of medicine, medicinal plants are used as raw or in powdered form for the extraction of biologically active components. Therefore, the demand for plant-based medicines, health products, pharmaceuticals, food supplements, cosmetics, etc. is increasing in both developing and developed countries (Kalia 2005).

2 Medicinal Plants: International Scenario

The majority of medicinal plants are grown in tropical countries of the world. Since these are high-value commodities, they are important source of valuable foreign exchange for many countries. The global medicinal plants market was US $9 billion in the year 2009. According to WHO estimates, the present demand for medicinal plants is ~ US $14 billion a year, and by the year 2050, it would be ~ US $5 trillion. The sale of herbal medicines is expected to get higher at 6.4 %, an average annual growth rate (Inamdar et al. 2008). Due to the contribution of numerous significant factors, the market of medicinal plants has grown at an expressive rate worldwide. Some of them are preference of consumers for natural therapies, concern regarding undesirable side effects of modern medicines and the belief that herbal drugs are free from side effects, great interest in alternative medicines, preference of population for preventive medicine due to increasing population age, and the belief that herbal medicines might be of effective benefit in the treatment of certain diseases where conventional therapies and medicines have proven to be inadequate (Calixto 2000).

According to an estimate, in 1991, the herbal medicine market in the European countries was about $6 billion, with Germany accounting for $3 billion, France $1.6 billion, and Italy $0.6 billion, while in other countries was 0.8 billion. In 1997, the European market alone reached about $7.0 billion and the German market about 50 % of the European market, i.e., about $3.5 billion. This market is followed by France, $1.8 billion; Italy, $700 million; the United Kingdom, $400 million; Spain, $300 million; and the Netherlands, about $100 million (Calixto 2000).

2.1 Medicinal Plants: Indian Scenario

India has often been referred to as the "medicinal garden of the world" (WHO 1977) because nature has bestowed India with an enormous wealth of medicinal plants. India has a unique position in the world where several plant remedies are being utilized for the health care of people in a number of recognized indigenous systems of medicine, viz., Ayurveda, siddha, Unani, homeopathy, yoga, and naturopathy. Medicinal plants are popular among rural and urban communities due to their therapeutic value. India has 2.4 % of world's area with 8 % of global biodiversity. Around 25,000 effective plant-based formulations are used in traditional and folk medicines. More than 1.5 million practitioners are using the traditional medicinal system for health care. It is estimated that more than 7800 manufacturing units are involved in the production of natural health-care products and traditional plant-based formulations, which require more than 2000 tons of medicinal plant raw materials annually (Aneesh et al. 2009). The export of medicinal plants has been quite substantial in the last few years. The turnover of medicinal plants in India as over-the-counter products, ethical and classical formulations, and home remedies of traditional systems of medicine is about $1.0 billion, and export of herbal crude extract is about $80 million (WHO 1977).

Medicinal plants may be associated with a variety of microorganisms and exert a significant impact on overall quality of herbal products and preparations. As plant materials are highly susceptible to microbial contamination (Czech et al. 2001), many medicinal plant products are failing to compete in international market due to high microbial load (Dubey et al. 2008). According to international requirement, "Microbial quality is extremely important to be achieved"—to make plant materials suitable for human use and commercialization.

3 Microbial Contamination of Medicinal Plants

The medicinal plants and their products are exposed to a wide range of microbial contaminants during their cultivation, harvest, processing, storage, distribution, and sale. Sources of microbial contamination include the macro environment (i.e., soil in which the plants are grown), dust, insects, fecal material, and contaminated water (Pafumi 1986; Chan 2003). Medicinal plants are known to be contaminated with heat-resistant bacterial spores and molds, and the microbial bioburden ranged from 10^3 to 10^8 $CFUg^{-1}$ (Rtmitchell 2003). Types and number of microorganisms present depend on many factors, e.g., the microbial characteristics of spices depend on their origin, nature of their processing, transportation, and storage condition (Mandeel 2005). Other factors like drying in open, inadequate packaging and storage result in contamination. In addition, microbial contamination can arise from poor handling and poor hygiene practices by farmers (McKee 1995; Oily and Muroki 2002; Banerjee and Sarkar 2004). Medicinal plants after harvesting are often sun-dried by spreading them on open field to reduce microbial load and then sold without any treatment (Andress et al. 2001; Fennell et al. 2004).

Most medicinal plants are produced in tropical and subtropical regions. The hot and humid atmosphere, the simple, unassuming production conditions, extended drying times, and often inadequate GAP (Good Agriculture Practices) to the farmers may cause significant hygienic and quality problems (Hudson and Hasell 1998). Quality is one of the most important and critical factors in the world market and medicinal plants are no exemption. Importers and exporters place increasing importance on "clean and safe" medicinal plants rather than "cleaned" and do not import those which are still contaminated after cleaning. Cleanliness properties are considered the most important factors when evaluating the quality of medicinal plants (Banerjee and Sarkar 2004).

Many medicinal plants are cultivated and harvested in countries with poor sanitary conditions. Thus, it is expected that medicinal plants traded in these areas are at more risk of microbial contamination (Kneifel et al. 2002). The microorganisms responsible for the deterioration of medicinal plants and the consequences to consumers are shown in Table 1.

Various factors which influence survival and multiplication of microorganisms in medicinal plants are categorized in four categories:

(a) Intrinsic physical characteristics

1. Water activity
2. Typical microbial content when stored in clean, sealed containers
3. Antimicrobial properties

(b) Types of the microbial population

1. Ability to form spores
2. Adaptability to dry conditions
3. Aerobic or Anaerobic

(c) Handling and storage condition

1. Agricultural and harvesting practices (e.g., handling practices, pest control)
2. Sanitation (e.g., water, equipment) and worker hygiene
3. Temperature and humidity
4. Isolation methods (e.g., packaging, storage containers)

Table 1 Microorganisms usually present in medicinal plants

Bacteria	Pathogenic bacteria	Yeasts and molds
Bacillus subtilis	*Staphylococcus aureus*	*Aspergillus flavus/parasiticus*
Bacillus megaterium	*Clostridium perfringens*	*Botrytis cinerea*
Bacillus pumilus	*Clostridium botulinum*	*Penicillium* ssp.
Bacillus cereus	*Salmonella* spp.	*Saccharomyces cerevisiae*
Halobacterium halobium	*Escherichia coli*	*Rhizopus stolonifer*
Bacillus brevis	*Listeria monocytogenes*	*Zygosaccharomyces rouxii*
Bacillus polymyxa	*Shigella* spp.	*Xeromyces bisporus*

(d) Use of microbial decontamination processes

1. Drying
2. Heating
3. Fumigation
4. Irradiation

4 Microbial Decontamination Techniques

As the medicinal plants are highly contaminated with the microorganisms, there is a need for their decontamination. Chemical and physical methods with varying degrees of success are available for decontamination. Fumigation of medicinal plants with ethylene oxide (ETO), methyl bromide (MB), or propylene oxide (PPO) is the main chemical method used commercially in several countries. Physical methods commercially used for microbial decontamination include steam and heat sterilization and irradiation (Ayres et al. 1980; Lee et al. 2004).

4.1 Fumigation

For the complete sterilization or for reducing microbial load, chemical treatment by fumigation is widely used (Farkas 1998). Ethylene oxide or propylene oxide, when applied either under vacuum or under pressure, with or without an inert gas diluent, can kill bacteria, yeasts, molds, and pathogens without the need for high temperatures.

Use of ethylene oxide is, however, prohibited in many countries such as Japan and some countries of the EU including the United Kingdom due to its tendency to react with organic components of medicinal plants and leave the harmful residues ethylene chlorohydrin and ethylene bromohydrin. Ethylene chlorohydrin is a known carcinogen that persists in the medicinal plants for many months, even after processing. Ethylene oxide is considered by the International Agency for Research on Cancer (IARC) to be a human carcinogen (Fowles et al. 2001). For this reason and because of worker safety issues, its use in the United States is reviewed by the Environmental Protection Agency (EPA), and 50 ppm is set as limit of residue level. ETO gas is currently used as a fumigant to disinfect medicinal plants potentially contaminated with pathogenic bacteria, in New Zealand, the United States, and Canada. However, in Europe, ETO is banned as a medicinal plants and food fumigant due to potential toxicological risks to workers and consumers.

When the standards for microbiological contamination of medicinal plants are set very low, as is seen with processors operating under HACCP or ISO standards, ETO can be very inconvenient. After the treatment, the medicinal plants are

allowed to outgas ETO (approximately 1 week), bacteriological tests are conducted to ensure the product meets the standards which take 2−3 days if the product does not comply with the microbiological requirement, and the treatment must be repeated. Some ETO processors try to circumvent this system simply by running the medicinal plants through the treatment twice, before even performing the bacteriological tests. This can easily result in unacceptably high levels of the chemical residues. Medicinal plants destined for ETO treatment must be packaged in materials that allow the gas to enter the package. Bulk barrels must be opened for treatment. Such packaging requirements allow for recontamination. ETO sanitation is not compatible with packaging materials that are impervious to gas or are heat-sealed plastics (Chmielewski and Migdal 2005).

4.2 Steam Treatment

Various forms of steam treatment are currently in use, and the choice between saturated, dry, and superheated steam depends on the technology and product to be treated. Steam treatment for dried medicinal plants is usually a function of time and temperature. Steam treatment can considerably reduce microbial counts. As with most of the decontamination techniques, spore-forming pathogens are more difficult to be killed with steam than are non-spore-forming pathogens. Steam treatment can be applied to medicinal plants either in whole or ground form. Treating the whole product is technically easy; the posttreatment handling needs to follow strict hygiene rules in order to limit the risks of recontamination during grinding, handling, storage, and packing. Treatment of ground spices has the advantage of limiting the risk of recontamination, as the product can be packed right after it is treated. Steam treatments often induce organoleptic degradation, loss of volatile oil contents, and severe damage to the active compounds of medicinal plants. Therefore, a steam treatment is not suitable more so because an increase in temperature is accompanied by reduction quality of medicinal plants.

4.3 Irradiation

In recent decades, irradiation has become one of the most discussed technologies for safety and shelf life of medicinal plant-based products by removal of or reduction in load of microorganisms causing medicinal value deterioration. For this purpose, both ionizing and nonionizing radiations have been considered for certain processes.

4.3.1 Nonionizing Radiations

The ultraviolet (UV) radiations inactivate microbes by destroying the DNA so that the microbes cannot reproduce. Irradiation to UV might be a preferred method because of its greater ability for inactivation of a broad spectrum of microorganisms, absence of heat generation, and chemical-free nature. As a method of decontamination, it is not effective because of low penetrating power of UV (Sharma and Demirci 2003), inaccessibility of the microbes on the surfaces, and the cost of commercial UV light generators (Sommers 2012).

Microwave sterilization process has been developed at Washington State University (WSU) and received Food and Drug Administration (FDA) clearance for processing of mashed potato (Harrington 2010). Microwave irradiation method was used by Flor et al. (2011) for drying leaves of medicinal plant, *Echinodorus macrophyllus* (Kunth), in order to evaluate its effect on pharmacochemical composition of the plant part. But this irradiation method has not been used for decontamination directly as drying in itself makes way for reduction of microbial load by not providing the congenial conditions for the growth of microorganisms. In fact microwaves are nonionizing radiations, which are not particularly antimicrobial in themselves; rather their killing effect is largely due to the heat that they generate. These radiations do not change the molecular structure of the compounds being treated. Microwave irradiation is not effective enough to reduce bacterial population during the treatment (Plessi et al. 2002).

Infrared rays also bring about sterilization by generation of heat. Medicinal plants to be sterilized are placed in a moving conveyer belt and passed through a tunnel that is heated by infrared radiators to a temperature of 180 °C. The articles are exposed to that temperature for a period of 7.5 min. Articles sterilized included metallic instruments and glassware (Sridhar Rao 2014). Infrared and microwave irradiation have proved to be of limited value because these methods are basically forms of heating and consequently have the same disadvantages of the use of heat.

4.3.2 Ionizing Radiations

The aim is to prolong the shelf life of medicinal plants kept under various conditions such as in shops and households and to eliminate pathogenic organisms that cause diseases and reduction in spoilage microorganisms.

Ionizing radiations are a part of the electromagnetic spectrum. These are highly penetrating and effective because of their relatively short wavelengths and high energy. Therefore, unlike other methods, ionizing irradiation processing can be applied to prepacked medicinal plant material to get the desired effect. Irradiation can prolong the shelf life of stored medicinal plants by decontamination which eliminates pathogenic organisms and reduces spoilage microorganisms, thereby diminishing the possible risk of diseases and deterioration of the medicinal plants.

Expert committee of WHO/IAEA/FAO has approved the following three types of ionizing radiations to be used in medicinal material irradiation (WHO 1998):

- Gamma rays: These are generated from radioisotopes Cobalt-60 or Cesium-137 and are most often used in irradiation application because of high penetrating power and low cost.
- Electron beam: These are generated from machine sources (accelerators) which are operated at or below an energy level of 10 MeV.
- X-rays: These are generated from machine sources which are operated at or below an energy level of 5 MeV.

When an ionizing radiation emitted from a radioactive source penetrated into a medium (e.g., medicinal plants), all or part of the radiation energy is absorbed by that medium. The quantity of energy absorbed by the medium is called the absorbed dose which is measured in "rad." It was defined as a unit equivalent to the absorption of 100 ergs/g of matter. The new unit used now according to the international system is the gray "Gy." It is equal to the absorption of 1 J/Kg.

1 Gy = 100 rad

1 KGy = 100 Krad

10 KGy = 10^6 rad = 1 Mrad

The amount of ionizing radiation energy absorbed in a unit of time is called the "dose rate" (WHO 1998).

Decontamination of medicinal plants by ionizing radiation is a safe, efficient, environmentally clean, and energy efficient process. Irradiation is used to inactivate spoilage microorganisms, to reduce quality losses during storage, and to guarantee the hygienic quality of medicinal plants (Moy and Wong 1996). Use of ionizing radiation as a physical method of microbiological decontamination of food products is approved by the Codex Alimentarius Commission. The experts agreed that these radiations do not cause any toxicological changes or activation of irradiated food products (Thorne 1991; Diehl 1992).

A large number of facilities in the world now use gamma irradiation for sterilization of medicinal plants. Irradiation using Cobalt-60 as a source of gamma radiations is a well-established industrial process in India. It is a very efficient and convenient technique for achieving a high-level sterility. There are several advantages with the use of gamma radiations compared to electron beam and X-ray machines. Cobalt-60 is a monoenergetic radiation source with a half-life of 5.27 years. It is readily available from simple nuclear reactions in nuclear reactors. The major difference in gamma radiation and electron beam lies in their penetration powers, where gamma radiations can penetrate deep inside the product and the electron beams do not have good penetration power.

Though X-rays in the energy ranges of 8–10 Mev have penetrations comparable to those of gamma rays, they are not yet very popular. Probably, at these ranges of energy, X-rays may also cause nuclear transformations and prohibit their use in commercial scale. The maximum energy of gamma radiations from Cobalt-60 is only 1.33 Mev, which is not sufficient to cause nuclear transformations of any type

in the products being processed. Gamma radiations from Cobalt-60 or Cesium-137 are therefore considered more useful for sterilization.

Gamma irradiation sterilization is a cold process, with a temperature rise of not more than a few degrees centigrade. The process is particularly suitable for industrial-scale sterilization of heat-sensitive products, enclosed in air and moistureproof packs in shipping cartons. The radiation dose required for the destruction of fungi and majority of bacteria varies from 1.5 to 15 kGy. Usually bacterial spores are more resistant than the vegetative forms. A radiation dose of 25 kGy is the officially accepted dose for medical product sterilization in many countries.

Gamma irradiation has been extensively studied as a means of reducing the microbial contamination of medicinal plants. Experiments indicate that medicinal plants with water contents of 5–12 % are very resistant to physical or chemical change when irradiated. The effect of irradiation on the microbial contamination of medicinal plants is dependent on irradiation dose, the type of microorganism present, and initial contamination level (Al-Bachir and Lahham 2003; Gupta et al. 2011). Gamma irradiation is now internationally recognized as an effective method to maintain the quality of medicinal plants for a long time.

5 Regulatory Aspects of Gamma Irradiation Processing

Before introducing this new technology, positive evidence and assurance had to be obtained that it would not have any hazardous side effects. The task of proving this was coordinated by the International Project in the Field of Gamma Irradiation. Data generated by this Project were periodically reviewed by joint FAO (Food and Agriculture Organization), IAEA (International Atomic Energy Agency), and WHO (World Health Organization) expert committees which represent the collective views of a group of international top-level experts and not just the views of individuals or organizations.

The irradiation of medicinal plants such as spices is allowed in most countries. International standards such as Codex have accepted it as a beneficial treatment. Major producing countries such as India allow the irradiation of spices; major exporting countries such as the United States and Canada both accept and use the process commercially. The FDA (USA) and UK panel have accepted the concept of dosimetric release for radiation sterilized medical devices manufactured under GMP (Good Manufactured Practices). No post sterility microbiological testing is required with radiation if dosimetric release procedures are followed. Radiation sterilization dose of 25 kGy provides such a high-safety factor wherein tests for sterility are generally considered redundant.

Many countries have approved irradiation of medicinal plants for microbial decontamination and for insect's disinfestations. Among these countries are Argentina, Brazil, France, India, South Africa, the United States, etc. (ICGFI 1991). Gamma irradiation results in a much lower level of microbial contamination and

is often the only treatment effective enough to meet standards set by processors operating under Hazard Analysis and Critical Control Points (HACCP) or the International Organization for Standardization (ISO) (WHO 1994).

6 Influence of Gamma Irradiation on Medicinal Plants

The gamma irradiation has the ability to ionize compounds, thereby creating highly reactive free radicals. The killing effect of irradiation can be attributed to breaking of chemical bonds of essential macromolecules such as DNA or to the ionization of water which results in forming highly reactive radicals such as H, OH, etc. These free radicals split carbon bonds of macromolecules in living organisms, thereby killing the microorganisms. Since no heat is generated in this form of destruction of microorganisms, radiation sterilization is commonly known as cold sterilization.

According to Arena (1971), ionizing radiations cause water molecules to lose an electron and produce OH^{-}. This product immediately reacts with other water molecules to produce a number of compounds, including hydroxyl radicals (OH•), molecular hydrogen, oxygen, and hydrogen peroxide H_2O_2. Hydroxyl radicals are very reactive and are known to interfere with the bonds between nucleic acids within a single strand or between opposite strands. Although biological systems have a capacity to repair both single-stranded and double-stranded breaks of the DNA backbone, the damage occurring from ionizing radiations was random and extensive (Razskazovskiy et al. 2003). Therefore, recovery processes in bacteria after their radiation damage are unlikely to occur.

The differences in sensitivity to ionizing radiation between microorganisms are related to the differences in their chemical and physical structure and in their ability to recover from the radiation injury. The amount of radiation energy required to control microorganisms in food, therefore, varies depending on the resistance of the particular species and the number of organisms present.

Gamma irradiation is suitable for decontamination of medicinal plants as the process does not affect the chemical or physical properties of the material and microbial population is reduced by at least 10^5 cfu g^{-1} at a dose of 10 kGy (Singh et al. 1998). The effect of different gamma irradiation doses on the microbial content of medicinal plants has been studied in detail, and it has been confirmed that doses between 10 and 20 kGy lead to complete sterilization of medicinal plants where the original level of contamination was of the order 10^7 cfu g^{-1} (IAEA 1992).

The radiation dose depends upon the initial level of contamination of the medicinal plants; it also depends upon the type of microorganisms present (Alam et al. 1994). In general, it was found that molds, fungi, and coliforms are eliminated by doses lower than those required for bacteria. Studies showed that minimum doses as low as 4–5 kGy eliminated these organisms, whereas some bacteria and yeasts required minimum doses of 10 kGy to reach count up to non-detectable level (Kiss 1982; Alam et al. 1994; Farkas 1998; Owczarczyk et al. 2000).

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Several authors have reported the treatment of medicinal plants with gamma irradiation (Renata et al. 2005) to prevent the microbial load. Various physiochemical and sensory characters of the plants were observed after irradiation, and the method was found to be suitable. Extensive research has shown that proteins, essential amino acids, minerals, trace elements, and most vitamins do not represent significant losses during irradiation even at doses over 10 kGy (Al-Jassir 1992). Studies showed that biologically active substances like flavonoids, anthocyanins, essential oils, glycosides, triterpene saponins, oleanosides, and mucus and pharmacological activity in medicinal plants did not change significantly after irradiation (Al-Jassir 1992; Gupta et al. 2010).

Toxicological and nutritional tests have confirmed the safety of foods irradiated at doses below 10 kGy (Thayer et al. 1996). The US Food and Drug Administration had set a limit for irradiation treatment of medicinal plants that must not exceed 30 kGy (Bendini et al. 1998; Olson 1998). The radiation pasteurization process for many medicinal plants has been approved or endorsed by many world agencies and associations such as the FDA, the WHO, the Codex Alimentarius Commission, the American Medical Association, the Institute of Food Technologists, and the health authorities in approximately 40 countries (Thayer et al. 1996).

7 Conclusion

Contamination of medicinal plants with microorganisms can lead to afflicted quality of the product due to disruption of the stability of the preparation and modification of physical characteristics and appearance and lead to inactivation of the active ingredients due to enzymatic activity of microorganisms. Gamma irradiation has been shown to be a safe and effective process in controlling microbial contamination, without losses of the biologically active substances of medicinal plants. It is obvious that more comprehensive study of effect of gamma irradiation of medicinal plants is needed in order to ensure the quality, an important concept for providing the desired safety and reliability for its use.

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Microbial Food Spoilage: Control Strategies for Shelf Life Extension

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Abstract Food spoilage can be defined as any sensory change in food flavor which the consumer considers to be unacceptable. Spoilage may arise from insect damage, physical damage (freezing, drying, etc.), chemical changes (usually involving oxygen), and indigenous enzyme activity in the animal or plant tissue. Spoilage is therefore complex and may occur at any stage along the food chain. Bacteria, fungi, yeast, and insects are the main cause for food spoilage. There are a wide range of metabolites produced during microbial spoilage including alcohols, sulfur compounds, hydrocarbons, fluorescent pigments, as well as organic acids, esters, carbonyls, and diamines. Preservation of food for its safety and long shelf life is dependent on the food type and properties (pH, water activity, nutrient content, antimicrobial constituents, etc.), initial microbial flora, and processing and storage conditions (heating, acidification, reduced water activity, storage atmosphere, chilled storage, etc.). This review deals with food spoilage, microbes causing food contamination, prevention of microbial spoilage, and preservation of foods.

1 Introduction

Food spoilage can be defined as any sensory change (tactile, visual, olfactory, or flavor) which is considered unacceptable by the consumer. Spoilage may occur at any stage along the food chain and may arise from (a) insect damage, (b) physical damage (bruising, freezing, drying, etc.), (c) indigenous enzyme activity, and (d) chemical changes (usually involving oxygen). Spoilage is, therefore, complex

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and involving physical, chemical, biochemical, and biological changes (Voysey 2007). The main reason for food deterioration is due to microorganisms that will cause spoilage and decay. These include Gram-positive and Gram-negative bacteria, yeasts, and molds. Gram-negative rod-shaped bacteria such as *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Alteromonas*, *Flavobacterium*, *Moraxella*, and *Achromobacter* may grow at cold temperatures and have been shown to contribute to the spoilage of chilled red meat, cured meats, poultry, fish, shellfish, milk, and dairy products (Voysey 2007). *Vibrio* spp. are halophilic bacteria (salt loving) that may therefore cause spoilage of seafish and cured meats. Microbial spoilage includes (a) visible mold growth, (b) production of gas, (c) diffusible pigment and enzymes which may cause softening and rotting (proteolysis), and (d) slime, off-odor, and off-flavor (Voysey 2007). There are a wide range of metabolites produced during microbial spoilage, including alcohols, sulfur compounds, ketones, fluorescent pigments, organic acids, as well as esters, carbonyls, and diamines.

Spoilage is generally most rapid in proteinaceous chilled fresh foods (e.g., red meats, poultry, fish, and dairy products) due to (a) high nutrition, high moisture content, and relatively neutral pH. Other fresh foods that may suffer significant spoilage are fruit and vegetables. Fruits are generally spoiled by yeast and molds, as the low pH prevents bacterial growth. Vegetables are frequently subjected to both bacterial and fungal spoilage. Suitably processed (and packaged) or ambient stable foods with a low moisture content will be expected to show little sign of microbial spoilage by yeasts or molds. On the other side, food loss, from farm to consumer, causes considerable environmental and economic effects. The USDA Economic Research Service estimated that more than 96 billion pounds of food in the USA were lost by retailers, foodservice, and consumers in 1995 (Doyle 2007; Kantor et al. 1997). Factors that changed food science and technology include (a) canning, where it revolutionized food preservation and made it more available; (b) commercial freezing and refrigeration which allowed preservation of meats; (c) food additives such as antimicrobials, antioxidants, benzoates, sorbitol, etc.; and (d) refrigerated railcars and trucks which increased the availability of fresh fruits, vegetables, and meats. This review deals with food spoilage, food poisoning, causal microorganisms, as well as preservation strategies for long shelf life of foods.

2 History of Food Preservation

Throughout the history of mankind, science, bringing new discoveries, allowed our lives to become healthier, more efficient, and safer, and at the same time, more dangers are possible. As early as the beginning of the nineteenth century, major breakthroughs in food preservation had begun. Soldiers and seamen, fighting in Napoleon's army, were living off salt-preserved meats. These foods, however, provided nutritional value, but frequent outbreaks of scurvy were developing (Harris and Von Lesecke 1973). To try and solve the problem, Napoleon began the search for a better mechanism of food preservation and offered 12,000 francs to

the person who devised a safe and dependable food preservation process for foods to remain fresh and wholesome for long periods of time. A Parisian confectioner named Nicolas Appert, who had been experimenting with food all his life, produced a satisfactory method and collected the prize in 1809 from the great Napoleon himself (Harris and Von Lesecke 1973). He observed that food heated in sealed containers was preserved as long as the container remained unopened or the seal did not leak. This became the turning point in food preservation history. Fifty years following the discovery by Nicolas Appert, another breakthrough had developed. Another Frenchman, named Louis Pasteur, noted the relationship between microorganisms and food spoilage. This breakthrough increased the dependability of the food canning process (Harris and Von Lesecke 1973). As the years passed, new techniques assuring food preservation would come and go, opening new doors to further research.

3 Food Spoilage

Food spoilage can be defined as a disagreeable change in a food's normal state. Food spoilage is a metabolic process that causes foods to be undesirable or unacceptable for human consumption due to changes in sensory characteristics. Such changes can be detected by smell, taste, touch, or sight (Sherratt et al. 2006). Spoiled foods may to be not hazardous and cause no illness if they are free of pathogens or toxins. However, changes in texture, smell, taste, or appearance cause them to be rejected. Changes in food, either through growth of microorganisms or enzyme deterioration of food, will lead to unsafety and spoilage of foods. The rate of deterioration depends on a variety of factors which must be controlled carefully (Food Poisoning. A fact of life 2009).

3.1 Factors Causing Spoilage

Some spoilage is inevitable, and a variety of factors cause deterioration of foods including: (1) Endogenous enzymes in plants oxidizing phenolic compounds (browning) or degrading pectins (softening). (2) Rodent and insect damage that may provide an entry point for microbial growth on foods. (3) Parasites, when visible, for example, in meat or fish, rendering food undesirable. (4) Microbes (bacteria, molds, yeasts) growing on and metabolizing foods. (5) Light causing degradation of pigments, fats, and proteins (off-flavors and off-odors) or stimulating pigment production (greening of potatoes). (6) Temperature, both excessive heat and freezing, physically affecting texture of foods and breaking emulsions. (7) Air, particularly oxygen, oxidizing lipids producing strong off-odors and off-flavors. (8) Too little moisture causing cracking, crumbling, or crystallization in contrast to moisture in excess causing sogginess, stickiness, or lumping (Voysey

2007; Doyle 2007). These factors are interrelated, as certain temperatures, oxygen, and moisture levels increase the activities of endogenous enzymes. Types of food decay are discussed below (Spoilage of Food. Home.pacific.nethk/ppleung/chem/preservation.htm).

3.1.1 Putrefaction

During this type, biological decomposition of organic matter with production of ill-smelling and ill-tasting products occurs, associated with anaerobic organisms (no oxygen present). Proteins are degraded by proteolytic microorganism, where hydrogen sulfide, amino acids, and ammonia are produced.

3.1.2 Fermentation

Through which chemical changes in organic substances are produced by the action of enzymes. This general definition includes virtually all chemical reactions of physiological importance, and scientists today often restrict the term to the action of specific enzymes, called ferments, produced by minute organisms such as molds, bacteria, and yeasts. For example, lactase, a ferment produced by bacteria usually found in milk, causes the milk to be sour by changing lactose (milk sugar) into lactic acid.

3.1.3 Rancidity

Rancidity includes three types: microbial rancidity, hydrolytic rancidity, and oxidative rancidity. Microbial rancidity occurs due to deterioration changes of fats with time, which result in undesirable flavors and odors. These changes in fats are given the term rancidity. Fatty acids are formed through hydrolysis of the lipid (fat) by the water it contains. Some of the liberated fatty acids are volatile, and some have very unpleasant odors and flavors. The oxidation of acylglycerols which occurs in air, without the presence of enzymes, is called autoxidation. Among the products of autoxidation are hydroperoxides which have no taste, but they decompose easily to form aldehydes, ketones, and acids, which give oxidized fats and oils their rancid flavors.

3.2 High-Risk Foods

Some foods are highly risky, as they provide the ideal conditions needed for growth of microorganisms. These foods include meat, meat products, milk, dairy products, as well as fruit and marine foods (fish, shrimp, crab, etc.). If such foods became

contaminated with food poisoning microorganisms, and conditions allow them to multiply, the risk of food poisoning increases. People at high risk are elderly people, babies, and pregnant as they need to be extra careful about the foods. People with low resistance to infection should avoid high-risk foods such as meat products, marine foods, and unpasteurized soft cheese (Food Poisoning. A fact of life 2009).

3.3 Detection of Food Spoilage

Spoilage is manifested by a variety of sensory cues such as off-colors, off-odors, softening of vegetables and fruits, and formation of slime. However, even before it becomes obvious, microbes have begun the process of breaking down food molecules for their own metabolic needs (Doyle 2007). Sugars and easily digested carbohydrates are used first; plant pectins are then degraded. Proteins are attacked producing volatile compounds with characteristic smells such as ammonia, amines, and sulfides. These odors start to develop in meat when there are about 10^7 CFU of bacteria/cm^2 of meat surface and are usually recognizable at populations of 10^8 CFU/cm^2 (Ellis and Goodacre 2006). Early detection of spoilage would be advantageous in reducing food loss because there may be interventions that could halt or delay deterioration, and on the other hand food that had reached the end of its designated shelf life but was not spoiled could still be used. Numerous methods for detection of spoilage have been devised with the goals of determining concentrations of spoilage microbes or volatile compounds produced by these microbes. However, many of these methods are considered inadequate because they are time-consuming or labor-intensive and/or do not reliably give consistent results (Doyle 2007).

Detection of volatile compounds produced by spoilage bacteria can be a less invasive and more rapid means for monitoring spoilage. Biogenic amines (putrescine, cadaverine, histamine, and tyramine) are commonly produced during spoilage of high protein foods and can attain levels that cause illness, particularly in spoiled fish. HPLC methods have been used to quantitate different amines in fish (Baixas-Nogueras et al. 2005), chicken (Balamatsia et al. 2006a), and cheese (Innocente et al. 2007). Combined concentrations of these amines are expressed as a biogenic amine index that is related to the extent of food spoilage and to the concentrations of spoilage organisms.

Other techniques being developed to detect microbes or chemicals associated with spoilage include (a) FT-IR (Fourier transform infrared spectroscopy) used with beef (Ellis et al. 2004) and apple juice (Lin et al. 2005), (b) visible and short-wavelength near-infrared spectroscopy to detect microbial load in chicken by diffuse reflectance (Lin et al. 2005), (c) ion mobility spectrometry for detecting trimethylamine in meat (Bota and Harrington 2006), and (d) gas chromatography-mass spectrometry for analyses of fish. It is expected that some advances in nanotechnology will improve the portability, sensitivity, and speed of detection systems.

3.4 Food Spoilage Microorganisms

Chemical reactions that cause offensive sensory changes in foods are mediated by a variety of microbes that use food as a carbon and energy source. These organisms include prokaryotes (bacteria), single-celled organisms lacking defined nuclei and other organelles, and eukaryotes, single-celled (yeasts) and multicellular (molds) organisms with nuclei and other organelles (Doyle 2007). Some microbes are commonly found in many types of spoiled foods, while others are more selective in the consumed foods; multiple species are often identified in a single spoiled food item, but there may be one species (a specific spoilage organism) primarily responsible for production of the compounds causing off-odors and off-flavors. Some microbes, such as lactic acid bacteria and molds, secrete compounds that inhibit competitors (Gram et al. 2002). Spoilage microbes are often common inhabitants of soil, water, or the intestinal tracts of animals and may be dispersed through the air and water and by the activities of small animals, particularly insects. It should be noted that with the development of new molecular typing methods, the scientific names of some spoilage organisms, particularly the bacteria, have changed in recent years, and some older names are no longer in use (Gram et al. 2002). Spoilage microorganisms are discussed below.

3.4.1 Yeasts

Fungi include molds and mushrooms. Yeasts are a subset of fungi and are generally single-celled facultative organisms (capable of growth with or without oxygen) and adapted for life in liquid environments. Unlike some molds and mushrooms, yeasts do not produce toxic secondary metabolites. Yeasts are well known for their beneficial fermentations that produce bread and alcoholic drinks. They often colonize foods with a high sugar or salt content and contribute to spoilage of pickles, fermented foods, and some dairy products. Fruits and juices with a low pH are another target, and there are some yeasts that grow on the surfaces of meat and cheese (Doyle 2007; Smits and Brul 2005). There are four main groups of spoilage yeasts: (a) *Saccharomyces* spp.: spoil wines and other alcoholic beverages by producing gassiness, turbidity, and off-flavors associated with hydrogen sulfide and acetic acid (Loureiro and Malfeito-Ferreira 2003; Restuccia et al. 2006; Siegmund and Pollinger-Zierler 2006).. (b) *Zygosaccharomyces*: are the usual spoilage organisms in foods such as salad dressings, dried fruit, jams, and soy sauce (Castro et al. 2003; Martorell et al. 2007; Meyer et al. 1989). They usually grow slowly, producing off-odors and off-flavors and carbon dioxide that may cause food containers to swell and burst. (c) *Candida* and related genera: are a heterogeneous group of yeasts, some of which also cause human infections. They are involved in spoilage of fruits, some vegetables, and dairy products (Casey and Dobson 2003; Fitzgerald et al. 2004). (d) *Dekkera*/*Brettanomyces*: are principally involved in spoilage of fermented foods, including alcoholic beverages and some

dairy products. They can produce volatile phenolic compounds responsible for off-flavors (Loureiro and Malfeito-Ferreira 2003; Couto et al. 2005).

3.4.2 Molds

Molds are filamentous fungi that do not produce large fruiting bodies like mushrooms. Molds are very important for recycling dead plant and animal remains in nature but also attack a wide variety of foods and other materials useful to humans. Molds are well adapted for growth on and through solid substrates, generally produce airborne spores, and require oxygen for their metabolic processes. Most molds grow at a pH range of 3–8 (Doyle 2007). Spores can tolerate harsh environmental conditions, but most are sensitive to heat treatment. An exception is *Byssochlamys*, whose spores have a D value of 1–12 min at 90 °C (Doyle 2007). Molds have a diverse secondary metabolism producing a number of toxic and carcinogenic mycotoxins. Some spoilage molds are toxigenic, while others are not (Pitt and Hocking 1997). Spoilage molds can be categorized into four main groups:

A. *Zygomycetes* are widespread in nature, growing rapidly on bread, simple carbon sources in soil, and plant debris. *Zygomycetes* generally require high water activities for growth. The most common spoilage species are *Mucor* and *Rhizopus*. Zygomycetes are not known for producing mycotoxins, but there are some reports of toxic compounds produced by a few species (Doyle 2007). Some zygomycetes are also utilized for production of fermented soy products, enzymes, and organic chemicals (Voysey 2007).
B. *Penicillium* and related genera are distinguished by their reproductive structures that produce chains of conidia. Although *Penicillium* spp. can be useful to humans in producing antibiotics and blue cheese, many species cause spoilage fruits and vegetables, including cereals (Doyle 2007). Some species produce potent mycotoxins (patulin, ochratoxin, citreoviridin, penitrem). *Penicillium* spp. cause visible rots on citrus, pear, and apple fruits and cause enormous losses in these crops. Other species can attack refrigerated and processed foods such as jams and margarine (Voysey 2007). A related genus, *Byssochlamys*, is the most important organism causing spoilage of pasteurized juices because of the high heat resistance of its spores (Voysey 2007).
C. *Aspergillus* and related molds are more resistant to high temperatures and low water activity than *Penicillium* spp. and tend to dominate spoilage in warmer climates. Many aspergilla produce mycotoxins: aflatoxins, ochratoxin, territrems, and cyclopiazonic acid. *Aspergillus* spp. spoil a wide variety of food and non-food items (paper, leather, etc.) but are probably best known for spoilage of grains, dried beans, peanuts, tree nuts, and some spices (Doyle 2007).

D. *Fusarium* spp. cause plant diseases and produce several important mycotoxins but are not important spoilage organisms. However, their mycotoxins may be present in harvested grains and pose a health risk (Doyle 2007).

3.4.3 Bacteria

Bacterial species comprise a wide range of Gram-positive and Gram-negative strains. Gram-positive bacteria include spore- and non-spore-forming species.

Gram-Positive Spore-Forming Bacteria

Spore producers are usually associated with spoilage of heat-treated foods because their spores can survive high processing temperatures. These Gram-positive bacteria may be strict anaerobes or facultative (Voysey 2007). **Thermophilic** spore-forming bacteria prefer growth at high temperatures as high as 55 °C. Some thermophiles (*Bacillus* and *Geobacillus* spp.) cause sour spoilage of high or low pH canned foods with little or no gas production (Pepe et al. 2003). **Mesophilic** anaerobes, grown at ambient temperatures, cause several types of spoilage of vegetables (*Bacillus* spp.); putrefaction of canned products, early blowing of cheeses, and butyric acid production in canned vegetables and fruits (*Clostridium* spp.); and medicinal flavors in canned low-acid foods (*Alicyclobacillus*) (Chang and Kang 2004). **Psychrotolerant** spore-forming bacteria produce gas and sickly odors in chilled meats and brine-cured hams (*Clostridium* spp.), while others produce off-odors and gas in vacuum-packed, chilled foods and milk (*Bacillus* spp.) (Chang and Kang 2004).

Gram-Positive Lactic Acid Bacteria

Lactic acid bacteria are a group of Gram-positive bacteria, including species of *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Oenococcus*, some of which are useful in producing fermented foods such as yogurt and pickles (Doyle 2007). However, under low oxygen, low temperature, and acidic conditions, these bacteria become the predominant spoilage organisms on a variety of foods. Undesirable changes caused by lactic acid bacteria include greening of meat and gas formation in cheeses (blowing), pickles (bloater damage), and canned or packaged meat and vegetables. Off-flavors described as mousy, cheesy, malty, acidic, buttery, or liver-like may be detected in wine, meats, milk, or juices spoiled by these bacteria. Lactic acid bacteria may also produce large amounts of an exopolysaccharide that causes slime on meats and ropy spoilage in some beverages (Doyle 2007).

Gram-Negative Enterobacteriaceae

Enterobacteriaceae are facultatively anaerobic bacteria that include a number of human pathogens such as *Salmonella*, *E. coli*, *Shigella*, and *Yersinia*, as well as a large number of spoilage organisms. These bacteria are widespread in nature in soil, on plant surfaces, and in digestive tracts of animals and are therefore present in many foods. *Erwinia carotovora* is one of the most important bacteria causing soft rot of vegetables in the field or those stored at ambient temperatures. Biogenic amines are produced in meat and fish by several members of this group, while others produce off-odors or off-colors in beer (*Obesumbacterium*), bacon and other cured meats (*Proteus*, *Serratia*), cheeses (several genera), coleslaw (*Klebsiella*), and shell eggs (*Proteus*, *Enterobacter*, *Serratia*). Temperature, salt concentration, and pH are the most important factors determining which, if any, of these microbes spoil foods (Doyle 2007). Many Gram-negative bacteria, including pseudomonads and enterobacteriaceae, secrete acyl homoserine lactones to regulate the expression of certain genes, such as virulence factors, as a function of cell density. These acyl homoserine lactones quorum-sensing signals may regulate proteolytic enzyme production and iron chelation during spoilage of some foods (Rasch et al. 2005).

Gram-Negative *Pseudomonas*

Pseudomonas and related genera are aerobic group of rod-shaped bacteria, some of which can degrade a wide variety of unusual compounds. They generally require a high water activity for growth, inhibited by pH values less than 5.4 and grown rapidly (psychrophilic) at 3–10 °C (Voysey 2007). Four species of *Pseudomonas* (*P. fluorescens*, *P. fragi*, *P. lundensis*, and *P. viridiflava*), as well as *Shewanella putrefaciens* and *Xanthomonas campestris*, are the main food spoilage organisms in this group. Soft rots of plant-derived foods occur when pectins that hold lyase enzymes are secreted by *X. campestris*, *P. fluorescens*, and *P. viridiflava* (Doyle 2007). These two species of *Pseudomonas* comprise up to 40 % of the naturally occurring bacteria on the surface of fruits and vegetables and cause nearly half of post-harvest rot of fresh produce stored at cold temperatures. The strains, *P. fluorescens*, *P. fragi*, and *P. lundensis*, cause spoilage of animal-derived foods (meat, fish, milk) by secreting lipases and proteases that cause formation of sulfides and trimethylamine (off-odors) and by forming biofilms (slime) on surfaces (Doyle 2007).

Other bacterial species are associated with spoilage of chilled, high protein foods such as meat, fish, and dairy products. They may not be the predominant spoilage organisms but contribute to the breakdown and decay of food components and may produce off-odors. Most species are aerobic although some grow at low oxygen levels and may survive vacuum packaging (Voysey 2007; Doyle 2007). Some examples include (a) *Acinetobacter* and *Psychrobacter*, which are predominant bacteria on poultry carcasses on the processing line and have been isolated from a variety of spoiled meat and fish. *Acinetobacter* grows at a pH as low as 3.3 and has

been detected in spoiled soft drinks. These two genera do not produce extracellular lipases, hydrogen sulfide, or trimethylamine (fishy odor) and so are considered to have a low spoilage potential (Doyle 2007). (b) *Alcaligenes* is a potential contaminant of dairy products and meat and has been isolated from rancid butter and milk with an off-odor. These bacteria occur naturally in the digestive tract of some animals and also in soil and water. (c) *Flavobacterium* is found widely in the environment and in chilled foods, particularly dairy products, fish, and meat. It uses both lipases and proteases to produce disagreeable odors in butter, margarine, cheese, cream, and other products with dairy ingredients (Voysey 2007). (d) *Moraxella* and *Photobacterium* are important constituents of the microflora on the surface of fish. *Photobacterium* can grow and produce trimethylamine in ice-stored, vacuum-packaged fish (Doyle 2007).

4 Food Poisoning

There are thousands of cases of food poisoning each year, many of which are not reported or recorded in official statistics. Food poisoning may result from poor domestic food preparation, or poor food processing in industry. This may result in loss of business and people's jobs if it is a serious outbreak. Microorganisms occur naturally in the environment; on cereals, vegetables, fruit, animals, people, water, and soil; and in the air (Food Poisoning. A fact of life 2009). Most bacteria are harmless, but a small number can cause illness. Food which is contaminated with food poisoning microorganisms missed normal taste and smell. Food poisoning can be mild or severe. The symptoms will be different depending on what type of bacteria is responsible. Common symptoms include severe vomiting, diarrhea, exhaustion, headache, fever, and abdominal pain (Food Poisoning. A fact of life 2009).

4.1 Causal Factors for Food Poisoning

Some common factors leading to food poisoning include (1) poor hygiene, (2) preparation of food too far in advance, (3) frequent cooling and thawing, (4) infected food handlers, (5) contaminated processed food, (6) improper warm holding, and (7) consuming infected raw food. Sources of spoilage microorganisms include soil, water, air, dust, animal hides, the gastrointestinal tract, plant products, as well as food handlers, food utensils, and processing equipment (Food Poisoning. A fact of life 2009).

4.2 *Food Poisoning Microorganisms*

Food poisoning can be caused by eating food contaminated with bacteria, viruses, chemicals, or poisonous metals such as lead or cadmium. Most food poisoning, however, is caused by bacteria. Food which has become contaminated with harmful bacteria does not always taste bad. Most of the time it looks, smells, and tastes like it normally does (Doyle 2007). When someone swallows bacteria that cause food poisoning, there is a delay (incubation period) before symptoms begin. This is because most bacteria that cause food poisoning need time to multiply in the intestine (Food Poisoning Bacteria—*Salmonella, Listeria, E. coli* 0157, *Campylobacter*. info@www.accepta.com). The length of the incubation period depends on the type of bacteria, where it sticks to the lining of the intestine and can destroy cells by production of toxins (poisons) which absorbed and cause damage. Because bacteria enter the body through the digestive system, symptoms will generally be nausea, vomiting, abdominal cramps, and diarrhea (Australian Government 2010). In some cases, food poisoning can cause very serious illness or even death. Most common types of food poisoning bacteria are discussed below.

4.2.1 *Clostridium botulinum*

These bacteria are found in the soil and in the inadequately processed canned meat, vegetables, and fish (faulty canning). Food poisoning caused by clostridium bacteria is important to know about because these bacteria are common in the environment. Food poisoning by clostridium can be gotten from poor food handling practices in the home, in the factory, or in a food outlet, especially relating to cooking and storage/refrigeration temperatures. *Clostridium* food poisoning symptoms occur about 12 h after eating a contaminated food (Australian Government 2010). Symptoms include stomach pains, diarrhea, drooping eyelids, and sometimes nausea and vomiting. Symptoms last about 24 h. One type of clostridium bacteria produces a very serious food poisoning disease called botulism. This disease is caused by eating food which is contaminated with an extremely poisonous toxin produced by the bacteria *Clostridium botulinum*. Unless properly treated, death occurs within 3–7 days or a slow recovery over months.

4.2.2 *Staphylococci*

These bacteria are found on the skin, in sores, in infected eyes, and in the nose, throat, saliva, and bowel of humans. There may be many of these bacteria in the yellow mucus (slimy substance) which comes from the nose or is coughed up when a person has a cold or a lung infection (Australian Government 2010). *Staphylococci* do not cause illness until they get onto food and grow and multiply. While they are doing this, they produce a toxin (poison). It is the toxin which causes the

illness. The toxin is not destroyed by cooking the food. Symptoms of *Staphylococcus* food poisoning usually appear between 1 and 8 h after eating the infected food.

4.2.3 *Campylobacter*

These bacteria are found in many animals including dogs, cats, cattle, and poultry. The sources of infection from these bacteria are usually contaminated food and water. Infection can occur from ingestion of contaminated food or water (especially undercooked chicken and creek or river water), contact with infected animals (especially puppies or kittens with diarrhea), or poor food handling (especially by using the same chopping boards, knives, and plates for raw and cooked chicken) (Australian Government 2010). Campylobacter food poisoning symptoms usually last from 2 to 5 days. These include diarrhea, severe abdominal pain, vomiting, and fever. It is a serious disease in indigenous communities because of the possibility of dehydration from diarrhea.

4.2.4 *Escherichia coli*

Escherichia coli is the most prevalent infecting organism in the family of Gram-negative bacteria known as enterobacteriaceae (Barry and Dori 2000). *Escherichia coli* bacteria were discovered in the human colon in 1885 by German bacteriologist Theodor Escherich (Feng et al. 2002). Dr. Escherich also showed that certain strains of the bacterium were responsible for infant diarrhea and gastroenteritis, an important public health discovery. Although *E. coli* bacteria were initially called *Bacterium coli*, the name was later changed to *Escherichia coli* to honor its discoverer (Feng et al. 2002). *Escherichia coli* is often referred to as the best or most-studied free-living organism (Barry and Dori 2000; James 2000). More than 700 serotypes of *E. coli* have been identified (Barry and Dori 2000; Griffin and Tauxe 1991). The "O" and "H" antigens on the bacteria and their flagella distinguish the different serotypes (Griffin and Tauxe 1991). Most strains of *Escherichia coli* are harmless, but those that produce verocytotoxin (called verocytotoxin-producing *E. coli*, or VTEC) can cause severe illness (Food Poisoning Bacteria—*Salmonella, Listeria, E. coli* 0157, *Campylobacter*. info@accepta.com).

E. coli bacteria normally live in the intestines of humans and animals. It is also found in high-risk foods such as raw meat and dairy products. Although most strains of these bacteria are harmless, several are known to produce toxins that can cause diarrhea. One particular *E. coli* strain signed 0157:H7 can cause severe diarrhea and kidney damage (Food Poisoning Bacteria—*Salmonella, Listeria, E. coli* 0157, *Campylobacter*. info@accepta.com). Diarrhea, which may contain blood, can lead to kidney failure or death. It is important to remember that most kinds of *E. coli* bacteria do not cause disease in humans. Indeed, some *E. coli* are beneficial, while some cause infections other than gastrointestinal infections, such as urinary tract infections (Barry and Dori 2000). The *E. coli* that are responsible for the

numerous reports of contaminated foods and beverages are those that produce Shiga toxin, so called because the toxin is virtually identical to that produced by *Shigella* dysenteriae type 1 (Griffin and Tauxe 1991). The best-known and also most notorious *E. coli* bacteria that produce Shiga toxin is *E. coli* O157:H7 (Barry and Dori 2000; Griffin and Tauxe 1991). Shiga toxin-producing *E. coli* cause approximately 100,000 illnesses, 3000 hospitalizations, and 90 deaths annually in the USA (Mead et al. 1999). Most reported STEC infections in the USA are caused by *E. coli* O157:H7, with an estimated 73,000 cases occurring each year (Mead et al. 1999). A study published in 2005 estimated the annual cost of *E. coli* O157:H7 illnesses to be $405 million, which included $370 million for premature deaths, $30 million for medical care, and $5 million for lost productivity (Frenzen et al. 2005).

The incubation period—that is, the time from exposure to the onset of symptoms in outbreaks of *E. coli* O157:H7 illness—is usually reported as 3–4 days, but may be as short as 1 day or as long as 10 days (Rangel et al. 2005). Infection can occur in people of all ages but is most common in children (Su and Brandt 1995). Unlike other *E. coli* pathogens, which remain on intestinal surfaces, Shiga toxin-producing bacteria, like O157:H7, are invasive (Clark M E coli food poisoning www about ecoli com). After ingestion, *E. coli* bacteria rapidly multiply in the large intestine and then bind tightly to cells in the intestinal lining (Su and Brandt 1995). This snug attachment facilitates absorption of the toxins into the small capillaries within the bowel wall (Siegler 1995; Garg et al. 2003). Once in the systemic circulation, Shiga toxin becomes attached to weak receptors on white blood cells, thus allowing the toxin to reach to the kidney where it is transferred to numerous avid (strong) receptors that hold on to the toxin (Siegler 1995). Inflammation caused by the toxins is believed to be the cause of hemorrhagic colitis, the first symptom of *E. coli* infection, which is characterized by the sudden onset of abdominal pain and severe cramps (Boyce et al. 1995). Such symptoms are typically followed within 24 h by diarrhea, sometimes fever. As the infection progresses, diarrhea becomes watery and then may become grossly bloody, that is, bloody to the naked eye (Clark M E coli food poisoning www about ecoli com). *Escherichia coli* symptoms also may include vomiting and fever, although fever is an uncommon symptom.

4.2.5 *Salmonella*

There are hundreds of different types of salmonella but not all are harmful to humans. Salmonella can cause food poisoning which can occur from poor food handling practices in home or in food outlets, seafood caught in polluted water, or eggs with dirty shells, meat, or poultry which has been contaminated by poor food handling before it gets to the food outlet, such as at the abattoir (Food Poisoning Bacteria—*Salmonella, Listeria, E. coli* 0157, *Campylobacter*. info@accepta.com). Salmonella food poisoning takes up to 48 h to develop after the food is eaten. Symptoms include nausea, stomach cramps, diarrhea, fever, and headache and may last between 3 and 21 days. It can cause death in very young, weak, or very old people. People who have cancer or are taking medication for serious health

conditions such as heart, kidney, or liver problems need to also be particularly careful that they eat safe food.

4.2.6 *Listeria monocytogenes*

Listeria monocytogenes is present all around in the environment. It has also been found in low numbers in many foods. In certain foods, such as soft mold-ripened cheeses and pâtés, it may be present in higher numbers (Food Poisoning Bacteria—*Salmonella, Listeria, E. coli* 0157, *Campylobacter*. info@accepta.com). Eating foods containing high levels of *L. monocytogenes* is generally the cause of illness. *L. monocytogenes* usually causes illness in vulnerable groups such as pregnant women, babies, the elderly, and people with reduced immunity (Food Poisoning. A fact of life 2009). Among these groups, the illness is often severe and life threatening. *L. monocytogenes* is found in high-risk foods such as unpasteurized milk and dairy products, cook-chill foods, pate, meat, poultry, and salad vegetables. Signs and symptoms of food poisoning by *L. monocytogenes* range from mild, flu-like illness to meningitis, septicemia, and pneumonia. During pregnancy, it may lead to miscarriage or birth of an infected baby.

4.2.7 *Vibrio cholerae*

The *Vibrio* bacteria live in contaminated water where they are transmitted to various species of fish and seafood such as prawns, oysters, and tuna. It causes the well-known fatal disease, cholera. These bacteria are shaped like a "comma" (a punctuation symbol) with a distinctive tail and are the direct cause of cholera in humans. *Vibrio cholerae* bacteria transfer to humans via consumption of infected food or contaminated water. The majority of these bacteria are killed by stomach acid, but a few manage to survive. These remaining bacteria access the small intestine. Their aim is to invade the soft lining of the intestinal walls, but to do so they produce a type of protein that helps for growing the distinctive curly tails which used to move through the thick mucus of these walls (Food poisoning guide: *V. cholera*. www.medic8.com). Once they access these walls, they thrive and multiply. Once inside the intestinal walls, they lose their curly tails and, instead, produce a mix of proteins which are pathogenic. This means that they have the ability to cause disease and infection. These toxins are responsible for causing watery diarrhea and other symptoms (Food poisoning guide: *V. cholera*. www.medic8.com). They produce copious amounts of fluid which is expelled from the body as diarrhea and often results in serious dehydration. There is also the risk of these bacteria infecting another person due to poor sanitation or if they have contact with infected water.

V. cholera, V. parahaemolyticus bacteria form part of the *Vibrio* genus of bacteria which are known to cause food poisoning, other gastrointestinal illnesses, and blood poisoning (septicemia). Symptoms include watery diarrhea, abdominal

pain, severe dehydration, vomiting, and shock (Food poisoning guide: *V. cholera*. www.medic8.com). The main risk is that of dehydration which can be fatal, so it is important that any treatment plan includes fluid replacement.

4.2.8 *Bacillus cereus*

Bacillus cereus is a type of bacteria that produces toxins. These toxins can cause two types of illness: one type characterized by diarrhea and the other, called emetic toxin, by nausea and vomiting (Food Poisoning. A fact of life 2009). These bacteria are present in foods and can multiply quickly at room temperature. *B. cereus* causes food poisoning where it is found in high-risk foods such as rice, meat, seafood, salads, potatoes, sauces, soup, and other prepared foods that have sat out too long at room temperature. Signs and symptoms are watery diarrhea, nausea, vomiting, and abdominal cramps with an incubation period of 1–6 h. This usually lasts less than 24 h after onset (Food Poisoning. A fact of life 2009).

5 Spoilage of Fruits and Vegetables

Food spoilage is not normally found on freshly collected food materials (except for some plants) but are extremely common in the environment and can cause contamination through airborne transmission. Yeast and mold are more resistant to low temperature, low pH, low water activity values, and the presence of preservatives than bacteria, and most are not heat resistant (Voysey 2007). Fungal spoilage may be characterized by highly visible, often pigmented growth, slime, off-odors, and off-flavors. Vegetables such as tomato, potato, cucumber, lettuce, as well as asparagus, carrot, and legumes are most affected by fungi (Table 1). Yeast spoilage of fresh fruits and vegetables is represented in Table 2 (Voysey

Table 1 Spoilage of fresh and stored vegetables by some genera of fungi

Vegetables affected by fungi	Genus	Type of spoilage
Most vegetables especially carrot, lettuce, celery, cabbage	*Botrytis*	Gray mold rot
Most vegetables especially carrot, lettuce, legumes, *Brassica* species	*Sclerotinia*	Watery soft rot
Legumes, carrot, *Brassica* species	*Rhizopus*	Soft rot
Tomato, cucumber, asparagus, potato	*Fusarium*	Dry rots
Tomato, potato, carrot	*Phytophthora*	Brown rots (blight)
Cucumber, legumes, potato	*Pythium*	Cottony leak
Onion, *Brassica* species	*Peronospora*	Downy mildews
Tomato, *Brassica* species	*Alternaria*	Black rots

Table 2 Yeast spoilage of fresh fruits and vegetables

Commodities	Yeast
Dates	*Saccharomyces* spp., *Candida quilliermondii*, *Hanseniaspora valbvensis*
Figs	*Candida krusei*, *Saccharomyces cerevisiae*, *Torulopsis stellata*, *Hanseniaspora valbvensis*, *Klorkera apiculata*
Strawberries	*Klorkera apiculata*
Tomatoes	*Pichia kluyveri*, *Klorkera apiculata*, *Nematospora coryli*
Legumes, coffee berries, nuts, citrus fruits	*Nematospora coryli*
Pineapple	*Candida* spp.

2007). Bacteria that cause soft rot of some fruits and most vegetables are *Erwinia* spp. and *Pseudomonas* spp. (Voysey 2007).

6 Food Preservation

Food preservation is a process involved in protection of food against microbes and other spoilage agents to permit long shelf life for future consumption. Shelf life of a food is the time during which it remains stable and retains its desired qualities (Doyle 2007). The preserved food should retain a palatable appearance, flavor, and texture, as well as its original nutritional value (Spoilage of Food. Home.pacific.nethk/ppleung/chem/preservation.htm). Basic important benefits of food preservation include the following: (1) protect food against microbes and other spoilage agents; (2) add a variety to the food and increase the food supply (examples are canned or dehydrated vegetables or other foods); (3) change raw foods into more stable form and thus increase the shelf life of foods, fruits, and vegetables to ensure safety of foods for future consumption; (4) decrease the wastage of food; (5) decrease dietary inadequacies; and (6) allow many foods to be available year-round, in great quantity and the best quality. Variety in diet is brought about with the aid of preserved food supply. For example, some Middle East countries do not grow any vegetables due to arid soil conditions (Spoilage of Food. Home.pacific.nethk/ppleung/chem/preservation.htm). This shortcoming is overcome through the import of fresh and preserved fruits and vegetables. Microorganisms, enzymes, and chemical reaction of food components are the main causes of food spoilage. So the principles of preservations are killing and inhibition of microbial growth, destroying enzymes, and retardation of chemical changes (Spoilage of Food. Home.pacific.nethk/ppleung/chem/preservation.htm).

6.1 Microbial Indicators of Product Quality

Spoilage microorganisms or their metabolic products that are present in a given food may be used as a predictive to assess the food quality and shelf life of a product. Microbial indicators that are highly correlated with food quality are represented in Table 3 (Voysey 2007). Metabolic products as indicators of a product quality include diacetyl (fruit juice concentrates), histamine (canned tuna), lactic acid (canned vegetables), trimethylamine and volatile nitrogen (seafish), and volatile fatty acids (butter). Food spoilage can be prevented by the following directives: (1) use of quality raw materials, (2) correct storage for the food type, (3) use of predictive methods, (4) hygiene of processing environment, (5) giving out appropriate shelf life, (6) usage of organic acids and antimicrobial agents, and (7) training and education.

6.2 Side Effects of Food Preservation

Principles of food preservation depend on heating up or cooking of foods such as milk, fruits, and vegetables. Heat kills microorganisms and their spores, alters protein structure, and destroys enzyme activity of microorganisms in food. Food preservation process used to inhibit microorganisms can be performed by heat treatment, smoking, irradiation, freezing, canning, meat curing, as well as salting, drying, and dehydration (Smith 2007). Some of these methods may show disadvantage such as canning where the containers that are not properly canned may result in botulism. On the other side, when foods are smoked, they absorb various chemicals from the smoke including aldehydes and acids. Aldehydes are carcinogenic, and people who eat a heavy diet of smoked foods suffer disproportionately from cancer of the mouth and stomach. Through meat curing, where strong salt solution containing $NaNO_3$, KNO_3, and spices is used, excessive intake of nitrites causes a fall in the level of hemoglobin in the blood. In long term, this leads to malnutrition and reduced lifespan. Nitrates are harmless, but when nitrates are

Table 3 Microbial indicators that are highly correlated with food quality (Voysey 2007)

Organism	Product
Acetobacter	Fresh cider
Bacillus	Bread dough
Clostridium	Hard cheeses
Pseudomonas putrefaciens	Butter
Leuconostoc mesenteroides	Sugar (during refinery)
Byssochlamys spp.	Canned fruits
Zygosaccharomyces bailii	Mayonnaise, salad dressing
Flat sour spores	Canned vegetables
Yeasts	Fruit juice concentrates
Lactic acid bacteria	Beers, wines

ingested in the diet, they are reduced to nitrites in the body. Then, nitrites may react to form nitrosamines (Sivasankar 2004). Disadvantage of freezing includes textural changes. Freezing involves the change of water contained in the food from a liquid to a solid (ice). When water freezes, it expands, and the ice crystals formed cause cell walls of food to rupture. As a result, the texture of the product will be much softer when it thaws. Textural changes are most noticeable in fruits and vegetables that have high water content and are not as apparent in products that are cooked before eating. Methods of heating include (a) blanching, usually applied before freezing of fruits and vegetables to denature enzymes; (b) pasteurization, used to destroy pathogenic microorganisms and extend the shelf life of a food; and (c) commercial sterilization, which destroys all pathogenic and toxin-forming organisms, as well as other types of organisms, which if present could grow in the food and cause spoilage under normal handling and storage conditions (Desrosier 1963). High temperatures, however, can diminish product appearance, texture, and nutrient quality of all forms of cooked food, milk sterilized by ultra-high temperature, beer, and wine (Sivasankar 2004).

7 Control Strategies of Spoilage Microorganisms

Spoilage microorganisms are not originally an essential part of foods but are widely present in water, soil, air, and other animals. Healthy living plants and animals can ward off bacteria and fungi, but as soon as they are slaughtered or harvested, their defenses deteriorate and their tissues become susceptible to spoilage microbes (Doyle 2007). The most important first step in delaying the spoilage process and that can prevent colonization by many, but not all, microbes is the good manufacturing practices with strict attention to sanitation and hygiene. Some strategies to ensure good control of microbial spoilage are discussed below (Doyle 2007):

1. Spoilage microorganisms require certain conditions for growth, and therefore management of the environment of foods can change these factors and delay spoilage. Many, but not all, microbes grow slowly or not grow at all at low temperatures, and deep refrigeration can prolong the lag phase and decrease growth rate of microbes.
2. Many microbes require a high water activity, and therefore keeping foods such as grains and cereal products dry will help to preserve them.
3. Some microbes require oxygen, while others are killed by oxygen, and some species are facultative. Managing the atmosphere during storage in packaging can retard or prevent the growth of some microbes. Several types of modified atmosphere packaging have been developed to retard growth of pathogenic and spoilage organisms (Balamatsia et al. 2006a; Charles et al. 2006; Ercolini et al. 2006; Tremonte et al. 2005).

However, due to the continuous changes of spoilage microbes, to overcome difficulties and any barriers against them, further strategies are utilized to extend shelf life of foods. Such controlling strategies must be assessed for compatibility with different foods to ensure absence of any significant organoleptic changes of foods caused by addition of preservatives (Doyle 2007).

7.1 Common Methods of Food Preservation and Processing

Preservation involves a change to the nature of a product that reduces the microbial load or limits the growth of microorganisms while maintaining the food item's nutritional value, texture, and flavor. Clean, high-quality ingredients are needed for effective preservation (Smith 2007). Use of unsound raw material is economically unwise due to the losses involved and the possible negative effect on finished product quality. The exact method of preservation used is dependent on the product, its effect on product safety, and the process facility in terms of power, space, equipment, and hygiene (Smith 2007). Food processing is often the set of methods and techniques used to transform raw ingredients into food for consumption. The *food processing industry* utilizes these processes. Food processing often takes clean, harvested, or slaughtered food components and converts them into attractive and marketable food products (Sivasankar 2004). Common methods of food preservation include canning, smoking, drying, salting, pickling, sterilization, and sugaring (Smith 2007). Some of these methods may show disadvantage as discussed above.

7.2 Common Methods of Shelf Life Extension

Shelf life extension relies on changing the storage conditions and/or the product packaging to inhibit microbial growth. Methods used for shelf life extension are freezing, chilling, vacuum packing, and controlling atmosphere packaging (Smith 2007).

7.2.1 Freezing

Freezing can be a simple process to implement and can extend the shelf life by years in some cases. Product quality is retained better by faster freezing speeds to avoid the smaller size of ice crystals formed. Numerous methods are available for freezing product, including blast freezing (cabinet, room, or spiral freezer, dependent on throughput), plate freezing (for blocks of meat, fish, or vegetables), or scraped surface heat exchangers for ice cream (Smith 2007). The exact method will depend on throughput and costs, e.g., a liquid nitrogen bath uses an expensive coolant but has a very high throughput and takes up less space than a tunnel.

7.2.2 Chilling

Chilling is done via a blast chiller cabinet or room; larger-scale production can use spiral chillers for continuous production. Liquids can be cooled using plate or scraped surface heat exchangers (Smith 2007).

7.2.3 Vacuum Packing

Vacuum removes oxygen (when reacts with food causing undesirable changes in color and flavor). Vacuum packing is relatively a simple way of extending shelf life, with refrigeration. It is often used for meat or fish products (Smith 2007). Use of contaminated material, however, can lead to growth of pathogenic anaerobic bacteria. This method does have risks, especially in leaky seals or damaged packaging. If there is contamination, the spoilage may not be readily visible.

7.2.4 Controlled Atmosphere Packaging

Controlled atmosphere packaging is used as a means of extending the shelf life of products such as chilled meat, fish, dairy, or poultry (Smith 2007). The atmosphere inside the packaging is modified or controlled to inhibit or reduce the rate of spoilage. This is an expensive process due to the heavy gauge packaging needed, the machinery setup required, and the food grade gas mix used (Smith 2007). A less aggressive version is gas flushing that has lower machinery costs but a lower throughput. These systems are used with careful temperature control (refrigeration).

8 Recent Advance in Food Preservation

Recent processing technologies, in addition to the thermal processing, being developed to kill spoilage microbes, are discussed below.

8.1 Irradiation

Some of the future methods of food preservation are irradiation and chemical additives. Although these methods are currently in use, they are expected to expand and develop further. Irradiation of food is the process of exposing food to ionizing radiation (Blum 2012). This process can alter the bacteria, microorganism, or virus' DNA, without harming the food. Irradiation is attractive because of its selective targeting. It is already used on non-food items. The molecular bonds in the

microbial DNA are the main targets of the irradiation, but DNA and RNA synthesis, denaturation of enzymes, and cell membrane alterations may also be affected (Blum 2012). The process of irradiation opens the possibility to process a large number of foods in great quantities; however, it can be expensive. The buildings for such a process require specific infrastructure and construction that is both expensive and time-consuming. The Food and Agriculture Organization, the International Atomic Energy Agency, and the World Health Organization concluded in their report that any food irradiated up to a maximum dose of 10 kGy is considered safe and healthy (Blum 2012). Essentially, three things were concluded in their report: (1) It will not lead to toxicological changes in the food that will negatively affect our health. (2) The technology will not increase the microbial risk of the consumer. (3) Irradiation will not lead to nutritional losses. However, most consumers are still radiation phobic despite these positive results. They are not willing to buy something that has, in their mind, been radiated (Blum 2012). Irradiation of fruit and meat is reported (Balamatsia et al. 2006b; Fan et al. 2006; Mahapatra et al. 2005).

8.2 Addition of Chemicals

Addition of chemicals is another process to preserve foods. Some familiar examples of food additives are sodium benzoate, benzoic acid, calcium, sodium propionate, propionic acid, calcium, potassium, and sodium sorbate, sorbic acid, and sodium and potassium sulfite. Consumers feel that this is a risk because of the unnatural state of chemicals. Chemicals are added to prevent spreading of mold or other microorganisms. Furthermore, addition of oxygen-scavenging chemicals (oxygen eliminators) is an effective preservative method because oxygen increases the possibility of spoilage to the food (Blum 2012). A special class of additives that reduce oxidation is known as the sequestrant, that is, compounds that capture metallic ions, such as those of copper, iron, and nickel, and remove them from contact with foods (World of Microbiology and Immunology 2003). The removal of these ions helps preserve foods because in their free state, they increase the rate at which oxidation of foods takes place. Some examples of sequestrants used as food preservatives are ethylenediaminetetraacetic acid, citric acid, sorbitol, and tartaric acid.

8.3 Antimicrobials

Recently, naturally occurring antimicrobials including chitosan, lysozyme, and nisin; various plant extracts such as tea, spices, and their essential oils; as well as phenolic compounds have been widely investigated and are recommended for food preservation (Rawdkuen et al. 2012). Food-borne illnesses that result from consuming food contaminated with pathogenic bacteria have been of serious public

concern worldwide. Food-borne illnesses associated with *E. coli* 0157:H7, *S. aureus*, *Salmonella enteritidis*, and *L. monocytogenes* are a major public health concern all over the world (Juneja et al. 2012). These bacteria are correlated with serious illnesses such as urinary tract infection, cholecystitis, or septicemia, post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis, and food poisoning. Consumer demand for naturally derived compounds such as antimicrobial substances has been increased (Rawdkuen et al. 2012; Yousef 2013). Antimicrobial substances have been used to inhibit food-borne bacteria and extend the shelf life of processed food by either reducing the microbial growth rate or by extending the lag phase of the target microbes. Bacteriocin, including nisin, can help control spoilage bacteria in dairy products, fish, juice, and vegetables (Mahapatra et al. 2005; Cha and Chinnan 2003; Deegan et al. 2006; Jamuna et al. 2005). Chitosan incorporated into foods or used as a coating for fruits and vegetables inhibits growth of some spoilage bacteria and yeasts (Altieri et al. 2005; Devlieghere et al. 2004). Many herbs, essential oils, and spices have demonstrated potential inhibitory activity against spoilage microbes in a variety of foods (Arici et al. 2005; Angelini et al. 2006; Kang et al. 2006; Matan et al. 2006; Oussalah et al. 2006; Sacchetti et al. 2005; Kaur et al. 2013).

8.4 High-Pressure Preservation

The most recent technology and safe process of food preservation is the high-pressure preservation. Balasubramaniam et al. (Balasubramaniam et al. 2008; Yousef and Balasubramaniam 2013) developed a process where food is attacked with 100,000 pounds per square inch of pressure. A process in which high-pressure processing works for food preservation was explained. The food is packaged in a plastic bag and then put into the first compartment. That compartment is also filled with water, to help with the pressure. The second compartment is filled with hydraulic fluid, which is used to press the piston between the two compartments. The water becomes more condensed, and as a result the pressure in the first compartment increases rapidly (Freedman 2011). After only a few minutes, any food can be ready to eat. In this process, no chemicals are added; there is no contamination or taste alteration to the food. The bacteria in the food remain intact; however, they die due to the pressure-induced dismantlement of their DNA structure. This process destroyed pathogens and spoilage organisms while keeping food chemistry basically intact. High-pressure technology enables pasteurization of foods with minimal effects on taste, texture, appearance, or nutritional value (Balasubramaniam et al. 2008; Yousef and Balasubramaniam 2013). Almost all foods are able to be pressurized in the machine. The only exception is some veggies and fruits, which get too crushed (Freedman 2011). This technology is so new, simple, and so good to reserve for large industrial food processing plants. High-pressure processing of fruits, juices, meat, and fish is reported (Hocking et al. 2006; Tahiri et al. 2006; Torres and Velazquez 2005).

9 Conclusion

Because evidence exists that microorganisms can acquire varying levels of resistance or tolerance to environmental stresses, there is some concern that this might provide protection for food-borne pathogens against antimicrobials and preservation processes. Development of resistance to manufacturing and processing treatments could occur in a food production system and could influence preservation treatment efficacy. Food preservation processes are designed to either inhibit the growth of or inactivate bacteria, depending upon the type and severity of the process used. Thus, food preservation exposes bacteria to a lethal stresses. Food preservation has become vital, and preservation has come from simple processes such as salting to more complex preserving methods such as irradiation and chemical additives. Looking into the future, high-pressure preservation seems to be the next logical step. In an increasingly health-conscious world, uncontaminated, well-preserved food source and low costs of production are the ultimate goals to ensure and enjoy a preservative food.

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Aspergillus and Ochratoxin A in Latin America

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Abstract Species within *Aspergillus* section *Nigri* and section *Circundati* are important sources of ochratoxin A (OTA) contamination in several foods and beverages such as cereals, grapes, spices, peanut, cocoa, wine, beer, and coffee worldwide. Few surveys showed the epidemiology, ecology, and distribution of black aspergilli occurring in crops from different Latin American regions. Within section *Nigri*, *Aspergillus niger* aggregate species are commonly the most prevalent, followed by *A. carbonarius* and *Aspergillus uniseriate*. *Aspergillus ochraceus* is the most important ochratoxigenic species included in the section *Circundati*. These species differ in their OTA-producing ability, since the percentage OTA-producer strains and the levels produced are variable. The OTA levels detected in grains and fruits also were variable, and it was shown that their damage affected the incidence of ochratoxigenic species and OTA contamination. Temperature and water activity were also key factors determining the *Aspergillus* section *Nigri* colonization and OTA accumulation in different commodities. The knowledge of the factors affecting preharvest and post-harvest contamination of foods by black aspergilla and OTA production is essential to improve the quality and safety of foods. Different strategies can be used to reduce the impact of *Aspergillus* and OTA accumulation. Alternative strategies to the chemical control can be biocontrol or the use of natural substances such as antioxidants and essential oils. The data presented in this review provide relevant information for using appropriate management strategies to reduce or prevent the development of ochratoxigenic species and OTA accumulation on different commodities relevant for Latin America.

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1 Introduction

Fungal genera most commonly isolated in foods and byproducts are *Aspergillus, Fusarium*, and *Penicillium*. Members of the *Aspergillus* genus are universally distributed, being ubiquitous in most environments, but more abundant in temperate and subtropical climates between latitudes 26° and 35° north or south of Ecuador (Klich 2002). Particularly, *Aspergillus* species occupy different ecological niches and are metabolically versatile (Frisvad et al. 2007). Therefore, given the variety of habitats in which they can be found, they are economically important due to their beneficial as well as harmful effects. One of the most relevant consequences of their presence is the mycotoxin contamination of foods and feeds which cause adverse health effects, in addition to leading to huge economic losses in producer and exporter countries.

Ochratoxin A (OTA) is a mycotoxin that has demonstrated to be nephrotoxic and has been associated with a fatal human kidney disease, referred to as Balkan endemic nephropathy (BEN) and with an increase in tumor incidence of the upper urinary tract (Marquardt and Frohlich 1992). It has also showed carcinogenic, immunotoxic, genotoxic, teratogenic, and possibly neurotoxic properties. The presence of OTA has been reported in a wide range of foods and beverages, in body fluids, and in kidneys of animals and humans (EFSA 2006). Natural occurrence of OTA in Latin America has been observed in coffee, wines, beers, grapes, dried grapes, species, cereals, and derivates destined for humans and animals (Iamanaka et al. 2005; Taniwaki 2006; Chulze et al. 2006; Magnoli et al. 2006a, 2007a, b; Kawashima et al. 2007; Rosa et al. 2009; Shundo et al. 2009; Ponsone et al. 2010; Vega et al. 2012; Vanesa and Ana 2013). The maximum limits for OTA are regulated in many countries but not in Latin America countries. However, several raw food and its products are exported to countries included in the European Union, which has established regulations for OTA in several food (European Commission 2006, 2010).

The most relevant *Aspergillus* species responsible for OTA contamination in food belong to section *Nigri* and *Circundati*, due to their incidence in foodstuffs and the number of strains to produce OTA. Moreover, they are always present in crops and can be isolated from different products during processing stages (Amézqueta et al. 2012). Within the section *Nigri*, *A. carbonarius* and *A. niger* have been identified as one of the main sources of OTA contamination and accumulation in grapes, raisins, and wines from Latin America (Díaz et al. 2009; Chiotta et al. 2013). These species also contributed to OTA contamination in coffee, cocoa, peanut, and cereals (Taniwaki et al. 2003; Magnoli et al. 2006a, b; Copetti et al. 2010). *Aspergillus ochraceus* is one of the most important ochratoxigenic species included in section *Circundati* and it was isolated frequently in coffee beans and less commonly in cereals (Taniwaki et al. 2003; Magnoli et al. 2006b).

Several studies have demonstrated that OTA contamination severity in food and feed chains depends on different factors such as agroclimatic conditions, farm location, and by-product processes system and storage. The knowledge of these

factors is essential to reduce OTA presence, not only to improve food and feed quality but also to maintain their safety. This chapter focuses on ochratoxigenic species of *Aspergillus* and OTA contamination in the more common substrates grown in Latin America, the factors that influence their accumulation and strategies to reduce or prevent the development of ochratoxigenic species and the subsequent OTA production.

2 State of Art of *Aspergillus* Section *Nigri* and *Circundati* Taxonomy

Differences in species number recognized by different authors have demonstrated the difficulties to establish clear criteria for the identification of species included in *Aspergillus* section *Nigri*. Abarca et al. (2004) conducted an extensive review of this section describing taxonomic problems and the importance of the various species described at the moment. Samson et al. (2004) described four new species, accepting a total of 15 taxa: *A. aculeatus, A. brasiliensis, A. carbonarius, A. costaricaensis, A. ellipticus, A. japonicus, A. foetidus, A. heteromorphus, A. homomorphus, A. lacticoffeatus, A. niger, A. piperis, A. sclerotioniger, A. tubingensis*, and *A. vadensis*. These authors based the differentiation between species in eight secondary metabolite profiles and some morphological characteristics. Other species have been described later, such as *A. ibericus, A. uvarum, A. sclerotiicarbonarius*, and *A. aculeatinus* (Serra et al. 2006; Noonim et al. 2008; Perrone et al. 2008). A classification summary of the section *Nigri* was proposed by Samson et al. (2007), and methods used in the diagnosis of known species were assembled and accepted. Some additional methods are included in Frisvad et al. (2007), who propose the classification of black *Aspergillus* based on morphological, physiological, and chemical characteristics as resulted of similar clusters previously obtained by β-tubulin sequencing (Samson et al. 2004; Perrone et al. 2007). For example, *A. carbonarius*, *A. sclerotioniger*, *A. ibericus*, and *A. sclerotiicarbonarius* species were grouped in the suggested series "Carbonaria" because they have relatively large rough-walled conidia, a relatively low growth rate at 37 °C, moderate citric acid production, and other characters in common besides belonging to the same clade according to β-tubulin sequencing. Varga et al. (2011) described four new species, *A. eucalypticola, A. neoniger*, *A. idologenus*, and *A. fijiensis*, and revalidated other species, such as *A. acidus* (Kozakiewicz 1989) and *A. violaceo-fuscus* (Gasperini 1887), including a total of 26 taxa divided into five major clades within *Aspergillus* section *Nigri*. *Aspergillus acidus* (previously known as *A. foetidus* var. *pallidus* and *A. foetidus* var. *acidus*) was also a valid species, while *A. foetidus* was considered a synonym of *A. niger* based on molecular and physiological data. Moreover, the species previously described as *A. coreanus* and *A. lacticoffeatus* were found to be color mutants of *A. acidus* and *A. niger*, respectively. In subsequent studies, Sørensen et al. (2011) described a novel species, *Aspergillus saccharolyticus* sp. nov., a uniseriate

Aspergillus species which is morphologically similar to *A. japonicus* and *A. aculeatus,* but with a totally different extrolite profile to any known species of *Aspergillus*. In another study, the species included in the "*A. aculeatus* clade" used in previous molecular studies was revised using combined molecular data. These data together with two different PCR-fingerprinting methods indicated that *A. japonicus* should be treated as a synonym of *A. violaceofuscus*. Similarly, *A. fijiensis* is reduced to synonymy with *A. brunneoviolaceus* (Hubka and Kolarik 2012). On the other hand, *A. floridensis* and *A. trinidadensis* were described as novel uniseriate species of *Aspergillus* section *Nigri*. *Aspergillus floridensis* was closely related to *A. aculeatus*, and *A. trinidadensis* was closely related to *A. aculeatinus* (Jurjević et al. 2012). Another study indicated that *A. luchuensis, A. kawachii*, and *A. acidus* are the same species, and *A. luchuensis* was selected as the correct name based on priority (Hong et al. 2013). They also showed that the name *A. awamori* was misapplied and regarded *A. awamori* as doubtable species. They renamed *A. awamori* as *Aspergillus welwitschiae* (Table 1).

Table 1 Classification of *Aspergillus* section *Nigri* species

Aspergillus section *Nigri*		Ochratoxin A
Clade *A. carbonarius*	*A. carbonarius*	+
	A. sclerotioniger	+
	A. sclerotiicarbonarius	−
	A. ibericus	−
Clade *A. heteromorphus*	*A. ellipticus*	−
	A. heteromorphus	−
Clade *A. homomorpha*	*A. homomorphus*	−
Clade *A. niger*	*A. niger* = *A. foetidus* = *A. lacticoffeatus*)	+/−
	A. welwitschiae (*A. awamori*)	+/−
	A. neoniger	−
	A. tubingensis	*
	A. luchensis (*A. acidus*—*A. kawachii*—*A. coreanus*)	−
	A. brasiliensis	−
	A. costaricaensis	−
	A. piperis	−
	A. vadensis	−
	A. eucalypticola	−
Clado A. aculeatus	*A. japonicus* (*A. violaceofuscus*)	−
	A. aculeatus	−
	A. uvarum	−
	A. aculeatinus	−
	A. indologenus	−
	A. fijensis (*A. brunneoviolaveus*)	−
	A. saccharolyticus	−
	A. floridensis	−
	A. trinidadensis	−

*Trace amounts of ochratoxin A production

Members of *Aspergillus* section *Circundati* have been taxonomically revised by Christensen and Raper (1970) and Christensen (1982). Varga et al. (1998, 2000a, b, c) presented molecular analysis of the genetic variability among species of this section. Rather few new species have been described: *A. sepultus* (Tuthill and Christensen 1986), *Neopetromyces muricatus* (Udagawa et al. 1994; Frisvad and Samson 2000), and *A. persii* (Zotti and Montemartini Corte 2002). Based on rDNA sequence data, Peterson (2000) indicated that same species originally placed in section *Circundati* belong to other sections, leaving a homogeneous series of very closely related fungi in the section, with only *A. robustus* being different from the remaining species. In 2004, Frisvad et al. based on a polyphasic approach using morphological characters, extrolites, and partial β-tubulin sequences observed that except for *A. robustus*, the species were phylogenetically and phenotypically strongly related. Moreover, seven new species were described, such as *A. cretensis, A. flocculosus, A. neobridgeri, A. pseudoelegans, A. roseoglobulosus, A. steynii,* and *A. westerdijkiae*. Currently, nineteen species are included within section *Circundati*, in addition to *A. robustus* (Samson et al. 2006) (Table 2).

Table 2 Classification of *Aspergillus* section *Circundati* species

Aspergillus section *Circundati*	Ochratoxin A
(A. robustus)	–
A. auricomus	–
A. bridgeri	–
A. cretensis	+
A. elegans	–
A. flocculosus	+
A. insulicola	–
A. melleus	*
A. neobridgeri	–
A. ochraceus	+/–
A. ostianus	*
A. persii	*
A. petrakii	*
A. pseudoelegans	+
A. roseoglobulosus	+
A. sclerotiorum	+/–
A. steynii	+
A. sulphureus	+
A. westerdijkiae	+
Neopetromyces muricatus	+

*Trace amounts of ochratoxin A production

3 Biodiversity Ochratoxigenic Species of *Aspergillus* in Latin America

Ochratoxigenic species are commonly isolated from a wide variety of grains and fruits grown in Latin America. They usually cause spoilage, damage, and contamination on agricultural products at different stages of production: pre- and post-harvest processing and handling. Moreover, their presence in food and sub-products is considered a real hazard due to their potential to produce OTA (Varga et al. 2004).

3.1 Coffee Samples

Studies on the mycoflora of coffee beans have shown that *Aspergillus ochraceus, A. carbonarius*, and *A. niger* aggregates are the main ochratoxigenic fungal species isolated. More recently, two new species producing large amounts of OTA consistently has been described, *A. westerdijkiae* and *A. steynii* (Taniwaki 2006; Noonim et al. 2008; Khoury and Atoui 2010; Gil-Serna et al. 2011). Coffee is extensively consumed worldwide and Brazil is the major coffee bean producer and exporter in the world. Urbano et al. (2001) and Martins et al. (2003) evaluated the incidence of fungal species in coffee samples from Brazil, and among OTA-producer species were isolated *A. ochraceus* and *A. niger*. A lower frequency of *A. ochraceus* isolation than *A. niger* was observed in both studies, but the percentage of ochratoxigenic strains was higher. Taniwaki et al. (2003) examined the presence of OTA-producer species in Brazilian coffee samples collected from four regions characterized by different climatic conditions, at different stages of maturation and processing. The species most responsible for OTA contamination of coffee was *A. ochraceus*. Although *A. carbonarius* was found only in Alta Paulista, the hottest region studied, and only from beans in the drying yard or during storage, their strains had a high potential to produce OTA. In this study, *A. niger* aggregate was more common than *A. ochraceus* and *A. carbonarius*, and also, only a low percentage of strains were able to produce OTA. Batista et al. (2003) studied the mycoflora of processed coffee bean samples from the southern regions of Minas Gerais state, Brazil. They found that the most important ochratoxigenic species isolated were *A. sulphureus* and *A. ochraceus*. Although section *Nigri* species *A. niger* var. *niger, A. niger* var. *awamori*, and *A. foetidus* were isolated, none of the strains produced OTA. Silva et al. (2008) studied the incidence and distribution of ochratoxigenic fungi during processing of coffee beans: fermentation, drying and storage. They found *A. niger* in the last day of fermentation/drying of beans and in beans stored in jute and polystyrene sacks. *A. ochraceus* was only found during the fermentation, not being detected during storage.

The incidence of ochratoxigenic species on coffee beans collected from other countries also was studied. Suárez-Quiroz et al. (2004) analyzed coffee cherries collected from Mexico during the coffee process stages. *A. ochraceus* and *A. niger* were the only OTA-producer species isolated. As regards the *A. ochraceus* isolation, no great difference was seen among the processes evaluated, but the dry method appears to stimulate the presence of *A. niger*. Magnoli et al. (2008) evaluated green and roasted coffee bean samples from Colombia marketed in Argentina. Only A. *niger* aggregate species were isolated among the ochratoxigenic species and the percentage of OTA-producer strains was higher than those reported previously in coffee cherries by other authors.

The species distribution on coffee beans was also evaluated using molecular tools. Schmidt et al. (2004) using AFLP markers identified *A. niger* and *A. carbonarius* species and developed species-specific PCR primers for the identification of *A. carbonarius* in coffee green samples. Magnani et al. (2005) by sequencing of the ITS regions and RFLP analysis identified *A. niger*, which was the most frequently isolated species, followed by *A. ochraceus* and *A. carbonarius*. Moreover, they reported the presence of *A. tubingensis* which was not described previously because only classical taxonomy has been used in other studies.

3.2 Grape Samples

Aspergillus section *Nigri* species were frequently isolated from South American vineyards showing different potential of OTA production. Studies on the red grape mycobiota using morphological criteria showed that species included within the *A. niger* aggregate were the dominant species and the main OTA producers in grapes from Argentina and Brazil (Rosa et al. 2002; Magnoli et al. 2003; Ponsone et al. 2007). Subsequent studies on grapes harvested in different grape-growing regions from Argentina showed that *A. niger* aggregate species were dominant, followed by *A. carbonarius* and *A. uniseriate*. *A. carbonarius* showed the highest percentages of OTA-producer strains and also the highest toxin level produced being mainly isolated from hotter regions, such as La Rioja and San Juan (Chiotta et al. 2009a). Using amplified fragment length polymorphism (AFLP), *Aspergillus* section *Nigri* species were grouped in four main clusters: *A. carbonarius, A. tubingensis, A. niger* "aggregate," and *Aspergillus* "uniseriate." The *A. tubingensis* cluster was the most prevalent group and was clearly separated from *A. niger* "aggregate" (Chiotta et al. 2011a). In another study, phylogenetic data corroborate the biodiversity of *Aspergillus* section *Nigri* populations. The sequencing results showed that the strains were grouped as *A. carbonarius, A. tubingensis, A. niger*, and *A. japonicus*. Also *A. homomorphus* was identified and its isolation was relevant because it was the first time that it has been found in vineyards and in Argentina (Chiotta et al. 2011b).

The presence of OTA-producing strains of *Aspergillus* on red and white grapes was also evaluated from Chilean vineyards. Based on morphological characteristic,

strains isolated were identified as *A. niger, A. carbonarius, A. wentii, A. niveus, A. versicolor*, *A. westerdijkiae*, and *A. paradoxus*. However, *A. carbonarius* and *A. niger* were the most frequently species, more isolated from red than in white grape cultivars. OTA-producing strains were only found among *A. niger, A. carbonarius, A. wentii*, and *A westerdijkiae* (Díaz et al. 2009).

3.3 Other Substrates

Cocoa is a very important crop from Central and South America and it is used as ingredient in several foods, such as cakes, biscuits, child foods, ice cream, and sweets. The most common *Aspergillus* section *Nigri* species found on cocoa beans collected from Brazil were *A. carbonarius* and *A. niger* aggregate. *A. melleus, A. westerdijkiae*, and *A. ochraceus* were also isolated but in lower percentages. Among species isolated, *A. carbonarius* was the main source of OTA in cocoa (Copetti et al. 2010). Sánchez-Hervás et al. (2008) evaluated the mycobiota of samples of cocoa stored from several origins including Ecuador and also revealed the predominance of *A. niger* aggregate and *A. carbonarius* ochratoxigenic species. Although the presence of black aspergilli in cocoa beans was low than in grapes and coffee, the OTA-producer species percentage and the levels produced were higher and *A. ochraceus* did not contribute to OTA contamination in cocoa beans.

Cereals and by-products are the main foods for human consumption throughout the world. Studies of corn kernels and corn-based feeds carried out in Argentina indicated that the most frequent *Aspergillus* section *Nigri* species are those belonging to *A. niger* aggregate, followed by uniseriate species. *A. foetidus* was isolated only from poultry feed. Within *Aspergillus* section *Circundati, A. ochraceus* was isolated but in low percentages. The species included in *A. niger* aggregate showed the highest percentage of OTA-producer strains (Dalcero et al. 2002; Magnoli et al. 2005, 2006b). In studies performed in Brazil, *A. niger, A. sydowii, A. versicolor, A. ochraceus, A. melleus*, *A. alutaceus*, and *A. carbonarius* species were isolated of corn and poultry feeds. *A. ochraceus* showed higher ability to produced OTA than other species isolated (Fraga et al. 2007; Rosa et al. 2006, 2009).

The species biodiversity within *Aspergillus* section *Nigri* on dried fruit samples from different South America origins was studied by Iamanaka et al. (2005) and Ferracin et al. (2009). Among species found, *A. niger* was predominant, followed by *A. ochraceus* and *A. carbonarius*. Within *A. niger* aggregate, *A. niger* sensu stricto, *A. tubingensis*, and *A. foetidus* species were identified. The identification of this species was confirmed by molecular analysis, such as sequencing (ITS1-5.8S-ITS2 region of the rDNA and β-tubulin sequences) and random amplification of polymorphic DNA (RAPD). The main ochratoxigenic species isolated were *A. ochraceus* and *A. carbonarius*. In another study carried out in Argentina, the mycoflora in raisins of different varieties was examined. In black raisins, the

predominant species isolated were *A. niger* var. *niger A. niger* var. *awamori*, and *A. carbonarius*. These species in white raisins were isolated but in lower frequency. *A. carbonarius* was the species that showed the highest percentages of ochratoxigenic strains (Magnoli et al. 2004).

Peanut (*Arachis hypogaea)* is an important food commodity worldwide and Argentina is the largest producer of peanuts in Latin America (SAGPyA 2013/14). Magnoli et al. (2006a) studied the occurrence of *Aspergillus* section *Nigri* and OTA in storage peanuts from Argentina. The predominant species isolated were *A. niger* var. *niger* and *A. niger* var. *awamori,* followed by *A. carbonarius* and *A. japonicus*. In another study, Magnoli et al. (2007a) analyzed storage peanuts samples but during a 3-month period. Within the section *Nigri*, the same species were isolated. Although *A. carbonarius* was present at low levels, it was the species with the highest percentage of OTA-producing strains.

Although yerba mate (*Ilex paraguariensis* L.) is a crop economically important in Latin America, few studies on the natural mycoflora in this crop have been done. In a study carried out in Argentina by Castrillo et al. (2013), the *Aspergillus* section *Nigri* incidence on different commercial branch of yerba mate was evaluated. Uniseriate species, *A. japonicus* var. *japonicus* and *A. japonicus* var. *aculeatus*, were more predominant than biseriate species, *A. foetidus, A. niger* var. *niger, A. niger* var. *awamori*, and *A. carbonarius*. The species percentage was higher in milled yerba mate (without aging) than elaborated yerba mate, which could be related to successive steps of development process of the product. None of the strains analyzed showed OTA production in vitro at detection level of the methodology employed. In a previous study, the same authors performed a phylogenic analysis of *Aspergillus* section *Nigri* isolated from yerba mate. The results showed that all isolated strains were included in *A. niger* and *A. carbonarius* clades (Castrillo et al. 2012). *A. niger* aggregate and *A. ochraceus* were also isolates from yerba mate samples obtained of Paraguay during the product process and the final products (Paiva et al. 2013).

4 Ecophysiology of Ochratoxigenic Species

Several studies from South America have shown the effect of water activity, temperature, and their interactions on *Aspergillus* section *Nigri* species growth and OTA production on different culture media and natural substrates (Astoreca et al. 2010). The growth of *A. niger* aggregate and OTA production were evaluated on corn, peanuts, coffee, and their different grain-based media (Astoreca et al. 2007a, b, 2009a, b, 2010). The optimum a_w level for growth ranged from 0.95 to 0.99 with optimal temperature of 25 or 30 °C. For OTA production, the same temperatures were optimal in a range of 0.97–0.99 a_w. Any of the strains evaluated were able to produce OTA on coffee-based medium and the concentrations reached on irradiated coffee beans were higher than those produced on the other irradiated substrates. These authors suggested that the chemical composition

of natural substrates determined the ability to produce OTA by these fungal species. *A. carbonarius* on dried grape extract medium and synthetic grape juice medium showed an growth optimal at 0.97 a and 0.98 a under all temperatures evaluated with optimal temperatures between 25 and 30 °C (Astoreca et al. 2007a, b; Chiotta et al. 2015). The production of OTA by *A. ochraceus* strains on coffee beans under different ecophysiological conditions was also studied (Palacios-Cabrera et al. 2004). These authors found that at 0.87 and 0.95 a_w at the constant temperature of 25 °C, OTA production was higher. Under alternating temperatures, OTA production was higher than at constant temperature, and alternating temperatures indirectly favored OTA production due to condensation and a subsequent rapid increase in moisture content and water activity of the coffee beans.

Other studies evaluated ecophysiological parameters in different synthetic media. Palacios-Cabrera et al. (2005) determined the influence of water activity, incubation time. and temperature on the growth of *A. ochraceus, A. carbonarius*, and *A. niger* on different culture media (CYA, DG18, and MY40G). For *A. carbonarius*, 30 °C was the best temperature for growth, while for *A. niger*, temperatures above 30 °C were better in all culture media. *A. ochraceus* presented good growth at 25 and 30 °C in all culture media, while at 35 °C, growth was slower, especially on CYA. The media with reduced a_w, such as DG18 and MY40G, were not a limiting factor for this species. Romero et al. (2007, 2010) determined the effects of a_w and temperature on lag-phase extension, radial growth rate, and OTA production by a cocktail inoculum of *A. carbonarius* in CYA medium. They found that the maximum growth rate was observed at 0.95 a_w and 30 °C. In general, growth rates increased with the increment of a_w. The highest OTA accumulation was observed at 15 °C and 0.95 a_w after 28 days of incubation. The amount of OTA produced by mixed inoculum was an intermediate level from that synthesized by the four individual strains. The authors postulated that it could be due to intraspecific interactions.

The knowledge of the optimal conditions for growing of ocratoxigenic species and OTA production will allow establishing preventive measures to ensure food safety. The appropriate management of these conditions is the key to improve the commodity storage and to keep at minimum loss in quality.

5 Impact of Ochratoxin A Production by *Aspergillus* Section *Nigri* on Food Safety

Ochratoxin A has been detected in coffee with variable levels of contamination among Latin American countries as well as within the same coffee-growing areas. Studies conducted in Brazil have shown that the percentage of coffee samples contaminated with OTA ranges from 7.5 % to 98.8 % and the OTA levels range from 0.10 to 7.30 ng/g and from 0.10 to >100 ng/g in green and processed coffee,

respectively (Table 3) (Prado et al. 2000; Taniwaki et al. 2003; Batista et al. 2003; Martins et al. 2003; Gollücke et al. 2004; Almeida et al. 2007; Batista et al. 2009). In coffee samples from Mexico, OTA occurrence were low and the OTA level detected on green coffee samples was 0.20 ng/g, while on processed coffee, samples varied between 0.10 and 0.30 ng/g (Suárez-Quiroz et al. 2004). In another study, green and processed coffee samples from Colombia were evaluated and OTA contamination were not detected in any sample analyzed (<1 ng/g) (Magnoli et al. 2008). In contrast, Diaz et al. (2004) found that green and processed coffee samples from Colombia were highly contaminated. The levels detected range from 0.90 to 19.40 ng/g and from 8.40 to 13.90 ng/g for green and processed coffee, respectively. Similar OTA contamination on imported coffee samples from Colombia and Brazil was found by Vanesa and Ana (2013). In Costa Rica, 99 % of processed coffee samples were contaminated with OTA level lower than 0.90 ng/g (Guzmán et al. 2007).

Wine and by-products contaminated with OTA have been reported in several countries from South American countries. In a study done by Rosa et al. (2004), wine, grape juice, and frozen pulp samples commercialized in Brazil were analyzed. In this study, 28.7 % of national and imported wine samples were contaminated with OTA, at concentrations ranging from 0.02 to 0.07 ng/mL. Out of the grape juice and frozen pulp samples, 25 % were contaminated with OTA at concentrations ranging from 0.02 to 0.10 ng/mL. In another study, OTA contamination was also evaluated in red wines and imported red wines from Brazil and the levels detected range from 0.10 to 1.33 ng/mL and from 0.03 to 0.32 ng/mL, respectively. Any of the grape juice samples analyzed were contaminated with OTA (Shundo et al. 2006). Wine samples collected at manufacturers' stock and retail markets of different regions from Argentina and Chile were analyzed to assess OTA contamination. However, none of the wine samples were contaminated (Pacin et al. 2005). In other study carried out in Argentina, OTA contamination on grapes and wine samples collected in different grape-growing regions from Argentina were evaluated (Chiotta et al. 2013). Although ochratoxigenic species were isolated from all regions, the OTA levels detected in grape and wine samples were low, ranging from 0.10 to 5.40 ng/g and from 0.01 to 4.82 ng/mL, respectively. Vega et al. (2012) evaluated the incidence of OTA on wines produced in Chile, covering a wide distance with different climates and growing conditions. They found that OTA was present in 1.7 % of wine samples in low levels (<0.35 ng/g). In red wine samples, the OTA incidence was twice than in white wine and the highest incidence was observed in the Merlot variety, followed Malbec variety.

The studies of OTA contamination on cereals in Latin America have been done with corn samples and corn-based feed. Dalcero et al. (2002) analyzed the natural incidence of OTA in corn-based feed, such as poultry, pig, and rabbit feed. The toxin was found in 38 % of the poultry feed samples with levels ranging from 25 to 30 ng/g. From rabbit feed samples, 25 % contained OTA and the levels ranged from 18.50 and 25.50 ng/g. Only 13 % of pig feed samples were contaminated with similar toxin levels to those found in poultry and rabbit feeds. Magnoli et al. (2005),

Table 3 Ochratoxin A levels detected in different substrates collected from Latin America

Substrate	Type of substrate	Origin	OTA-positive samples (%)	Range of OTA (ng/g)	References
Coffee	Green coffee	Brazil	7.5	>5.0	Taniwaki et al. (2003)
	Green coffee	Brazil	33.3	0.2–7.3	Martins et al. (2003)
	Green coffee	Brazil	97.3	0.2–6.3	Gollücke et al. (2004)
	Green coffee	Brazil and Colombia	100.0	0.2–20.3	Vanesa and Ana (2013)
	Green coffee	Colombia	ND	–	Magnoli et al. (2008)
	Green coffee	Colombia	14.3	0.9–19.4	Diaz et al. (2004)
	Green coffee	Mexico	51.1	0.1–0.3	Suárez-Quiroz et al. (2004)
	Processed coffee	Brazil	12.5	0.6–4.1	Batista et al. (2003)
	Processed coffee	Brazil	56.0	0.1–> 100.0	Batista et al. (2009)
	Instant coffee	Brazil	98.8	0.2–6.3	Almeida et al. (2007)
	Processed coffee	Brazil and Colombia	54.1	0.1–5.8	Vanesa and Ana (2013)
	Soluble coffee	Brazil and Colombia	77.3	0.2–13.6	Vanesa and Ana (2013)
	Roast coffee	Colombia	ND	–	Magnoli et al. (2008)
	Soluble coffee	Colombian	100.0	8.4–13.9	Diaz et al. (2004)
	Processed coffee	Mexico	71.4	0.2	Suárez-Quiroz et al. (2004)
	Processed coffee	Costa Rica	99	<0.9	Guzmán et al. (2007)
Grape by-products	Red wine	Brazil	28.7	<0.1	Rosa et al. (2004)
	Grape juice and frozen pulps	Brazil	25.0	<0.1	Rosa et al. (2004)
	Red wines	Brazil	31	0.1–1.3	Shundo et al. (2006)
	Wine	Argentina and Chile	–	–	Pacin et al. (2005)
	Grapes	Argentina	29	0.1–5.4	Chiotta et al. (2009a, 2013)
	Red wine	Argentina	37	0.1–4.8	Chiotta et al. (2013)
	Red and white wine	Chile	1.7	<0.35	Vega et al. (2012)

(continued)

Table 3 (continued)

Substrate	Type of substrate	Origin	OTA-positive samples (%)	Range of OTA (ng/g)	References
Cereals	Corn and corn-based feed	Argentina	13–38	18.5–30	Dalcero et al. (2002)
	Corn and corn-based feed	Argentina	10–15	15–25	Magnoli et al. (2005)
	Corn-based feed	Brazil	100	17–197	Fraga et al. (2007)
	Corn-based feed	Brazil	100	1.3–80	Rosa et al. (2006)
	Corn-based feed	Brazil	44	42–224	Rosa et al. (2009)
	Corn kernel	Argentina	–	–	Magnoli et al. (2006a, 2007b)
Other	Beer	Brazil	4	1–18	Kawashima et al. (2007)
	Cocoa	Brazil	94	1.1–1.4	Copetti et al. (2013)
	Peanut	Argentina	50	5.6–130	Magnoli et al. (2006a, 2007a)
	Paprika	Brazil	85.7	0.2–97.2	Shundo et al. (2009)

in a subsequent study with these substrates, detected OTA levels in 10 %, 15 %, and 12 % of poultry, pig, and rabbit feed samples, respectively. The mean levels detected ranged from 15 to 25 ng/g from three feeds. Fraga et al. (2007) and Rosa et al. (2006) detected that 100 % poultry corn-based feed samples were contaminated with OTA with levels from 17 to 197 ng/g and from 1.30 to 80 ng/g, respectively. Rosa et al. (2009) observed on swine corn-based feeds that 44 % were positive for OTA contamination with levels between 42 and 224 ng/g. In contrast, other studies not detected OTA in any of the commercial corn kernel samples analyzed (Caldas et al. 2002; Magnoli et al. 2006b)

Few studies regarding OTA occurrence in other substrates are available from Latin America. Kawashima et al. (2007) evaluated OTA content on beers produced in different locations in the states of Paraná, São Paulo, Rio de Janeiro, Paraíba, and Pará. Ochratoxin A was found in 4 % of the samples and the concentrations ranged from 1 to 18 ng/mL. Although the number of contaminated samples was low, the OTA contamination was not restricted in terms of region. Copetti et al. (2013) assessed the natural occurrence of OTA from different cocoa fractions obtained during the technological processing. The study showed that 94 % of the samples were contaminated, the highest levels of toxin were detected in the cocoa shell,

cocoa powder and cocoa cake, with means of 1.10, 0.90 and 1,40 ng/g, respectively. Magnoli et al. (2007a) evaluated OTA contamination on storage peanut samples and 50 % of the samples showed mean levels ranging from 5.6 to 130 ng/g. The mean value of OTA obtained after the first month of storage was significantly higher from those obtained after the second and third month (mean 30, 6.50, and 13 ng/g, respectively). These results were similar to the ones obtained in a previous work on occurrence of OTA in peanuts (Magnoli et al. 2006a). Shundo et al. (2009) analyzed OTA contamination on paprika samples and the toxin levels were detected in 85.7 % of the samples at levels ranging from 0.24 to 97.20 ng/g.

In summary, the natural occurrence of OTA in wines was not relevant in comparison to the other substrates evaluated in Latin American countries, but it could contribute to OTA exposure of human populations. The high frequency of OTA occurrence in coffee and corn-based feed samples showed the importance of an effective control of these products by farmer and industries.

6 Strategies to Reduce the Impact of *Aspergillus* Section *Nigri* and Ochratoxin A Accumulation in the Food Chains

The isolation of ochratoxigenic species, especially *A. carbonarius* and *A. ochraceus,* on grapes, raisins, cocoa, coffee, dried fruits, etc., represents an OTA contamination risk. The development of effective prevention systems and diagnostic tools could be effective in the control of OTA occurrence on food. Several studies have showed that the contamination with OTA can occur in both preharvest and post-harvest (Magan 2006; Taniwaki 2006; Magan and Aldred 2007). Weather conditions prior to harvest, type and amount of fungicide used, ecology of the ochratoxigenic fungal species, removal of inoculum sources, mechanical and insect damages on crops, and moisture content of crops at harvest are all key aspects which need to be considered in determining the level of fungal and toxin contamination. Moreover, post-harvest storage, transport, and processing are all important stages in the food chain which need to be monitored.

6.1 Preharvest Strategies

6.1.1 Management Practices During Crops

The knowledge of the factors that favor the growth of OTA-producer fungi is one of the first strategies for controlling the entry of this mycotoxin in the food chain. In the present item, only coffee and grape studies are discussed because in Latin America, few studies on other substrates were performed.

In coffee crops, it has been observed that the soil is an important source of inoculum. Previous studies showed that the ochratoxigenic species are isolated more frequently of coffee fruits taken from the soil and from different processes in which the coffee remains in contact with the soil during a period of time (Taniwaki et al. 2003; Morae and Luchese 2004; Batista et al. 2009). However, the cropping system, either conventional or organic, was not an influential factor on the isolation of the ochratoxigenic species and OTA contamination on coffee (Fátima Rezende et al. 2013). The state of maturity of the grains was a key factor on OTA contamination, since more OTA on overripe and damaged cherries than on green and ripe cherries was observed (Teixeira et al. 2001; Bucheli and Taniwaki 2002). Climatic conditions particularly the geographic region also determined the distribution of the ochratoxigenic species, with *A. niger* and *A. ochraceus* being the most common species isolated (the latter species isolated in lower frequency comparatively hot regions) and *A. carbonarius* the species isolated in lower frequency and in hotter regions (Pitt et al. 2001; Taniwaki et al. 2003).

On grapes collected from South American vineyards, *A. niger aggregate* and *A. carbonarius* were the prevalent ochratoxigenic species, increased from veraison to harvest stage in comparison to 1 month after setting (Ponsone et al. 2007; Díaz et al. 2009; Chiotta et al. 2013). The soil and weeds represented an important inoculum source and the species were present on weeds at most of the grape stages (Chulze et al. 2006; Chiotta et al. 2009b). On red grapes, these species were more frequent than on white grapes, being Bonarda, Syrah, and Cabernet Sauvignon varieties with the highest *A. carbonarius* percentage (Díaz et al. 2009; Chiotta et al. 2013, 2015). The skin-damaged berries by insects to increased the isolation percentages of *Aspergillus* section *Nigri* species and OTA contamination in grapes (Chiotta et al. 2010). Cultural management such as the trellis system and drip irrigation on vineyards also influenced the frequency of fungal species isolation. Altitude, longitude, and latitude have shown some influence on the species distribution. The incidence of *A. niger* aggregate species increases towards the south and west of grape-growing area, while the incidence of *A. carbonarius* and *A. uniseriate* was higher in the northern regions. Data showed that among the possible prevention strategies that could be used to reduce fungal and OTA contamination are to minimize weeds on soil and pest damage on crops and to harvest crops on time. A decision support system could be considered through the crop production system.

Data showed that among the possible prevention strategies that could be used to reduce fungal and OTA contamination are to minimize weeds on the soil and pest damage on crops, to harvest crops on time at the mature stage. Decision support systems could be considered through the crop production chains. One of the strategies that is important to mention to reduce *Aspergillus* rot and OTA production is the use of biocontrol. Yeasts are considered one of the most potent biocontrol agents due to their biology and nontoxic properties. Recently, Ponsone et al. (2011) described two epiphytic strains of *Lachancea thermotolerans* that were able to control the growth and OTA accumulation of ochratoxigenic fungi both "in vitro" and "in situ." The data reported until now indicate that the yeasts that occur naturally on grapes are promising ecological fungicides, because they can survive

and colonize grape berries in the vineyards and also maintain the equilibrium of the natural environment.

6.2 Post-Harvest Strategies

The different storage conditions and processing techniques affect OTA contamination on the final products. Previous studies showed that the drying stage of coffee and cocoa is one of the more relevant control critical points (Romani et al. 2003; La Pera et al. 2008; Batista et al. 2009; Copetti et al. 2010; Vanesa and Ana 2013). The studies showed that in by-products obtained from different sun-drying processes in which the beans were in contact with the soil, higher levels of OTA contamination were detected. In contrast, the thermal treatment used during the process of roasting coffee and cocoa showed a reduction in the OTA concentration (Copetti et al. 2010; Castellanos-Onorio et al. 2011). Therefore, an effective sun-drying in combination with mechanical dehydration was an effective control system for OTA contamination. The fate of OTA content from must to wine during the red wine-making process in a pilot-scale vinification also showed a high percentage of OTA reduction. It was observed that the fermentation process, either alcoholic or malolactic, was responsible of OTA decreasing (Ponsone et al. 2009).

Since the risk of OTA contamination decreases during the processing, a good sanitary stage of beans and grapes at harvest time could favor the prevention of OTA contamination. However, the lower quality of the raw material together with inappropriate techniques of drying, fermentation, and storage could favor OTA accumulation and other strategies could be employed. One of strategy to reduce the entry of ochratoxin A in the food chain is the use of natural treatments to reduce fungal growth and toxin production. The effect of antioxidants and its combinations on grains was studied in vitro and during storage (Passone et al. 2007, 2009; Barberis et al. 2009a, b, 2010a, b). The results showed that the phenolic antioxidant controls both, growth and OTA production at different a_w levels and temperatures. Moreover, combinations of antioxidants at lower concentrations showed that the growth rate of all the ochratoxigenic strains was inhibited completely and OTA production was reduced significantly under all the environmental conditions assayed. The effect of essential oils on the reduction of *A. niger* aggregate and *A. carbonarius* growth and OTA accumulation also was observed by Passone et al. (2012).

7 Conclusion

This review will lead to understand the biodiversity of ochratoxigenic *Aspergillus* from Latin America and provides strategies to manage its incidence and the risk of ochratoxin A production in different substrates. The OTA natural occurrence was

observed mainly in coffee and corn-based feed. The biodiversity of ochratoxigenic *Aspergillus* varied according to the area and the substrate evaluated. *A. ochraceus* have been identified as one of the main sources of OTA contamination in coffee beans but *A. carbonarius* and *A. niger* were isolated frequently in grapes, raisins, and wine. These species also contributed to OTA contamination in coffee, cocoa, peanut, and cereals. The environmental conditions, mainly temperature, development in crops, and storage and processing techniques of food, affect OTA contamination. It is important to consider this aspect since although low OTA levels were detected in same substrates, ochratoxigenic species were frequently isolated. Therefore, if the conditions for OTA production are appropriated, the presence of ochratoxigenic species could result to a risk of contamination. Preharvest control strategies such as removal of inoculum sources from soil, to control mechanical and insect damages and to harvest the crops with the optimal moisture content, could be included during crops. In case contamination occurs, the hazards associated with OTA must be managed through post-harvest strategies. Chemical control could be used, but due to the increasing number of fungal strains resistant to chemical fungicides and the impact of these pesticides on the environment and human health, maximum levels of chemical residues have been regulated in many products. Thus, alternative methods are necessary to substitute or complement treatments with fungicides to control fungi under field or storage conditions. Natural products derived from plants, essential oils, antioxidants, and biocontrol have been evaluated.

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Listeria monocytogenes in Milk Products

Kieran Jordan, Karen Hunt, and Marion Dalmasso

Abstract Milk and milk products are frequently identified as vectors for transmission of *Listeria monocytogenes*. Milk can be contaminated at farm level either by indirect external contamination from the farm environment or less frequently by direct contamination of the milk from infection in the animal. Pasteurisation of milk will kill *L. monocytogenes*, but post-pasteurisation contamination, consumption of unpasteurised milk and manufacture of unpasteurised milk products can lead to milk being the cause of outbreaks of listeriosis. Therefore, there is a concern that *L. monocytogenes* in milk could lead to a public health risk. To protect against this risk, there is a need for awareness surrounding the issues, hygienic practices to reduce the risk and adequate sampling and analysis to verify that the risk is controlled. This review will highlight the issues surrounding *L. monocytogenes* in milk and milk products, including possible control measures. It will therefore create awareness about *L. monocytogenes*, contributing to protection of public health.

1 Introduction

1.1 Listeria monocytogenes *in Milk and Milk Environment*

As a food, milk is nutritious and contains protein, fats and micronutrients that can contribute to the daily diet. Aseptically drawn raw milk is practically sterile. But during the process of milking, it can become contaminated by bacteria which come from clinical and subclinical infections of the udder or from the farm environment, entering the milk via the teat skin, the milking equipment and the milker. Milk processing can introduce another opportunity for bacteria to contaminate the milk product, i.e. cross-contamination from the processing environment. Most of these bacterial contaminants are harmless but a minority can be disease-causing bacteria, like *Listeria monocytogenes*.

L. monocytogenes is the causative agent of listeriosis in humans that can affect susceptible populations such as newborn children, the elderly and

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immunocompromised persons (Lorber 1997; McLauchlin et al. 2004). Although not very frequent, listeriosis has a mortality rate of 20–30 % (Sleator et al. 2009), making it a pathogen of concern for the dairy industry (Table 1).

1.2 L. monocytogenes *Virulence*

Currently, the regulations assume that all *L. monocytogenes* are equally pathogenic. This is reasonable as at present there is no test to distinguish between *L. monocytogenes* of different pathogenicity. *L. monocytogenes* expresses about 50 molecules, including the virulence genes listeriolysin O and two major internalins, InlA and InlB, to promote its cell infection cycle (Camejo et al. 2011). Studies have shown that a significant proportion of *L. monocytogenes* isolates from foods carry unique virulence-attenuating mutations in the gene *inl*A or other virulence factors (Nightingale et al. 2008; Témoin et al. 2008; Van Stelten and Nightingale 2008; Camejo et al. 2011; Chen et al. 2011). In the case of *inl*A, these mutations can be the cause of a premature stop codon which leads to the production of a truncated and secreted InlA but can also completely abolish InlA production, reducing the ability of *L. monocytogenes* to invade human cells (Nightingale et al. 2008). In the future, it may be possible to determine a risk level associated with strains that are lacking certain genes or in which the presence of virulence-attenuating mutations diminishes the virulence potential of the strains. At present such risk assessment is not practiced and it is understood that all strains of *L. monocytogenes* are capable of causing disease.

1.3 Regulations for L. monocytogenes *in Foods*

In order to protect public health, authorities have set regulations on the maximum number of *L. monocytogenes* that can be present in food. In the USA, there is 'zero tolerance' of *L. monocytogenes*, where any occurrence in food is considered an offence. In the European Union, there is a more lenient approach which is based on the fact that *L. monocytogenes* is ubiquitous in the environment and that <100 cfu/g is insufficient to cause illness. There is a 'zero tolerance' for special dietary foods and foods intended for infants. The regulations also differentiate between foods that can and cannot support growth of *L. monocytogenes*. For foods that cannot support growth, up to 100 cfu/g are allowed in the food during its shelf life. For foods that can support growth, absence is required. It is the responsibility of the food business operator to demonstrate that a food is unable to support growth; otherwise the assumption is that growth will occur and the regulation of absence is applied. In New Zealand and Australia, the absence of *L. monocytogenes* in 25 g or millitres for five samples is required throughout the shelf life for raw milk and raw milk products and cheeses supporting *L. monocytogenes* growth (Anonymous 2012).

Table 1 Outbreaks of food-borne listeriosis

Year	Place	No. of cases (no. of deaths)	Type of milk product	Serotype	Reference
USA					
1979	Maryland	20 (3)	Raw milk cheese	4b	Ho et al. (1986)
1983	Maryland	49 (14)	Pasteurised milk	4b	Fleming et al. (1985)
1985	California	142 (48)	Mexican-type cheese	4b	Linnan et al. (1988)
1986–1987	Pennsylvania	36 (44)	Ice cream, brie	4b, 1/2b, 1/2a	Schwartz et al. (1989)
1987	California	11	Butter		FDA (2003)
1994		45	Chocolate milk	1/2b	Dalton et al. (1997)
2000	North Carolina	12 (5)	Queso Fresco cheese	4b	CDC (2001), MacDonald et al. (2005)
2003	Texas	13 (2)	Mexican-type fresh cheese		Swaminathan and Gerner-Smidt (2007)
2003–2007	Texas + seven states	74 (10)	Queso Fresco		Smith (2008)
2007	Massachusetts	5 (3)	Pasteurised milk		CDC (2008)
2008	Multistate	8	Mexican-style cheese		Jackson et al. (2011)
2009	Washington	2	Cheese		Anonymous (2009a)
Europe					
1981	England	11 (5)	Dairy products	1/2a	FDA (2003)
1983–1987	Switzerland	122 (33)	Soft cheese	4b	Bula et al. (1995)
1986	Austria	28 (5)	Unpasteurised milk		Allerberger and Guggenbichler 1989)
1989–1990	Denmark	26 (6)	Blue-mould cheese/hard cheese	4b	Jensen et al. (1994)
1993	Scandinavia	1	Goat's milk cheese		Eilertz et al. (1993)
1995	France	37 (11)	Soft cheese	4b	Goulet et al. (1995)
1997	France	14	Soft cheese	4b	Jacquet et al. (1998)
1998–1999	Finland	25 (6)	Butter	3a	Lyytikainen et al. (2000)
1999	England	2	Cheese	4b	Kimball (2006)

(continued)

Table 1 (continued)

Year	Place	No. of cases (no. of deaths)	Type of milk product	Serotype	Reference
2001	Sweden	120	Soft cheese	1/2a	Carrique-Mas et al. (2003)
2001	Belgium	2	Frozen ice cream		Yde and Genicot (2004)
2005	Switzerland	10 (3)	Soft cheese	1/2a	Bille et al. (2006)
2006	Czech Republic	75	Cheese	1/2a	Vit et al. (2007)
2006–2007	Germany	189	Acid curd cheese		Koch et al. (2010)
2009–2010	Austria, Germany, Czech Republic	Two outbreaks: 14 (5) and 20 (3)	Quargel cheese	1/2a (2 clones)	Fretz et al. (2010)
Canada					
2001	Manitoba	7	Cream		Pagotto et al. (2006)
2002	British Columbia	47	Cheese	4b	Pagotto et al. (2006)
2002	British Columbia	86	Pasteurised cheese	4b	Pagotto et al. (2006)
2002	Quebec	17	Soft and semihard cheese	4b	Gaulin et al. (2003)
2008	Quebec	38 (2)	Cheese	1/2a	Gilmour et al. (2010)
Asia					
2001	Japan	38	Washed-type cheese	1/2b	Makino et al. (2005)
South America					
2008	Chile	119 (5)	Brie		Anonymous (2009b)

1.4 Scope of the Chapter

The scope of this chapter is to assess current knowledge on *L. monocytogenes* occurrence in milk and milk products. It also focuses on the occurrence and persistence of *L. monocytogenes* in milk processing environment and the strategies to prevent and control it.

2 *L. monocytogenes* Contamination of Milk

Generally, where *L. monocytogenes* is detected in raw milk, the source is not identified, although it most likely comes from the environment post-milking. Studies of *L. monocytogenes* contamination of raw milk found that animal faeces, poor-quality feed and general lack of hygiene on the dairy farm are factors associated with contamination (Sanaa et al. 1993; Nightingale et al. 2005; Schoder et al. 2011). Direct excretion of *L. monocytogenes* into the milk, i.e. from clinical or subclinical mastitis, is rare although cases where subclinical mastitis in cows can go undetected have been reported (Gitter et al. 1980; Van Daelen and Jaartsveld 1988; Fedio et al. 1990; Bourry et al. 1995; Schoder et al. 2003; Winter et al. 2004) (Fig. 1).

2.1 *Occurrence of* L. monocytogenes *in Milk*

The reported incidence of *L. monocytogenes* in milk varies. In Ireland the incidence was 3 % (Rea et al. 1992) and 6 % (Fox et al. 2011a), in Northern Ireland 8.8 % (Harvey and Gilmour 1993) and in the USA 19.7 % (Latorre et al. 2009), 1 % (Waak et al. 2002), 4.1 % (Rohrbach et al. 1992) and 0 % (D'Amico and Donnelly 2010). Other reported incidences include 1.7 % in Iran (Mahmoodi 2010), 13 % in Mexico (Vázquez-Salinas et al. 2001) and 2.2 % in Turkey (Aygun and Pehlivanlar 2006). Seasonal variation in reported incidence has been observed, although there is

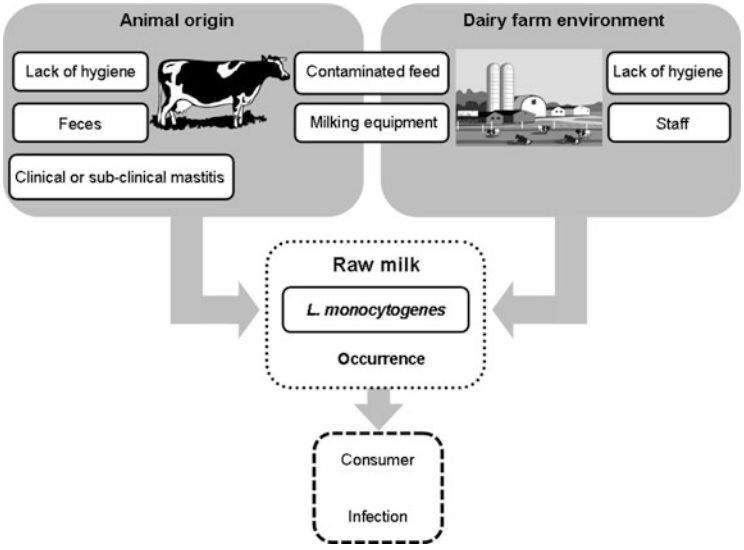

Fig. 1 Flow chart of contamination of raw milk with *L. monocytogenes*

no consistency in the variation observed. Waak et al. (2002) reported higher incidence in winter and Vázquez-Salinas et al. (2001) reported a spring/summer peak. Because of the variety of analytical methods used, and the improvements in detection methodologies over the years, it is difficult to compare these reported incidences.

2.2 Indirect Contamination of Milk from the Farm Environment

L. monocytogenes is widespread in the farm environment. Fox et al. (2009) found that an average of 19 % (57/298) of farm samples were contaminated, while Latorre et al. (2009) found *L. monocytogenes* in 7.3 % (22/303) of samples. Many foods originate at the farm so there is potential for cross-contamination of food with *L. monocytogenes*.

Milk is one of the foods that originate on the farm and while pasteurisation will inactivate *L. monocytogenes*, consumption of raw milk, especially by farm families, is widespread. In addition, there are many producers of artisanal dairy products, including ice cream, cheese and yoghurt, using unpasteurised milk and increasing interest at farm level in selling unpasteurised milk for consumption (Oliver et al. 2009).

2.3 Direct Contamination of Milk from Animals

Infection by *L. monocytogenes* has been confirmed in a wide variety of host animals including more than 40 species of domestic and wild animals. The most susceptible domestic species are sheep, goats and cattle. Listeriosis manifests itself clinically in ruminants as neonatal mortality (abortion), as septicaemia and most commonly as encephalitis. In general, small numbers are affected (8–10 % of the herd) with the animals surviving from 4 to14 days. In animals, susceptibility to infection with *L. monocytogenes* has been attributed to decreased cell-mediated immunity associated with advanced pregnancy (Quinn et al. 2011).

Udder infection with *L. monocytogenes* is most commonly reported in sheep and goats (Low and Donachie 1997). *L. monocytogenes* bovine mastitis is less commonly reported where subclinical mastitis in cows can go undetected (Gitter et al. 1980; Van Daelen and Jaartsveld 1988; Fedio et al. 1990; Bourry et al. 1995; Schoder et al. 2003; Winter et al. 2004). In such cases, milk often remains visually unchanged, and with no clinical signs of infection, contamination can persist even after treatment (Fedio et al. 1990).

A recent case study demonstrated direct excretion of *L. monocytogenes* into cow's milk (Hunt et al. 2012). Levels of hygiene on the farm involved in that study were visually very good, and all non-milk contact dairy environmental swabs were

negative, indicating the source of contamination was direct excretion into the milk. Following a visual inspection, none of the cows in the herd had any physical signs of infection, but an indistinguishable *L. monocytogenes* isolated was obtained from one cow and from the milk.

2.4 Growth in Milk and Raw Milk Consumption

L. monocytogenes has the ability to grow at 4 °C and therefore has the potential to grow in bulk-tank milk or during the distribution of unpasteurised milk. A risk assessment study has shown that a greater risk of listeriosis was associated with consumption of unpasteurised milk obtained from retail and farm stores as compared with unpasteurised milk obtained from bulk tanks (Latorre et al. 2011). This was probably due to additional time-temperature combination steps in the retail and farm store models, which increased the chances for growth of *L. monocytogenes* in the unpasteurised milk. In Greece, a probabilistic model to evaluate the growth of *L. monocytogenes* during the chill chain of pasteurised milk (including transport, retail storage and domestic storage) predicted that in 44.8 % of the milk cartons released to the market, the pathogen could grow until the time of consumption (Koutsoumanis et al. 2010). However, based on an initial contamination level of 1 cell/1 litre carton, the predicted percentage of milk cartons in which the pathogen would exceed the safety criterion of 100 cells/ml at the time of consumption was 0.14 %. In addition, the milk environment and storage conditions have an influence on *L. monocytogenes* virulence. It has been shown that incubation in pasteurised milk at 4 °C resulted in a higher invasion and intracellular proliferation of *L. monocytogenes* strains compared to raw milk when put into contact of human cells in vitro (Pricope-Ciolacu et al. 2013). The level of fat in milk also significantly affected the in vitro virulence of *L. monocytogenes*, whereas the contents in caseins and lactose did not.

3 Occurrence in Milk Products

Lower prevalence of *L. monocytogenes* has been reported in dairy products than in other ready-to-eat foods. However, cheeses appear to be contaminated with *L. monocytogenes* more frequently than milk, and retail cheeses, particularly soft cheeses, have been found to be contaminated in several instances (Lianou and Sofos 2007).

3.1 Occurrence and Growth in Cheese

L. monocytogenes has been detected in a wide variety of cheeses all over the world and results for some of these surveys are presented in Table 2. Even if the presence

Table 2 Examples of occurrence of *L. monocytogenes* in cheese

Year	Country	Type of cheese	Type of milk	Heat treatment	No. of positive samples/total samples tested (%)	Origin of contamination	Reference
2012–2013	Ireland	Farmhouse Cheddar cheese	Cow	Raw	12/20 (60)	Yard (outside environment)	Dalmasso and Jordan (2013a)
2011	Jordan	Soft to semihard brined white cheese		Raw or pasteurisation	39/350 (11)		Osaili et al. (2012)
2011	Mexico	Fresh cheese	Cow	Raw	18/200 (9)		Torres-Vitela et al. (2012)
2010	Croatia	Fresh cheese	Cow		2/60 (3)		Frece et al. (2010)
2007	Italy	Gorgonzola, blue-veined mould-ripened cheese	Cow	Pasteurisation	2/18 (11)	Maturing shelves	Cocolin et al. (2009)
2007	Ireland	Farmhouse cheeses mould ripened, blue mould, smear-ripened, fresh, semisoft and hard cheeses	Cow, goat, ewe	Raw or pasteurisation	21/351 (6)		O'Brien et al. (2009)
2005	Brazil	Minas frescal cheese, Latin-style soft cheese	Cow	Pasteurisation	6/10 (60)	Storage coolers	Brito et al. (2008)
2005	UK	Unripened, ripened soft and semihard cheeses	Cow, goat, ewe, other	Pasteurisation	4/2618 (0.15)		Little et al. (2008)
2004	UK	Unripened, ripened soft and semihard cheeses	Cow, goat, ewe, other	Raw or thermisation	17/1819 (1)		Little et al. (2008)

1991	England and Wales	Soft, ripened cheese	Cow	Pasteurisation	63/769 (8.2)		Greenwood et al. (1991)
1989	Italy	Soft cheese	Cow	Raw milk	2/21 (1.6)		Massa et al. (1990)

of *L. monocytogenes* is not detected in cheese, some authors consider that the presence of *Listeria* spp. other than *L. monocytogenes* indicates that hygiene whether at milking or during cheese making could be insufficient (Arrese and Arroyo-Izaga 2012).

In the EU, regulations specifically refer to the ability of food to support growth of *L. monocytogenes*, with maximum numbers of 100 cfu/g if food cannot support growth and absence if it can. Therefore, it is important to be able to assess the ability of food to support growth. Predictive modelling, based on experiments in laboratory media, can be used to make an initial assessment of growth ability; however the results from predictive modelling are not always accurate. Schvartzman et al. (2011) studied the effect of pH, water activity and inoculum level on the growth/no growth boundary of *L. monocytogenes* in cheese. They showed that in 40 % of cases, predictive modelling did not accurately predict growth. Similar results were obtained by Rosshaug et al. (2012). Therefore, challenge studies to determine the ability of a food to support growth of *L. monocytogenes* are essential. Thus, D'Amico et al. (2008) demonstrated that a 60-day period of ripening did not prevent the growth of *L. monocytogenes* in artificially contaminated surface-mould-ripened soft cheeses made from raw milk. Alternatively, growth in naturally contaminated cheese can be studied. Dalmasso and Jordan (2013a) determined that there was no growth in Cheddar cheese naturally contaminated with < 10 cfu/g of *L. monocytogenes*.

3.2 Occurrence in Yoghurt and Other Fermented Milk Products

Fresh or traditional milk products obtained from unpasteurised milk in many countries are highly susceptible to *L. monocytogenes* and have the potential to support its growth until consumption of the products. *L. monocytogenes* has been shown to survive in products such as cultured buttermilk, butter and even yoghurt (Choi et al. 1988; Farber and Peterkin 1991) (Table 3). Kassaify et al. (2010) reported the occurrence of *L. monocytogenes* in 42 % of Middle Eastern coagulated cream products.

Even if fermentation processes can prevent *L. monocytogenes* from growing in fermented milk products, it has also been shown that some strains can adapt to acid conditions and that acid adaptation enhances the survival of *L. monocytogenes* in acidified dairy products, including cottage cheese, yoghurt and whole-fat Cheddar cheese (Gahan et al. 1996). One mechanism employed by *L. monocytogenes* for survival at low pH is the adaptive acid tolerance response where a short adaptive period at a nonlethal pH induces metabolic changes that allow the organism to survive a lethal pH (Smith et al. 2012). Such survival of acid conditions by *L. monocytogenes* is likely to involve a variety of regulatory responses.

Table 3 Examples of occurrence of *L. monocytogenes* in milk and milk products (cheese not included)

Year	Country	Type of milk product	Heat treatment	Type of milk	No. of positive samples/ total samples tested (%)	Reference
2010	Croatia	Cream of raw milk	Raw	Cow	5/60 (8.3)	Frece et al. (2010)
2010	Croatia	Milk	Raw	Cow	4/60 (6)	Frece et al. (2010)
2009–2010	USA	Milk	Raw	Cow	107/21 (4.50)	Jackson et al. (2012)
2007–2008	New Zealand	Milk	Raw	Cow	2/295 (0.7)	Hill et al. (2012)
2007	Lebanon	Qishta, Middle Eastern coagulated cream product	Powdered or pasteurised liquid milk	Cow	13/31 (42)	Kassaify et al. (2010)
2003–2004	Algeria	Milk	Raw	Cow	10/233 (4)	Hamdi et al. (2007)
2002–2003	Portugal	Milk	Raw		2 /105 (2)	Kongo et al. (2006)
2001–2002	USA	Milk	Raw	Cow	2/248 (0.8)	Jayarao et al. (2006)
1991	England and Wales	Milk	Pasteurisation	Cow	(1.1)	Greenwood et al. (1991)

4 *L. monocytogenes* in the Processing Environment

L. monocytogenes is ubiquitous in the environment (Fox et al. 2011a) and has been isolated from a wide variety of ready-to-eat foods, not only from dairy products but also from meat and fish products, among others (Lianou and Sofos 2007). Its occurrence in the environment may pose the threat of *L. monocytogenes* transfer from the environment to the milk product, even though the routes of contamination are not always clearly identified. Although the control of *L. monocytogenes* in food is essential, it is also crucial to control *L. monocytogenes* occurrence in food processing environment to avoid cross-contamination (Fig. 2).

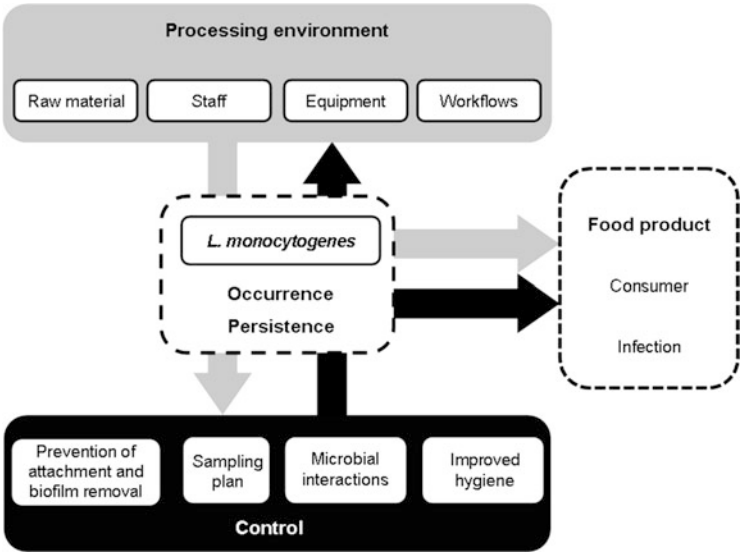

Fig. 2 Flow chart of contamination of milk product processing environment with *L. monocytogenes*

4.1 Occurrence in the Processing Environment

In many cases, the foods are contaminated at the processing facilities, e.g. at the slicing or packaging step, as *L. monocytogenes* has a remarkable ability to survive and persist in food processing environments. The bacterium is ubiquitous in the environment and may therefore be transmitted into the processing facilities by the raw materials, workers, trucks, tools, cleaning materials or machines (Reij et al. 2004) or it may hide in the factory and survive the cleaning and disinfection procedures, forming biofilms and then occasionally cross-contaminating food products.

In cheese processing facilities with associated dairy farms, it is essential that physical barriers, such as an efficient footbath, exist between the external environment and the inside of the processing facility, as the dairy farm environment is known to be a potential source of *L. monocytogenes* contamination (Ho et al. 2007; Fox et al. 2009). Nonfood contact surfaces, such as drains, cracks on floors, wheels of mobile equipment, etc., in cheese making facilities and outside the processing area have been reported as the main risk to food contamination with *L. monocytogenes* (Dalmasso and Jordan 2013a), which indicate that efforts to reduce processing environment contamination are worthwhile.

Risk assessment analysis for *L. monocytogenes* in the dairy sector is increasingly being used to help understand and control the risks and ensure food safety. For example, a qualitative risk assessment for microbial hazards in Swiss dairy products that considered multiple hazards and products from different types of dairies along

the food chain has recently been described (Menéndez González et al. 2011). The results obtained helped to make recommendations on the design of the national risk-based monitoring programme of dairy products. In the case of the production of a soft cheese made from pasteurised milk, a quantitative risk assessment analysis helped to identify the main factors and effects having an impact on the final risk, to determine intervention strategies at different food processing steps and to orientate specific research (Tenenhaus-Aziza et al. 2013).

4.2 Tools to Monitor Routes of Contamination

Subtyping of *L. monocytogenes* strains is essential to identify isolate relatedness and track routes of contamination throughout the food system, which is very important for developing control strategies. It also provides insights into the ecology of *L. monocytogenes,* the population genetics and the epidemiology (Wiedmann 2002). Several subtyping methods, not exclusively dedicated to monitoring routes of contamination like serotyping, ribotyping, phage typing or MLST, exist and have been recently reviewed (Jadhav et al. 2012). This section specifically focuses on some methods used to track *L. monocytogenes* strains and to understand routes of contamination throughout the processing environment.

4.2.1 PFGE

Pulsed-field gel electrophoresis (PFGE) is the 'gold standard' method for assessing *L. monocytogenes* strain interrelatedness (Goering 2010) and for monitoring the putative routes of contamination in food processing environments (Fox et al. 2011a). PFGE was first developed by Schwartz and Cantor at Columbia University in 1984 (Schwartz and Cantor 1984). It has made possible the separation of large DNA fragments. In conventional agarose gel electrophoresis, DNA molecules bigger than 40–50 kb in size fail to migrate efficiently and appear in a gel as a single large diffuse band due to their size-independent co-migration known as reptation (Singh et al. 2006; Slater 2009). PFGE is based on periodical changes in the direction of the electrical field, termed pulsed field, during migration of DNA in an agarose gel and allows the separation of DNA molecules over 1000 kbp. Briefly, the principal of PFGE is as follows: bacteria are embedded in agarose plugs in which cells are lysed in order to release DNA into the agarose plug. DNA is then digested and cleaved into large fragments by restriction enzymes and separated in a horizontal agarose gel using pulsed-field migration. This results in DNA fragment patterns or pulsotypes which differ from one strain to the other depending on the number and size of DNA fragments obtained from the digestion. Pulsotypes are used to identify and to compare bacterial strains such as *L. monocytogenes* after analysis of the gel image using specific software. The PulseNet International network proposes several standardised PFGE protocols for the study of food-

borne pathogenic bacteria including *L. monocytogenes* (PulseNet USA 2009). This allows the creation of databases for the comparison of strains worldwide (Martin et al. 2006; Fox et al. 2012).

For example, PFGE was used to identify the route of contamination of farmhouse Cheddar cheese from the outside to the inside of the cheese processing facility and finally to the cheese (Dalmasso and Jordan 2013a). The transfer of *L. monocytogenes* strains from dairy farms to associated cheese processing facility has also been demonstrated using PFGE (Ho et al. 2007).

4.2.2 Random Amplified Polymorphic DNA (RAPD)

The RAPD method is based on the amplification of unspecified genomic sequences with arbitrary sequences used as single primers in order to generate a band profile. The main weakness of RAPD is that it has a low reproducibility because of the low annealing temperatures used in the PCR protocol which may generate different amplification patterns in different laboratories (Tyler et al. 1997; Jadhav et al. 2012). Although less discriminative than PFGE, RAPD is a cost-effective, rapid and useful method for comparing strains (Gravesen et al. 2000). It has been widely used to type dairy isolates. For example, Chambel et al. (2007) used RAPD to compare more than 200 strains from Portuguese dairy factories and evaluate the pattern of contamination throughout time.

4.2.3 Other Methods for Tracking Strains

Amplified fragment length polymorphism (AFLP) and fluorescent AFLP (fAFLP) are based on the digestion of the genomic DNA by restriction enzymes, followed by PCR amplification and capillary gel electrophoresis (Graves et al. 2007; Roussel et al. 2013). Lomonaco et al. (2011) compared two AFLP methods and PFGE in regard to discriminatory power, typeability and concordance. They showed a very similar discriminatory power between the two AFLP methods and PFGE and suggested that AFLP may be used as part of routine surveillance in production plants in order to reveal sources and routes of transmission.

REP (repetitive element palindromic) and ERIC (enterobacterial repetitive intergenic consensus) repeats are imperfect palindrome sequences in bacterial genomes which can form stem and loop structures. REP palindromic sequences are found in the extragenic regions of the genome in direct or reverse orientation, whereas ERIC sequences generally consist of a central highly conserved inverted repeat sequences (Jadhav et al. 2012). REP- and ERIC-PCR are based on the amplification by PCR of these small sequences resulting in REP- or ERIC-PCR band patterns that are used to compare strains depending on the size and the number of bands on the profile. REP-PCR subtyping of *L. monocytogenes* has a lower discrimination power than PFGE but is still a fast low-cost molecular subtyping

method for the routine monitoring of *L. monocytogenes* in processing environments and could present an advantageous complement to PFGE (Zunabovic et al. 2012).

4.3 Persistence in the Processing Environment

Persistence means that particular types of microorganisms survive for prolonged periods of time in certain habitats. The mechanisms of persistence are the topic of much interest and debate and have been largely reviewed (Carpentier and Cerf 2011; Halberg Larsen et al. 2014). Persistence of *L. monocytogenes* relies on many factors, such as the physical and microbial natural habitat, transmission routes and genetic determinants. Owing to its elaborate physiological adaptation mechanisms, *L. monocytogenes* can survive and even proliferate under adverse environmental conditions such as low pH, high salinity and low temperature (Khelef et al. 2006).

In addition to strains persisting at larger-scale cheese production facilities (Lomonaco et al. 2009), persistence has also been documented at smaller artisan facilities (Fox et al. 2011a; Dalmasso and Jordan 2013a, b).

Various factors have been studied for their role in the persistence of *L. monocytogenes* strains, including disinfectant and desiccation resistance (Aase et al. 2000; Holah et al. 2002; Kastbjerg and Gram 2009; Vogel et al. 2010), differences in gene expression (Fox et al. 2011b) and biofilm formation (Norwood and Gilmour 1999; Lunden et al. 2000; Djordjevic et al. 2002).

4.3.1 Disinfectant Tolerance

It has been suggested that persistence could be due to harbourage sites that are not sufficiently sanitised and thus lead to recontamination events by strains resident in these sites (Carpentier and Cerf 2011). These sites include slots, drains, slicers, conveyer belts and packaging machines. Inadequate cleaning and disinfection procedures or other factors like food debris and biofilm formation can significantly reduce the efficiency of disinfectants against *L. monocytogenes* and consequently contribute to the maintenance of some strains in the processing environment. Some persistent strains have been shown to be more resistant to disinfectants such as quaternary ammonium compounds (QACs) than non-persistent strains (Aase et al. 2000; Lunden et al. 2003; Fox et al. 2011b). This could be due to the presence of genetic elements in persistent strains such as a plasmid-based gene cassette that confers increased resistance to a commonly used QAC compounds such as benzalkonium chloride (Elhanafi et al. 2010). It has also been shown that sublethal concentrations of benzalkonium chloride increased the proliferation of *L. monocytogenes* in the host cell (Pricope et al. 2013).

4.3.2 Attachment and Biofilm Formation

The attachment of *L. monocytogenes* cells to surfaces and the formation of biofilms in the food processing environment are well-known phenomena and have been recently reviewed (Valderrama and Cutter 2013). Biofilm formation is presumed to protect bacteria against environmental stresses (Ronner and Wong 1993) and so to facilitate persistence. For example, the examination of strains from bulk milk and milking equipment, and examination of biofilm on the milking equipment on a dairy farm, supported the view that the ability of *L. monocytogenes* to form biofilm is important in persistence of strains (Latorre et al. 2009). Persistent strains are believed to have better attachment abilities than non-persistent strains (Norwood and Gilmour 1999) even if in some cases, some non-persistent strains have shown a high aptitude to colonise surfaces (Lunden et al. 2000). A recent study also found that persistent strains from the dairy environment demonstrated better adherence than sporadic strains (Latorre et al. 2011). Higher biofilm formation among persistent compared to non-persistent strains from bulk milk samples was also described (Borucki et al. 2003). Food composition also seems to influence the adhesion of *L. monocytogenes* to solid surfaces during dynamic flow conditions (Skovager et al. 2013).

Moreover, multispecies biofilms, as commonly found, are believed to increase the protection of pathogenic bacteria, and especially *L. monocytogenes*, against disinfection (Norwood and Gilmour 2000; Van der Veen and Abee 2011). The presence of bacteria producing extracellular polysaccharide inside the biofilm may play a role in *L. monocytogenes* persistence by limiting the efficacy of sanitisers (Bremer et al. 2001; Carpentier and Cerf 2011).

4.3.3 Other Factors Potentially Involved in Persistence

Another phenomenon believed to contribute to persistence is the possible internalisation of *L. monocytogenes* inside protozoa, which can encyst to survive harsh conditions and thus protect *L. monocytogenes* from a hostile environment (Greub and Raoult 2004). Indeed, *L. monocytogenes* has been shown to survive after ingestion by protozoa and to be released after lysis of the protozoa sometime later (Ly and Muller 1990). However, there is some limitation of this mechanism as it has been demonstrated that *L. monocytogenes* was unable to persist in *Acanthamoeba* (Doyscher et al. 2013).

Integration of prophage DNA into the *com*K gene of *L. monocytogenes* was also proposed to lead to the persistent phenotype (Verghese et al. 2011). Persistent strains also seem to up-regulate genes implicated in the utilisation of carbon sources, possibly conferring to these strains competitive advantage and thus promoting persistence (Fox et al. 2011b).

Persistence of *L. monocytogenes* is a complex issue and could possibly be due to the contribution of different factors, which can vary from strain to strain (Fox et al. 2011b).

5 Control of *L. monocytogenes* in the Processing Environment

The control of *L. monocytogenes* in the processing environment is essential in contributing to food safety. The challenge for food manufacturers is to direct efforts to prevent the entry and establishment of *L. monocytogenes* within the processing environment. Good Manufacturing Practices (GMP) and employee training will facilitate this. In addition, an adequate Hazard Analysis Critical Control Plan (HACCP), or similar type plan, is necessary.

5.1 Sampling Plan

Environmental sampling is an effective way to assess hygiene and prevent future contamination events (Tompkin 2002). It is important to focus sampling to sites where occurrence of *L. monocytogenes* is expected or where it may be present and may contaminate food. Published guidelines exist for processing environment sampling (European Union Reference Laboratory for *Listeria monocytogenes* 2012). In their work, Dalmasso and Jordan (2013b) have demonstrated that processing environment sampling plans in a cheese processing facility were effective to assess hygiene, implement corrective actions in order to prevent contamination events, limit *L. monocytogenes* occurrence in food processing facilities and consequently improve the safety of the cheese. Being aware of the occurrence of *L. monocytogenes* in the processing environment is important as this occurrence can be dealt with. Having an action plan in the case of a positive result from sampling as part of the hygiene procedures is essential (Halberg Larsen et al. 2014).

5.2 Prevention of Attachment and Biofilm Removal

In the prevention of bacterial attachment to surfaces and biofilm formation, the choice of material regarding the physical properties of their surface is crucial as persistence is increased with porous materials, which should therefore be avoided (Mead and Scott 1994). Stainless steel has proven to be a material with low adherence of bacteria and easy to clean (Midelet and Carpentier 2002; Somers and Wong 2004). Many materials have incorporated bacteriostatic or bacteriocidal

agents which can reduce the attachment of bacteria to surfaces. However, these properties can be diminished or impaired with the presence of protein residues (Chaitiemwong et al. 2010; Møretrø et al. 2011).

Before any step of disinfection, a cleaning procedure has to be carried out. It contributes to removal of biofilm. A robust disinfection routine should include alternation between chemical disinfectants with different properties and mechanisms of action or between chemical and physical disinfection such as heat or UV. Standardised tests have been defined in order to ensure that disinfectants meet the required efficacy criteria (CEN 1997, 2002). As the composition of biofilm, the cleaning and disinfection procedures used and the compounds used vary from one food company another, there is currently no ideal/standard method for the complete removal of biofilms, as shown in several studies (Belessi et al. 2011; Cruz and Fletcher 2012).

5.3 Microbial Interactions

Microbial interactions have great potential to improve safety in the food chain and thus reduce the associated public health risk. These microbial interactions have been extensively reviewed (Jordan et al. 2014). Fermentation processes involved in the production of cheese and yoghurt are known to have antimicrobial properties against food spoilage microorganisms but also pathogens such as *L. monocytogenes*. Therefore, using traditional food fermentation processes with potentially hazardous raw materials (like raw milk) may be used for production of food with improved quality and increased safety (Adams and Mitchell 2002). Many studies have shown that lactic acid bacteria (LAB) involved in fermentation processes can control and inhibit pathogenic bacteria by different mechanisms like nutrient competition, immunostimulation, competition for binding sites and production of antimicrobial substances such as organic acids or hydrogen peroxide. Moreover, some LAB can also produce proteinaceous bacterial toxins, bacteriocins, which can be used as natural antibacterial preservatives for food (Gálvez et al. 2007). LAB bacteriocins usually exhibit activity against Gram-positive pathogens such as *L. monocytogenes* (Sobrino-Lopez and Martin-Belloso 2008) and have proven to be effective in food (Mills et al. 2011). Currently, two LAB bacteriocins, nisin and pediocin PA-1, are commercially available and have applications in food systems (EFSA 2006). Research on the use of bacteriocins in the food chain has been extensively carried out (Cotter et al. 2005) and developments in bacteriocins continue.

The use of bacteriophages for control of *L. monocytogenes*, although not strictly microbial interaction, is also of importance. Bacteriophages are good candidates for use as natural preservatives because of their ability to specifically target their host bacterium, their self-perpetuation and their stability during prolonged storage (Coffey et al. 2010). In dairy products, bacteriophages have been successfully used to control the presence and reduce the growth of *L. monocytogenes* (Greer

2005). In the USA, the Food and Drug Administration approved the use of a bacteriophage mixture that could be sprayed on specific food products to reduce the presence of *L. monocytogenes*, under the category of 'food additives'. A similar product can be used in the EU but under the category 'processing aid'.

6 Concluding Remarks and Future Perspectives

More knowledge on the *L. monocytogenes* strains contaminating processing facilities and the genetic and physiological factors that allow persistence is needed. To obtain this, more relevant models simulating niches in the food production environments combined with analytical tools to investigate the composition as well as genetic and physiological responses of complex microbiota should be developed (Malley et al. 2013). This will provide more relevant background information needed to develop new eradication strategies. Examples of new principles for combating biofilms that could be further investigated include the use of bacteriophages, targeting cell-to-cell communication, or the iron pathway or enzymes attacking specific biofilm components or new antibacterial materials. With new and fast techniques for strain characterisation, differentiation of *L. monocytogenes* strains with respect to virulence will be possible. Thus, virulence of a particular isolate may be taken into account in risk assessment.

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Listeria Species: Reemerging Pathogen in Drinking Water Utilities

Gulab Pandove, Parampal Sahota, and Neelam Garg

Abstract *Listeria monocytogenes* is recognized worldwide as one of the most important foodborne pathogens of concern for the food industries. It is a ubiquitous microorganism, and it is commonly isolated from foods of animal origin, mainly meat and milk products, but a few *Listeria* outbreaks have occurred in which these pathogens have been isolated from water systems and implicated as source of infection. The bacteria are aerobic and facultative anaerobic and non-spore and non-capsule forming, with optimal growth temperature of between 30 °C and 37 °C. They can, however, grow and reproduce at temperatures between 0.4 °C and 45 °C and pH 4.5–9.6. In the present chapter, occurrence of *Listeria* spp. in ground, surface, and bottled drinking water along with survival of *Listeria* spp. in oligotrophic and copiotrophic environments and antibiotic susceptibility, molecular detection, pathogenicity, and virulence of *Listeria* spp. have been discussed in detail.

Listeria monocytogenes cause listeriosis, abortion, human meningitis, infection during the perinatal period, granulomatosis infantiseptica, sepsis, diarrhea, pyelitis, and "flu-like" symptoms. The mortality rate of listeriosis is ~30 %. It is a major concern for food and water microbiologists.

There are currently no suitable microbiological criteria in India for the detection of emerging pathogens. There is need to revaluate the effectiveness of traditional indicators for risk management due to the emergence of pathogens. This confirmed the need for development of better microbial monitoring for assessing the safety of drinking water.

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1 Introduction

Listeria monocytogenes is recognized worldwide as one of the most important foodborne pathogens of concern for the food industries. It is a ubiquitous microorganism, and it is commonly isolated from foods of animal origin, mainly meat and milk products (Schuchat et al. 1991), but it can be also found in fresh produce, such as salads (Berrada et al. 2006). However, human listeriosis outbreaks are most often associated with ready-to-eat food products that are consumed without prior cooking (Ryser 1999). Ingestion of foods contaminated with *L. monocytogenes* can result in listeriosis, a severe infectious disease characterized by meningoencephalitis, abortion, septicemia, and a high fatality rate (30 %). Listeriosis predominantly affects certain risk groups, including pregnant women, newborns, elderly people, and immunocompromised patients (Kathariou 2002). However, recent reports of a noninvasive form of listeriosis that causes febrile gastroenteritis clearly indicate that persons with no predisposing conditions may be affected (Franciosa et al. 2001). The food safety regulations of most of the countries tolerate no *L. monocytogenes* in ready-to-eat foods (Gallagher et al. 2003), although the minimal infection dose is generally higher than 100 viable cells (Roberts et al. 1996). A few *Listeria* outbreaks have occurred in which these pathogens have been isolated from water systems and implicated as source of infection.

2 Historical Background and Taxonomy of *Listeria* spp.

Pirie (1940) isolated the organism from gerbilles (*Tetra lobengulae*) near the Tiger River in South Africa and named his isolate *Listerella hepatolytica*, the generic name being dedicated in honor of a British surgeon, Sir Joseph Lister. Pirie changed the name of the bacterium to *Listeria monocytogenes* in 1940, and in 1948 the organism was recognized by the same name in the sixth edition of *Bergey's Manual of Determinative Bacteriology* (Breed et al. 1948); however, to date six other species have been identified (Brugere-Picoux 2008).

3 Ecology and Host Range of *Listeria* spp.

Listeria is widely distributed in nature. The bacteria is an ubiquitous saprophyte that lives in plant-soil environments and has been isolated from about 42 species of domestic and wild mammals and 22 species of birds, as well as fish, crustaceans, insects, sewage, water, feedstuffs, milk, cheese, meconium, feces, and soil (Kirkan et al. 2006).

Listeriosis is a disease condition commonly associated with food and caused by pathogenic bacteria of the genus *Listeria* (*L. monocytogenes*, *L. ivanovii*, *L. innocua*,

L. seeligeri, *L. welshimeri*, *L. grayi*, and *L. murrayi*); only two, *L. monocytogenes* and *L. ivanovii*, are pathogenic; the former is responsible for disease in both humans and animals, while the latter causes disease mostly in ruminants but also in other animals (Brugere-Picoux 2008). There are reports however of *L. seeligeri* and *L. ivanovii* causing illness in humans (Cocolin et al. 2002) and *L. innocua* occasionally associated with encephalitis in ruminants (Walker et al. 1994).

Listeriosis is reported to be largely foodborne (Mead et al. 1999); however, nosocomial infections and person-to-person transmission (Jacobs and Murray 1986) have been reported. The clinical syndromes of the disease include invasive listeriosis, noninvasive gastrointestinal disease, as well as local skin and eye symptoms (Maijala et al. 2001). Invasive listeriosis causes meningoencephalitis, encephalitis, sepsis, and abortions and has a high mortality rate (20–30 %). On the other hand, noninvasive listeriosis causes fever, diarrhea, muscle pain, headache, nausea, vomiting, and abdominal pain in healthy adults (Lunden et al. 2004).

4 Factors Affecting Growth of *Listeria* spp.

The bacteria are aerobic and facultative anaerobic and non-spore and non-capsule forming, with optimal growth temperature of between 30 °C and 37 °C. They can, however, grow and reproduce at temperatures between 0.4 °C and 45 °C and pH 4.5–9.6 (Brugere-Picoux 2008); the bacteria exhibit a characteristic tumbling motility using peritrichous flagella at 20°–25 °C. On nutrient agar (24-h culture), *Listeria* colonies are round, 0.5–1.5 mm in diameter, and exhibiting bluish-gray color by normal illumination but a blue-green sheen under oblique light. *Listeria* species are catalase positive, oxidase negative, methyl red positive, and Voges-Proskauer positive.

Listeria survives wide ranges of salt concentrations (up to 10 %) (Roberts and Wiedmann 2003); the ability to survive and multiply under conditions frequently used for food preservation makes the bacteria particularly problematic to the food industry (Roberts and Wiedmann 2003).

Numerous studies have shown that *L. monocytogenes* is capable of adhering and forming biofilm on metal, glass, rubber, and plastic surfaces. Therefore they can be found in a variety of environments, including soil, sewage, silage, water, effluents, and foods.

5 Occurrence of *Listeria* spp. in Ground and Surface Drinking Water

L. monocytogenes was isolated from 21 % of a variety of surface waters in the north of the Netherlands, noting higher contamination rates (67 %) in waters near sewage treatment plant effluents (Dijkstra 1982). Colburn et al. (1990) reported isolating *Listeria* species from 81 % of river waters in California. They also found that *L. monocytogenes* was the most frequently isolated species, being present in 62 % of water samples. The authors suggested that there was a correlation between the *L. monocytogenes* from water and the potential that water was contaminated with bacteria of fecal origin from farming or municipal sources. Luppi et al. (1986) isolated *Listeria* spp. from 11 of 50 (22 %) of water samples from the river Po.

Frances et al. (1991) analyzed water samples from ponds and lakes. *Listeria* species were isolated on eight (27 %) occasions; six of these isolates were *L. seeligeri*, and one was *L. innocua* and one *L. welshimeri*.

Twenty-nine strains of *Listeria* were isolated from fresh surface water. Isolates mainly identified were *Listeria monocytogenes* (72.4 %); *Listeria innocua*, *Listeria grayi*, *Listeria ivanohovii*, and *Listeria welshimeri* were also present. 74.4 % of samples contained *Listeria* in mean concentrations of between 2 MPN/100 ml and 1320 MPN/100 ml in fresh surface water and untreated sewage, respectively (Bernagozzi et al. 1994).

Shaban and El-Taweel (1999) examined the presence of *Listeria* group from different aquatic environments using MPN and surface plate counts with the selective media and found that *L. monocytogenes* in wastewater varied from few cells to about 10^5 cfu/100 ml; the surface plate technique gave an order of magnitude higher count than the selective enrichment MPN technique. Variations in counts by both methods were rather small in case of chlorinated sewage effluent.

6 Occurrence of *Listeria* spp. in Bottled Drinking Water

Tagoe et al. (2011) analyzed 11 different sachet water brands from 11 separate vendors in Cape Coast Metropolis of Ghana bimonthly for 6 months. Bacterial counts ranged between 2.8×10^3 and 5.9×10^5 cfu ml^{-1} in all sachet water brands with different bacterial isolates which included *E. coli*, *coagulase-negative Staphylococcus*, *S. aureus*, *E. faecalis*, *K. aerogenes*, *M. catarrhalis*, *B. cereus*, *L. monocytogenes*, *Enterobacter* sp., etc. The degree of resistance of isolates to the antibiotics ranged from 50.0 % to 87.5 %, with multiple drug resistance to 4–7 antibiotics.

7 Gastrointestinal Epidemic Incidences by *Listeria* spp.

Barbuddhe and Chakraborty (2009) reported outbreak of gastroenteritis caused by *L. monocytogenes* in otherwise healthy individuals and more severe invasive disease in immunocompromised patients. Common symptoms include fever, watery diarrhea, nausea, headache, and pains in joints and muscles. The intestinal tract is the major portal of entry for *L. monocytogenes*, whereby strains penetrate the mucosal tissue either directly, via invasion of enterocytes, or indirectly, via active penetration of the Peyer's patches. *L. monocytogenes* has evolved species-specific strategies for intestinal entry by exploiting the interaction between the internalin protein and its receptor E-cadherin or inducing diarrhea and an inflammatory response via the activity of its hemolytic toxin, listeriolysin. The ability of these bacteria to survive in bile-rich environments, and to induce depletion of sentinel cells such as Paneth cells that monitor the luminal burden of commensal bacteria, suggests strategies that have evolved to promote intestinal survival. Preexisting gastrointestinal disease may be a risk factor for infection of the gastrointestinal tract with *L. monocytogenes*. Currently, there is enough evidence to warrant consideration of *L. monocytogenes*, as a possible etiology in outbreaks of febrile gastroenteritis, and for further studies to examine the genetic structure of *Listeria* strains that have a propensity to cause gastrointestinal versus systemic infections.

At least seven outbreaks of foodborne gastroenteritis due to *L. monocytogenes* have been reported. Illness typically occurs 24 h after ingestion of a large inoculum of bacteria and usually lasts 2 days. Common symptoms include fever, watery diarrhea, nausea, headache, and pains in joints and muscles. *L. monocytogenes* should be considered to be a possible etiology in outbreaks of febrile gastroenteritis when routine cultures fail to yield a pathogen (Ooi and Bennett Lorber 2005).

Riedo et al. (1994) isolated identical strains of *L. monocytogenes* from blood samples from two febrile pregnant women and from the stool samples of a person with diarrhea; they proposed the possibility that *L. monocytogenes* might cause gastroenteritis.

Convincing evidence that *L. monocytogenes* could cause gastrointestinal illness came from an outbreak of febrile gastroenteritis that was associated with the consumption of contaminated chocolate milk (Dalton et al. 1997). Symptoms developed in 75 % of persons (45 of 60) who drank chocolate milk.

The largest documented outbreak (Aureli et al. 2000) occurred in 1997, when 1566 students and staff members from two primary schools in northern Italy developed febrile gastrointestinal illness after eating cafeteria food. Cultures of one blood sample and 123 stool samples from hospitalized patients yielded *L. monocytogenes* strains that were identical to strains isolated from food and environmental specimens at the catering plant.

Guillet et al. (2010) reported *L. ivanovii*-associated gastroenteritis and bacteremia in a man. This isolate was indistinguishable from prototypic ruminant strains. *L. ivanovii* is thus an enteric opportunistic human pathogen.

8 Survival of *Listeria* spp. in Oligotrophic Environment

The ability of *Listeria monocytogenes* to survive and grow in different water systems from Mangalore Coast, Gurupur Estuary of Mangalore, and drinking water of Mangalore city was investigated. The water samples were inoculated with *L. monocytogenes* at a level of 10^7 cfu/ml and incubated at room temperature of 28.2 °C. Within 1 day, reduction in listerial counts was observed in all the samples, though the reduction was drastic in freshwater, followed by estuarine water and seawater. The survival of *L. monocytogenes* was higher in estuarine water (20 days) than in seawater (15 days). Freshwater did not support the growth of *L. monocytogenes* for long (5 days). The survival was very low in the unsterilized water samples (Jeyasekaran et al. 2001).

9 Survival of *Listeria* spp. in Copiotrophic Environment

Zúñiga-Estrada et al. (1995) studied the survival of *Listeria monocytogenes* in sterile skim milk during the fermentation with a yogurt starter culture and during storage at refrigeration temperature. Sterile skim milk was inoculated with 10^3, 10^5, and 10^7 cfu/ml of *L. monocytogenes* and with 10^6 cfu of lactic acid bacteria. Inoculated milks were fermented for 8 h at 42 °C, followed by refrigeration at 4 °C. Samples were taken at 2-h intervals during fermentation and at 2-day intervals during storage. Acidity and pH were measured, as well as viable count of lactic acid bacteria and pathogen. *L. monocytogenes* survived 8 h, 10 days, and 32 days in the fermented milk, when the inocula were 10^3, 10^5, and 10^7 cfu/ml, respectively. Inhibition of the pathogen was associated with a decrease of pH below 4.0 and increase in acidity. It was demonstrated that this pathogen is able to survive several weeks in milk fermented with a starter culture, contrary to the general belief which considered it very difficult due to the low pH. Therefore fermented milks may play an important role in the transmission of these bacteria.

Pasteurized whole ewe's milk was inoculated to contain ca. 1.0 times 10^{-6} to 2.0 times 10^{-6} *Listeria monocytogenes* Scott A or California (CA). Inoculated milk samples of 200 ml in sterile Stomacher bags were frozen at −38 °C and stored at −18 or −38 °C for 7.5 months. Inoculated milk was also made into Feta cheese curd, according to a standard procedure. After 5 h of drainage, curd samples of 200 g in sterile Stomacher bags were frozen at −38 °C and stored at −18 or −38 °C for 7.5 months. The pH values of the ewe's milk and Feta cheese curd before freezing were 6.70 and 5.43, respectively. At 15-day intervals, samples were thawed at 35 °C and tested for numbers of *L. monocytogenes* cells by surface plating on tryptose agar (TA) and tryptose salt agar (TSA) for ewe's milk samples or on lithium chlorite phenylethanol moxalactam agar (LPMA) for curd samples. A high percentage (ca. 95 %) of *L. monocytogenes* Scott A cells survived during storage of frozen ewe's milk at −18 or −38 °C for 7.5 months. The population of

L. monocytogenes CA decreased by ca. 50 and 40 % during storage of frozen ewe's milk for 7.5 months at −18 and −38 °C, respectively. The death rate of *L. monocytogenes* increased after repeated freeze-thaw cycles of ewe's milk at −18 or −38 °C. Populations of *L. monocytogenes* Scott A decreased by ca. 40 % in the center of the cheese curd samples, but the rate of death was less than ca. 17 % on the surface of the frozen cheese curd samples during storage at −38 °C for 7.5 months. Populations of strain Scott A decreased by ca. 57 % in the center of the cheese curd samples and by ca. 22 % on the surface of the frozen cheese curd samples during storage at −18° for 7.5 months. Populations of *L. monocytogenes* CA decreased by ca. 98 % for samples both at the center and the surface of the frozen curd during storage at −38 or −18 °C for 7.5 months (Papageorgiou et al. 1997).

10 Possible Reasons for Occurrence of *Listeria* spp. in Different Water Utilities

Further the reason for occurrence of *Listeria* spp. in drinking water utilities may be fecal wastes from domestic animals which may enter a water system by direct contamination of the water or through seepage or surface runoff. Domestic and wild animals contaminate water by defecation in unprotected surface water or through runoff and as a result of seepage of water through soil, resulting in contamination that may result in the incident of *Listeria* spp. in groundwater.

Another reason of contamination of groundwater may be poorly processed sewage effluents, malfunctioning of septic tanks, seepage from sanitary landfills, failure of the disinfection, and infiltration of contaminated water through cross-connection in the distribution system.

Broken or leaking pipes or leaking valves, joints, or seals may also provide a pathway for potential entry of microbes which could then become entrapped in the biofilm. Even in systems using good sanitary practice, main breaks may result in contaminant entry. Probably within seconds of entering the distribution system, some particles, including microorganisms, adsorb to clean pipe surfaces. The organisms take advantage of macromolecules attached to the pipe surface for protection and nourishment. The water flowing past carries nutrients that are essential for the organism's survival and regrowth.

The operating pressure in the water distribution system fluctuates over time and location, depending on several factors such as pipe elevation, operating set points for booster pumps, system valves, storage tank water level, and water demand variations. Pressure losses may also occur in the distribution system as a result of certain events including flushing, main breaks, and power outages and thus may result in the entry and multiplication of *Listeria* spp. in the distribution system.

Corrosion may also provide a protective surface for microorganisms. In iron pipes, electrochemical reactions at the pipe surface dissolve the metal to form pits

(releasing free ferrous ions) at one point while building a tubercle or nodule (composed of ferric hydroxide) at a remote spot. These pits and nodules so formed catch and concentrate nutrients and provide the organism with protection from water shear.

There is a general belief that the larger population of bacteria species grows as adherent to surfaces in all nutrient-sufficient aquatic ecosystems and that these sessile bacterial cells differ profoundly from their planktonic counterpart (Costerton et al. 1978). It has also been reported that the existence of pathogens, as free-living or plankton-associated cells, is critical to their survival in the environment as well as their transmission from one host to another (Donlan and Costerton 2002). Several studies have revealed the preponderance of *Listeria* species to exist as biofilms attached to surfaces such as stainless steel, glass, and propylene (Mafu et al. 1990) and PVC (Djordjevic et al. 2002). Some reports also (Paillard et al. 2005) indicate that *Listeria* species very easily survive conventional wastewater treatment processes and suggest that wastewater effluent could play a significant role in the epidemiology of the pathogen in the population.

11 Antibiotic Susceptibility of *Listeria* spp.

The widespread use of antimicrobials in human and veterinary medicine, as well as in animal production, has accelerated the development of drug resistance in a variety of pathogenic bacteria. *Listeria monocytogenes* are important gram-positive pathogens of public health concern. Antibiotic resistance in *L. monocytogenes* is mainly due to acquisition of three types of movable genetic elements: self-transferable plasmids, mobilizable plasmids, and conjugative transposons (Charpentier and Courvalin 1999). *Enterococci* and *Streptococci*, in particular, represent a reservoir of resistance genes for *L. monocytogenes*. The gastrointestinal tract of humans is considered the most probable site where the acquisition, by *Listeria* spp., of conjugative plasmids and transposons from *Enterococcus* and *Streptococcus* takes place (Doucet-Populaire et al. 1991).

Rota et al. (1996) found that a higher percentage of *L. innocua* were resistant to antibiotics than *L. monocytogenes*. The genetic basis for these differences is unclear, although the presence of plasmids encoding antibiotic resistance in *L. innocua* has been reported (Slade and Collins-Thompson 1990). Charpentier et al. (1995) have reported the presence of plasmid-mediated antibiotic resistance in *L. monocytogenes*.

Walsh et al. (2001) examined the susceptibility of 1001 food isolates of *Listeria* species to eight antibiotics; resistance to one or more antibiotics was exhibited in 0.6 % of *Listeria monocytogenes* isolates compared with 19.5 % of *Listeria innocua* isolates. Resistance was not observed in *Listeria seeligeri* or *Listeria welshimeri*.

David and Odeyemi (2007) found that erythromycin was the most effective antibiotic against the isolates of *L. monocytogenes* from environmental samples (cow manure, agricultural soil, and common vegetable) with the least resistance

(28.1 %), while chloramphenicol proved to be least effective with resistance of 52.29 %. The multiple-antibiotic resistant pattern of the isolates showed Augmentin/amoxicillin (33.3 %), Augmentin/erythromycin (24.18 %), and co-trimoxazole/chloramphenicol/amoxicillin (28.8 %) to be most prominent. The least value was observed in cloxacillin/co-trimoxazole/gentamicin with 15.34 %. The modal values of the minimum inhibitory concentrations (MICs) of the antibiotics to the isolates range between 4.0 and >16.0 μg/ml. Co-trimoxazole and gentamicin recorded the highest MIC compared with other antibiotics.

Mauro et al. (2007) studied the susceptibility of 38 *L. monocytogenes* strains isolated from 542 food and food-processing environmental samples to 22 antibiotics currently used in veterinary and human therapy. At least 97.4 % of strains showed resistance to oxacillin, lincomycin, flumequine, and clindamycin, regardless of both source and serotype. Sulfafurazole was active against environmental isolates and fish isolates (63.7 % vs. 41.2 % and 30 %, respectively).

Adetunji and Adegoke (2008) isolated 40 isolates of *L. monocytogenes* from cheese and found them sensitive to Augmentin, streptomycin, Claforan, erythromycin, gentamicin, Septrin, Tarivid, and Rocephin and highly resistant to nitrofuran, Fortum, Zinnat, and tetracycline.

Listerial resistance to antimicrobial therapy was also reported to be mediated by certain resistance genes which code for proteins that function in ways that inhibit or reduce the effects of antimicrobials on the pathogen (Davis and Jackson 2009).

12 Molecular Detection of *Listeria* spp.

A total of 30 strains of *Listeria monocytogenes* isolated from different foods (16 of different kinds of sausage, 14 cheese) and purchased at groceries of São Paulo City were ribotyped and analyzed for the presence and expression of hemolysin gene and production of phosphatidylinositol-specific phospholipase C—PI-PLC enzyme. The *L. monocytogenes* strains were differentiated into six ribotype classes. A total of 13 (43.3 %) from these strains belong to the same ribotype (ribotype I) and was coincident to the ribotype of the standard *L. monocytogenes* prototype strain (ATCC-15313). The hemolytic activity was observed in 29 (96.7 %) strains when incubated at 37 °C but not at 4 °C. The direct colony hybridization method for hemolysin gene detection showed a positive reaction with all 30 *L. monocytogenes* strains and a negative reaction with other *Listeria* spp. The PI-PLC was produced by 27 (90 %) of the strains analyzed. There was no correlation between the six identified ribotypes and the virulence factors (hemolysin and PI-PLC) studied (Pimenta et al. 1999).

A total of nine pairs of primers have been assayed for PCR detection of *Listeria monocytogenes* in food. They have been tested for specificity on a total of 72 strains including reference and food isolates belonging to *L. monocytogenes* and other species in the genus. First of all, a polyphasic approach has been carried out in order to establish a reference strain collection. They were biochemically and genetically

characterized by API-Lis and randomly amplified polymorphic DNA PCR (RAPD-PCR), with M13, T7, and T3 universal primers, and a data bank was created to compile the RAPD patterns of all the analyzed strains. The UPGMA cluster analysis of RAPD profiles with primer M13 showed eight clusters at 72.3 % similarity. Clusters 2 and 7 corresponded to *L. monocytogenes*. Clusters 1 and 6 grouped *L. ivanovii* strains. Clusters 3, 4, 5, and 8 corresponded to *L. grayi*, *L. innocua*, *L. welshimeri*, and *L. seeligeri*, respectively. Pattern analysis revealed the existence of misidentified reference strains which was confirmed by 16S rDNA sequence analysis. RAPD-PCR is a rapid genetic test which helped to confirm strain identity. On the basis of PCR specificity results, primers LM1–LM2 were the best combination for the detection of *L. monocytogenes* since they only amplified the specific fragment in strains that had been genetically and biochemically assessed as belonging to the species (Aznar and Alarcón 2002).

Almost full-length *iap* (invasion-associated protein) gene sequences were determined for 69 *Listeria monocytogenes* strains of all 13 known serotypes. A comparison of these sequences revealed that the *L. monocytogenes* strains can be grouped into three distinct genotypes. These clusters correlate well with distinct serotypes. Thus, strains of serotypes b and d belong to genotype I, a and c to genotype II, and 4a and 4c, which are rarely isolated from humans, group together within genotype III. These results could be corroborated by further comparative sequence analysis of genes encoding two phospholipases—plcA and plcB. Based on the *iap* gene sequences, a highly specific and reproducible competitive PCR detection method was developed. Primer pairs targeting genotype-specific regions of the *iap* gene were designed. The amplification of nonspecific PCR products from DNA of nontarget strains was prevented by adding competitive primers (Schmid et al. 2005).

Ingianni et al. (2007) isolated and identified *L. monocytogenes* in processed meat samples by a combined cultural-molecular method, hybridization technique with a specific DNA probe directed to the listerial internalin gene. The specificity of this method was found to be 100 % and sensitivity was as low as 1 cfu/2.5 g of food sample. A total of 278 meat samples were tested in comparison with PCR and conventional cultural assays. A total of 42 (15.4 %) *L. monocytogenes* were detected. PCR analysis gave three false-negative results and culture failed to detect the *Listeria* in five cases. With this cultural-molecular method, the identification and quantitative detection of *L. monocytogenes* were achieved within 36 h and no false-positive or false-negative tests were obtained, thus fitting most food industry requirements.

Navas et al. (2006) reported an increase in sensitivity of detection of *L. monocytogenes* by qPCR when a secondary enrichment is performed. From a value of 37 % positive samples after the primary enrichment, the percentage increased to 70 % after the second enrichment, suggesting the possibility to use a second enrichment step when very low numbers of *L. monocytogenes* are expected.

Rantsiou et al. (2008) described the development of a quantitative PCR (qPCR) technique to detect, quantify, and determine the viability of *L. monocytogenes* in foods. The method was based on the amplification of the intergenic spacer (IGS)

region between the *16S* and *23S rRNA* genes. A panel of more than 100 strains of *Listeria* spp. and *non-Listeria* was used in order to verify the specificity of the primers and TaqMan probe, and amplification signals were obtained only when *L. monocytogenes* DNA and RNA were loaded in the qPCR mix. Standard curves were constructed in several food matrices (milk, meat, soft cheese, fermented sausage, cured ham, and ready-to-eat salad). The quantification limit was of 10^3–10^4 cfu/g or ml, while for the determination of viability it was 10^4–10^5 cfu/g or ml. After an overnight enrichment in BHI at 37 °C, also 10 cfu/g or ml could be detected in all the matrices.

Azab El-Lathy et al. (2009) investigated 30 samples collected from the inlet and outlet of the treatment plants using PCR, MPN, and surface plate techniques. *Listeria* group was detected in 29 samples (97 %) using PCR technique, in 28 samples (93 %) using surface plate technique, and in 27 samples (90 %) using MPN technique. Statistical analysis (Student *t*-test) showed that there were significant differences between MPN and surface plate techniques in revealing the pathogenic bacteria.

13 Pathogenicity and Virulence Characteristic Features of *Listeria* spp.

The genus *Listeria* comprises six well-characterized species, among which *L. monocytogenes* is of particular importance as a pathogen causing serious foodborne infections in animals and humans. Newborns, pregnant women, and immunocompromised individuals are at increased risk for infection with virulent strains of *L. monocytogenes*. Such strains are able to display an intracellular life cycle within nonprofessional phagocytes as well as macrophages and monocytes.

Several proteins have been recognized as essential for the virulence of *L. monocytogenes* (Kuhn and Goebel 1995). Among these, the 60-kDa extracellular p60 protein, encoded by the *iap* (invasion-associated protein) gene, has been identified to be not only required for efficient adhesion to certain mammalian cell types but also essential for cell metabolism of *L. monocyt*ogenes due to its murein hydrolase activity (Wuenscher et al. 1993). It has been shown that all *Listeria* species secrete a p60 protein, albeit with genus-specific conserved N- and C-termini and species-specific variable internal portions (Bubert et al. 1992).

Pathogenic and nonpathogenic species can be differentiated by their hemolysin or PI-PLC activities. Hemolysis is the key character to distinguish the two species most frequently isolated, i.e., *L. monocytogenes* (hemolytic) and *L. innocua* (nonhemolytic). The virulence gene *plcA*, present on *L. monocytogenes*, *L ivanovii*, and *L. seeligeri*, encodes the synthesis of a phosphatidylinositol-specific phospholipase C (PI-PLC) (Gouin et al. 1994) which is generally employed for the differentiation of hemolytic and nonhemolytic *Listeria* (Notermans et al. 1991). Cleavage of L-α-phosphatidylinositol (PI) by PI-PLC resulted in the production of water-

insoluble fatty acids and the formation of an opaque white halolike zone of precipitation around the colonies of the hemolytic species. D-Xylose and L-rhamnose fermentation can also be used to differentiate *L. monocytogenes* (D-xylose-negative and L-rhamnose-positive) species from the other two hemolytic species *L. ivanovii* and *L. seeligeri* (D-xylose positive and L-rhamnose negative). Alanyl peptidase is an enzyme produced by all the *Listeria* species except for *L. monocytogenes*.

14 Future Perspective

L. monocytogenes cause listeriosis, abortion, human meningitis, infection during the perinatal period, granulomatosis infantiseptica, sepsis, diarrhea, pyelitis, and "flu-like" symptoms. The mortality rate of listeriosis is ~30 %. It is a major concern for food and water microbiologists.

Studies have shown that there is no correlation between occurrence of coliforms and indicator organisms (e.g., *E. coli*, thermotolerant coliforms) and nonoccurrence of emerging pathogens like *Listeria* spp. in same water sample (Pandove et al. 2013). The occurrence is suggestive of inadequate chlorination, potential biofilm formation in pipes, and contamination with human and animal waste.

There are currently no suitable microbiological criteria in India for the detection of emerging pathogens. Firstly, these contaminants should be proposed in contamination candidate list and as indicators of distribution system integrity. Secondly, the need of the hour is simultaneous detection of *E. coli*, coliforms, and emerging pathogens in drinking water. Hence there is need to revaluate the effectiveness of traditional indicators for risk management due to emergence of pathogens. This further confirmed the need for development of better microbial monitoring for assessing the safety of drinking water.

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Listeria monocytogenes: A Dangerous and Insidious Pathogen in Seafood

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Abstract *L. monocytogenes* is the bacterium that causes an infection known as listeriosis. Although rare, the disease has an important impact, with a mortality rate of 20–30 % among groups such as immunocompromised individuals, the elderly, infants and pregnant women. Contaminated seafood products are among the foods that cause the transmission of listeriosis, which occurs primarily by consuming infected food. Refrigerated ready-to-eat fish products such as smoked salmon and semi-preserved fish, in particular, are the foods in which *L. monocytogenes* is most commonly found.

1 Introduction

1.1 General Features

Listeria sp. is a facultatively anaerobic, non-spore-forming, psychrophilic Gram-positive rod-shaped bacterium, which is capable of multiplying in substrates at activity water (a_w) values up to 0.92. It can grow in the presence or absence of oxygen and at temperatures ranging from 1 °C to 45 °C. It is a halophile that in order to multiply requires salinity values up to 10 ppt and 25 ppt to survive (Ahmed 1991). *Listeria* is ubiquitous and is found in soil, water, sediments and sewage sludge. Animals, especially mammals and birds, are the reservoirs where *Listeria* dwells as a saprophyte at the enteric level. It is not uncommon to isolate *Listeria* from the stool samples of healthy individuals: in adults, this microorganism inhabits roughly 5 % of normal intestinal or vaginal flora (Aureli 2001). In the last 15 years, this microorganism has been considered a major causative agent of food-borne disease. The first case of *Listeria* infection in humans dates back to 1981, in Canada, triggered by the consumption of coleslaw that had been contaminated by wastewater, infected by sheep (Lennon et al. 1984).

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1.2 Listeria monocytogenes *in Fish Products*

L. monocytogenes contamination in humans is generally caused by consuming contaminated foods. The pathogen can be present in raw or processed products, which may have been contaminated during and/or after heat treatment. Because *L. monocytogenes* survives and multiplies even at a temperature of 2–4 °C, it can be contained in chilled ready-to-eat (RTE) foods having a relatively long shelf life. This category includes processed fishery products stored under refrigeration (as smoked salmon, semi-preserved fish and seafood salads). Modified atmosphere and vacuum packaging do not inhibit its growth. According to a study by Gombas et al. (2003), the prevalence of *L. monocytogenes* is higher in fish products than any other types of foods, especially in seafood salads, which have a contamination rate of 4.70 %, and smoked salmon of 4.31 %. Prevalence is much higher than, for instance, that of fresh soft cheeses (0.17 %), bagged salads 0.74 % or even canned meat (0.89 %). *L. monocytogenes*, together with *Aeromonas*, *Clostridium botulinum* (non-proteolytic types B, E and F), *Plesiomonas shigelloides* and halophilic *Vibrio* spp., belongs to a group of bacteria that are generally found in aquatic environments, so it's included in the so-called indigenous fish flora. *L. monocytogenes* is present in freshwater of lagoon environments and in the sea, only near the seashore, particularly after rainfall events (Croonenberghs 2000; Miettinen 2006). Monfort et al. (1998) found that there is an association between *Listeria* spp. (*L. monocytogenes*, *L. innocua and L. seeligeri*) and the presence of faecal coliforms. *Listeria* can be found in fish, molluscs and crustaceans, whether fresh or frozen, raw or undercooked fish products (Embarek 1994; Jørgensen and Huss 1998; Destro 2000; Pagadala et al. 2012). The contamination routes of *Listeria* in processing plants include surfaces (skin, mucus, tail and head) and the intestinal cavity of the fish. Among the various processed products, the ones that are most "at risk" are ready-to-eat (RTE) foods (Garrido et al. 2009; Herrera et al 2006) as, for instance, cold-smoked and marinated fish (Meloni et al. 2009). Smoked fish, in particular, has been accountable for several cases of human infection (Brett et al. 1998; Miettinen et al. 1999; Tham et al. 2000). Other sporadic cases involved the consumption of crabmeat (Faber et al. 2000) and shellfish (Croonenberghs 2000). Smoked salmon, especially when stored more than 2–3 weeks and owing to the ability of *L. monocytogenes* to multiply at low values of temperature and a_w, has caused a number of cases (Rørvik 2000; Duffes 1999; Dalgaard and Jørgensen 1998; Schiavo 1996; Dillon et al. 1992; Fuchs and Nicolaides 1994; Jemmi and Keusch 1992; Hudson and Mott 1993). Traditional Japanese cuisine, which foresees the consumption of plenty of raw fish products as sushi and sashimi, has been prone to a high prevalence of *L. monocytogenes* contained in various RTE products, particularly minced tuna and cod roe (12.1 % and 9.1 %, respectively) (Miya et al. 2010). Fishery products fall into two categories, based on risk assessment. The high-risk products include shellfish; raw fish eaten without any heat treatment; fish products that are lightly preserved, as salted, marinated or smoked fish; or any seafood product that has undergone a bland heat treatment (pasteurisation or hot

smoking). The low-risk ones include: semi-preserved fish with an NaCl content greater than 6 %, with a pH lower than 5 or which have the addition of sorbates, benzoates or NO_2; products that have undergone high enough heat treatments such as sterilisation; dried products; and fresh or frozen products (Rocourt et al. 2000). In EFSA's 2013 report, the prevalence of *L. monocytogenes* in fish samples across the European Union was 10.3 %; 1.7 % of these had levels exceeding 100 cfu/g at the end of shelf life (EFSA/2013). The serotypes of *L. monocytogenes* that were most frequently isolated from fish products are serotypes 1/2a and 1/2b and serotypes 3a and 3b, which have rarely been isolated in human clinical cases (Miya et al. 2010; Handa et al. 2005).

2 Contamination of Fish Products

Although *L. monocytogenes* has been reported in freshwater and seawater (especially in delta areas after precipitation), in the intestines of warm-blooded animals (human, domestic and wild animals and birds) and in wastewater, its presence in high concentrations has never been detected in fishery products; this is true with the exception of animals with chitin (shrimps, lobsters and crabs): because of the chitinase activity of *L. monocytogenes*, it can ecologically adapt itself to the exoskeletons of these animals. Consequently, particular attention must be paid when cleaning and sanitising the surfaces of the tanks that contain these animals as they are easily contaminated. Delta environments that are contaminated with *L. monocytogenes* help to explain why this pathogen is found in bivalve molluscs, which filter water, building up the pathogen. This, for instance, happens with oysters (Colburn et al. 1990), although their short shelf life makes it impossible for *L. monocytogenes* to multiply and reach a sufficiently high concentration to trigger the disease. In addition, *L. monocytogenes* is also significantly affected by the presence of the natural flora of live bivalves. The greatest risk factor of contamination of fish products remains the seafood-processing environment since *L. monocytogenes* is a psychrophilic pathogen that is capable of forming very resistant biofilms on work surfaces and equipment (Rørvik et al. 1995; Miya et al. 2010). Although there is not always a correlation between the pathogenicity of the strains isolated in humans and those isolated from seafood products (Norton et al. 2001), some studies have revealed a high number of virulent strains in processing environments (Gudmundsdóttir et al. 2006).

2.1 Listeriosis in Humans

L. monocytogenes is an intracellular pathogen known to cause a disease called listeriosis. The disease has no distinct clinical signs as its course depends on the health state of the host. The groups that are most at risk are infants, the elderly (over

65), pregnant women and immunocompromised individuals (AIDS patients, alcoholics, patients with neoplastic disease, diabetes, cirrhosis and so forth). The above-mentioned groups fall into what is known as YOPI (an acronym for: the young, old, pregnant, immunocompromised) (De Cesare et al. 2006; De Cesare and Manfreda 2002). If contaminated food is ingested, *L. monocytogenes* can penetrate the intestinal endothelial barrier and cross the placenta or the blood-brain barrier (Goldfine and Shen 2007; Ramaswamy et al. 2007) as the internalin family of surface proteins, e.g. InlA and InlB, that permit to adhere to the gastrointestinal epithelium, the placenta and the liver. Symptoms in adults may include meningitis, meningoencephalitis, encephalitis, septicaemia, endocarditis, pulmonary infections, arthritis, hepatitis and osteomyelitis. Milder forms include a self-limiting, febrile gastroenteritis, in generally healthy subjects. This condition may be underestimated, as the symptoms that characterise it are nonspecific (Franciosa et al. 2001). At times veterinarians may experience a form of skin eczema, caused by the direct contact with aborted foetuses (Swaminathan and Gerner-Smidt 2007). Women who contract the disease during pregnancy may have no symptoms or a mild flu-like illness, whereas the foetus can develop a congenital infection that may lead to a miscarriage, premature birth or even death (Jay et al. 2009). Newborns may have conjunctivitis with a purulent discharge, pneumonia, skin rash, vomiting, hyperexcitability and fever. Having an incubation period that ranges from 2 to 70 days, it is very difficult to isolate the food that may be responsible for the infection. Although the minimum infective dose has still not been identified, its range spans from 1,000 to 10,000 *Listeria*/dose (Faber and Peterkin 1991). However, the onset of the infection may even be caused by low doses of bacteria, especially among the high-risk groups. Contamination levels of roughly 100 have been also correlated to clinical cases. The infectious dose can vary depending on the virulence of the strain and on the host, as illustrated by the fact that only 3 out of the 13 known serotypes of *L. monocytogenes* (1/2a, 1/2b and 4b) cause most of the cases in humans (more than 90 %) and animals. Serotype 4b is mostly associated with outbreaks, whereas serotypes 1/2a and 1/2b are associated with sporadic infections. Serotypes 4a and 4c are rarely involved in cases of human listeriosis (Liu 2006). Still it is not easy to explain why in cases of frequent contamination the illness develops only rarely. As a matter of fact, listeriosis is considered a rare disease, with a very low incidence of 0.1–11.3 cases per million (Swaminathan and Gerner-Smidt 2007). Presumably, many isolated strains of *L. monocytogenes* are not pathogenic and the population has become somewhat immune (Kathariou 2002). According to the USFDA (1987), seafood products may reduce the virulence of *Listeria*, while other products, as milk and vegetables, enhance virulence. Being a bacterial disease, listeriosis, in both adults and children, can be treated with antibiotic therapy. For cases involving the immunocompromised however, for whom recovery is more difficult than the immunocompetent group, the therapy may not be sufficient. In pregnant women, a course of antibiotics, administered in early pregnancy, can prevent the disease from being transmitted to the foetus.

2.2 Cases of Listeriosis

Invasive listeriosis has become an important public health concern; in Europe the infection which calls for hospitalisation is over 90 % of the cases and its case fatality rate is 20–30 %. Its incidence (4 cases per million) is lower than the incidence of salmonellosis and campylobacteriosis which, in Europe, have a notably high incidence (237 and 456 cases per million inhabitants, respectively) (Pontello et al. 2012).

Several cases were documented, particularly in France (De Valk et al. 2001), Finland (Maijala et al. 2001), Switzerland (Bille et al. 2006), England (Maijala et al. 2001), Belgium (Yde et al. 2010) and Ireland (Jensen et al. 2010). The incidence in Britain ranges from 1.6 to 2.5 cases per million inhabitants, even though the cases associated with pregnancy have fallen (Rocourt et al. 2000). Regarding the distribution of listeriosis in other parts of the world, sporadic cases have been reported in the USA in 1989, linked to the consumption of shrimp, and in Australia and New Zealand in 1991–1992 where smoked mussels were the cause. In the USA, the incidence, in 1990–1993, fell from 7.7 cases per million populations to 3.7 cases in 1996. The average incidence, in 2009–2011, was 2.9 cases per million populations (CDC 2013). Their worst listeriosis outbreak was in 2011, infecting 147 people, with a death toll of 33 and 1 miscarriage among residents across 28 states. The outbreak was linked not to the consumption of a fish product but of tainted melons produced by a single company (CDC 2011).

2.3 EU Regulations on the Maximum Permissible Levels in Foods

Currently, the levels of *L. monocytogenes* permitted in foods are established by EC Regulation n. 2073/2005, modified by EC Regulation n. 1441/2007, setting the tolerance levels for *L. monocytogenes* for the various kinds of RTE products.

Various aspects were taken into account by experts when developing EC Regulation 2073/2005, for instance, the physiology and microbial ecology of *L. monocytogenes*, as the pathogen cannot actively duplicate itself in certain conditions as pH and/or a_w (of the substrate). Adverse conditions to the growth of *L. monocytogenes* are $a_w \leq 0.92$ or pH ≤ 4.4 or $a_w \leq 0.94$ and pH ≤ 5. The diagram below illustrates the conditions envisaged by the Regulation in relation to *L. monocytogenes* parameters (Fig. 1).

The safety standards on *L. monocytogenes* in ready-to-eat foods vary depending on their destination. Great attention goes into the food preparations, which are intended for infants and for special medical purposes; the standard applied is the

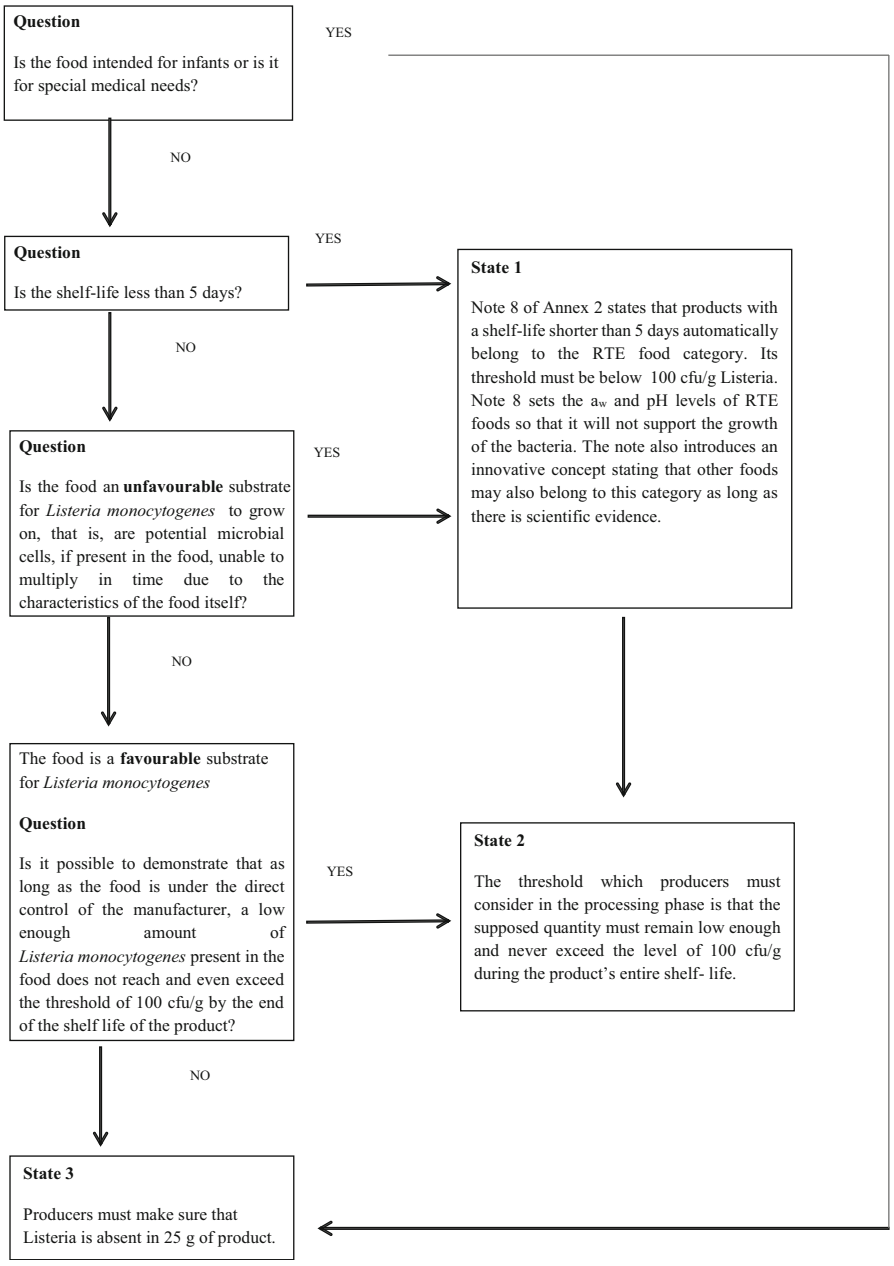

Fig. 1 Flow chart about *Listeria monocytogenes* parameters

absence of 1 cfu of *L. monocytogenes* in 25 g of ten sample units. Experts have divided ready-to-eat foods intended for all consumers into two other subgroups:

1. Foods that may be substrates for *L. monocytogenes* to grow on.
2. Foods that, for various reasons, do not support the growth of *Listeria*. In the first case, food business operators (FBO) must comply with more restrictive measures: monitoring the production process to be sure that each production batch will not have even one *L. monocytogenes* isolated in 125 g of food, testing five 25 g sample units while the product is still under their control, before its distribution and sale. In the second case, however, the food limit is up to 100 cfu of *L. monocytogenes* per gramme of food in five sample units, throughout commercial shelf life.

3 Transmission and Prevention in the Food Industry

As previously mentioned, *L. monocytogenes* is a pathogenic microorganism that is widespread in the environment, making repeated contamination in the food processing plants easy. Its routes of entry can vary and include raw materials, employees and the means of transportation. Once the organism is in, it colonises the different sites in the processing plant, especially conveyor belts, drains, floors, walls, pipes, transporting equipment and the refrigeration systems, as well as packing machines, slicers, work surfaces and utensils, like knives and cutting boards which often result positive when tested for *L. monocytogenes*. Cross-contamination can spread the pathogen from the processing equipment to the surfaces, then to the products, all the way through to the final product, until reaching the consumer. On the one hand, all possible points of entry of *Listeria* into the food processing facilities must be limited as much as possible. On the other hand, the growth and multiplication of this pathogen has to be prevented or at least inhibited when it has reached the products and equipment (Suslow and Harris 2000). The following preventive measures are pivotal:

1. Applying strict cleaning and sanitation procedures of the premises, the work surfaces and all equipment.
2. Implementing appropriate maintenance, cleaning and sanitising programmes of refrigeration and ventilation systems.
3. Applying good manufacturing practices by employees, with particular focus on preventing cross-contamination, and on time and temperature control. It is worth noting that the low temperatures in processing environments are not an inhibitory factor for the growth of *Listeria* since it can replicate even at refrigeration temperatures. To prevent cross-contamination, areas must be distinct, and thus, one area should be for incoming raw materials, another for conservation, yet another for the processing of the product and one for the storage of the final product. The number of steps involved, from one area to the other, has to be minimised and must flow in a preset direction. Effective cleaning and sanitising

involve more than just using the right sort of detergents and disinfectants for the particular surface that is being treated or using them in the right concentration and allowing them enough time to do their to work. It implies that the surfaces that need sanitising must be reachable and that any worn out, damaged, rusted tool be replaced. Furthermore, to control and manage the risk of *L. monocytogenes* contamination in processing environments, it is important not to disregard the pathogen's ability to form a biofilm: this microorganism adheres to surfaces, generating layers of bacterial cells of varying thickness. The presence of extracellular polymeric substances (EPS) keeps disinfectants from carrying out their bactericidal action. A greater EPS production leads to a greater resistance to disinfectants, therefore becoming a more persistent environmental pathogen (Nakamura et al. 2013). Biofilms may be found on different kinds of surfaces such as stainless steel, glass, plastic, polycarbonate, polystyrene and other materials that regularly are exposed to food (Di Bonaventura et al. 2008; Gandhi and Chikindas 2007). Biofilm formation is influenced by several factors such as the environmental temperature, the type of surface and the *L. monocytogenes* strain (serotype). In vitro analyses have shown that lowering the temperature of the environment reduces the production of biofilm, regardless of the strains of *L. monocytogenes*. Moreover, the presence of a nutrient-poor culture medium enhances its formation and also speeds up the production process compared to a richer medium (Kadam et al. 2013). Currently, there is still no evidence on the correlation between the formation of *L. monocytogenes* biofilm and food contamination that may lead to food-borne disease (Valderrama and Cutter 2013). Many studies have been dedicated to exploring the most effective chemicals and the strategies to eliminate biofilms in food processing plants. Testing evaluated the efficacy of products containing chlorine used alone or in tandem with detergents, quaternary ammonium compounds, hot water and even biological methods that are based on competing microorganisms (Gandhi and Chikindas 2007). Recently, Torlack and Sert have been examining the efficacy of benzalkonium chloride and ultrasonic waves to inactivate biofilm on plastic surfaces. Their results highlight that to inactivate the bacterial cells that make up the biofilm, a combined use of benzalkonium chloride and ultrasonic waves is much more effective than any single treatment (Torlack and Sert 2013). Another study, instead, focused on the effects of ozone and open-air factor (OAF) on *L. monocytogenes* biofilms formed on stainless steel, polypropylene and granite (Nicholas et al. 2013). Routine use of chemical agents to sanitise processing plants can lead to the risk of selection of bacterial strains resistant to active ingredients, thus persistent in the environment. Some authors therefore recommend rotating the use of at least two sanitising agents (Mereghetti et al. 2000).

4 Resistance and Growth Inhibition in Foodstuffs

L. monocytogenes is a Gram-positive bacterium that, compared to other non-spore-forming bacterial pathogens as *Vibrio parahaemolyticus*, *Salmonella* spp. and other Gram-negative bacteria, has an unusually high resistance. *L. monocytogenes* can withstand a wide range of temperatures (from 2 °C to 45 °C), and their ability to survive and multiply at refrigeration temperatures makes it difficult to control this pathogen. Furthermore, the lipid composition of its cell membrane can be modified, becoming sufficiently fluid to adapt even at low temperatures. Generally, a temperature of about 70 °C deactivates *L. monocytogenes* (Mackey and Bratchell 1989) although the potential variability of the different strains employed, the interaction with substrates, the presence of acids and the growth phase need to be taken into account each time (Doyle et al. 2001). Being resistant to very low-pH environments, it is known to survive in acidic foods and to cross the gastric barrier through various mechanisms that involve adaptation, as, for instance, the glutamic acid decarboxylase (GAD) system (Gandhi and Chikindas 2007). Examples of inactivation of *L. monocytogenes* in fishery products are reported in Table 1.

Table 1 Inactivation of *L. monocytogenes* in fishery products: physical and chemical methods

Method applied	Condition	Fish product	Source
Pasteurisation (product's core temperature is recorded)	60 °C/4.48 min	Fresh salmon	Embarek and Huss (1993)
	65 °C/0.87 min		
	70 °C/0.07 min		
	60 °C/1.98 min	Fresh cod	Embarek and Huss (1993)
	65 °C/0.28 min		
	70 °C/0.03 min		
High hydrostatic pressure (HHP)	450 MPa/10 min +35 days/5 °C No growth	Fresh salmon	Medina et al. (2009)
Pulsed UV light	5.6 J/cm^2 (3 pulses/second) for 60 s 8 cm apart	Fresh salmon	Ozer and Demirci (2006a)
Ionised water	2 min immersion in 50 ppm chlorine at 22 °C	Fresh salmon	Ozer and Demirci (2006b)
Sodium acetate	Washed in sol 4 M	Crabmeat	Degnan et al. (1994)
Sodium lactate and sodium diacetate	2.4 % lactate solution + 0.125 % diacetate solution on the surface, stored at 4 °C	Smoked salmon	Neetoo et al. (2008)
Trisodium phosphate	Immersion in 20 % solution	Fresh trout	Mu et al. (1997)

In foods, *L. monocytogenes* is affected by the presence of competitive microorganisms, which, even if nonspecific, compete for substrate. Several studies pointed how, for example, lactic bacterial flora hinders the growth of this pathogen (Huss 2003; Caplice and Fitzgerald 1999; Andrighetto et al. 2009). Known as the Jameson effect, this phenomenon could be considered as a potential "hurdle" in choosing the product's shelf life (Ross et al. 2000). Extensive research has been carried out to identify some of the pathogen's antagonists and to include them in the packaging material or apply them directly onto the product. For instance, the inhibitory activity of bacteriocins was considered, e.g. nisin (Nilsson 1999; Nilsson et al. 2000; Nykanen et al. 2000), as well as other bacteriocins (Brillet et al. 2004); the inhibitory activity of chitosans (Cruz et al. 2006) and the bactericidal power of specific bacteriophages (Soni and Nannapaneni 2010) were also evaluated. Essential oils like oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) inhibit the growth of *L. monocytogenes*; they can be used as ionising treatments in foods or incorporated in protective packaging film (active packaging) (Valderrama Solano and de Rojas Gante 2012).

5 Seafood and Predictive Microbiology

Lately, to gain greater insight into the behaviour of *L. monocytogenes* in fish products, predictive microbiology was developed. It has proven to be a useful tool to predict whether the substrate of fish favours the growth of *Listeria*. The first reports were by Dalgaard. His studies focused on smoked salmon inoculated with *L. monocytogenes*, with different growth kinetics (Dalgaard 1997). Other studies followed and include Cornu et al. who developed a model with variables such as temperature, salinity and phenolic content (Cornu et al. 2006). Subsequently in 2007, Mejlholm and Dalgaard explored the effects of lactate and diacetate on *L. monocytogenes* in modified atmosphere or vacuum-packed salmon, in marinated salmon and in cold-smoked halibut, finally designing a software program (SSSP) that featured seven variables and their interaction: temperature, CO_2, pH, salinity, lactates, diacetates and phenols. Finally, Dalgaard's group also developed a software program that includes the competitive action of lactic acid bacteria. This validated model has been extended to include 12 parameters (Mejlholm and Dalgaard 2007, 2009; Mejlholm et al. 2009, Dalgaard 2009). From the DTU (Technical University of Denmark) website, http://sssp.dtuaqua.dk/, the SSSP software is available, free of charge. It predicts the shelf life and growth of bacteria in different fresh and lightly preserved seafood, e.g. the effect of product temperature profiles recorded during storage and distribution by data loggers. Some of the predictive models in SSSP are equally useful for other types of food. With SSSP it is possible to know the *L. monocytogenes* growth and growth boundary model for the effect of temperature, salt ($NaCl/a_w$), pH, CO_2, smoke intensity, nitrite and organic acids (acetic/diacetate, benzoic, citric, lactic and sorbic acid). This model has been successfully validated for both seafood and meat products.

6 Laboratory Diagnosis

6.1 Diagnosis in Humans

In symptomatic patients, diagnosis is confirmed only after isolating *L. monocytogenes* from a blood sample, the cerebrospinal fluid in the case of infections of the nervous system or amniotic fluid and placenta in the case of pregnant women. Stool samples are rare. Serological tests are not commonly carried out as they lack sensitivity and specificity. The bacteria are detected by applying molecular methods such as real-time PCR.

6.2 Diagnosis in Food Samples

Generally, isolation techniques that are applied in foods comply with the ISO 11290-1:1996/Amd 1 2004 standards which foresee selective enrichment broth (Half Fraser and Fraser) and the use of a selective medium (Oxford agar and PALCAM agar or ALOA *Listeria* agar). To confirm any suspected colonies, subculture in blood agar and Tryptone Soya Agar with yeast extract is used, and specific tests such as catalase, Gram stain and the Christie-Atkins-Munch-Petersen (CAMP) test are carried out; traditional biochemical tests or miniaturised biochemical identification systems (i.e. API ® Listeria bioMerieux or Microgen Listeria ID Microgen Bioproducts) (Favretti et al. 2009) can also be used.

Traditional quantification of *L. monocytogenes* complies with the ISO 11290-2:1998/Amd 1 2004 standards. The growth of *L. monocytogenes* has demonstrated to be sensitive to changes in pH, refrigeration temperature and a_w; so the microorganism is present in the cultivable and detectable form only at certain environmental conditions. Differently from classical microbiology methods, the molecular methods can detect its presence even under conditions that do not encourage it to grow (Gambarin et al. 2012). For this reason, lately, new molecular identification techniques have arisen (Gambarin et al. 2012; Kérouanton et al. 2010) along with analytical techniques as MALDI-TOF MS (matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry) (Jadhav et al. 2012). To assess the potential virulence of *L. monocytogenes* strains, the following typing methods can be used: ribotyping (Pagadala et al. 2012), PFGE typing (Jensen et al. 2010) or multilocus sequence typing (MLST) (Jadhav et al. 2012).

Additional strain typing methods *for L. monocytogenes* include serotyping, phage typing, MEE (multilocus enzyme electrophoresis), the MLVA (multiple-locus variable number of tandem repeat analysis) and the microarray technique (Liu 2006). The target genes for the diagnosis in molecular biology include 16S rRNA, 23S rRNA, the genes for listeriolysin (hly), internalin (inlA, InlB), actin polymerisation (ACTA), phospholipases (PLCA, PLCB), metalloproteinase (mpl), virulence regulator (prfA) and others (Gasanov et al. 2005).

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Prevalence and Persistence of *Listeria monocytogenes* in Dairy and Other Ready-to-Eat Food Products in Africa

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Abstract Worldwide, *L. monocytogenes* bacteria are frequently associated with illness in humans, where immunocompromised individuals and pregnant women are at high risk of contracting listeriosis. The pathogen most often spreads through food consumption, so it is a major concern in most food-processing environments. In Africa, however, research on *L. monocytogenes* is scarce, making data on listeriosis limited. This is problematic because in Africa, traditionally produced foods have poor microbial quality. On average, 5.1 % of dairy and ready-to-eat foods are contaminated with *L. monocytogenes*. The pathogen has been isolated from milk, local cheese ("wara"), yogurt, ice cream, "kunu," and ready-to-eat meat products during and after processing. Furthermore, it is resistant to one or more antibiotics and can also form biofilms on various surfaces that contact food. So, while *L. monocytogenes* is persistent in food-processing environments in Africa, the serotypes of its circulating strains are largely unknown. This study therefore expounds the characteristics of *L. monocytogenes* and listeriosis associated with consuming contaminated diary and ready-to-eat foods in Africa. We also present the prevalence and persistence of the pathogen in most food environments as well as the safety measures that can limit its ability to contaminate foods/surfaces and spread.

1 Introduction

L. monocytogenes, a bacterium that causes listeriosis, is continuously viewed as a threat to global public health and is gaining important attention among stakeholders in food safety. Listeriosis affects all species of domestic animals and humans. The human infection is usually severe, resulting in high hospitalization rates and

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mortality, especially for "at-risk" populations, and causes abortions, meningitis, meningoencephalitis, spontaneous peritonitis, septicemia, etc. (Farber and Peterkin 1991; Rocourt 1996; Mead et al. 1999; Acha and Szyfres 2003). At-risk individuals are those with low immunity, such as HIV/AIDS patients, children under 5 years old, pregnant women, and elderly people (Farber and Peterkin 1991; Acha and Szyfres 2003; Borucki and Call 2003; McLauchlin et al. 2004; Liu 2006; Kuhn et al. 2008). *L. monocytogenes* is ubiquitous in nature and can survive harsh environmental conditions such as low pH, water activity (Nolan et al. 1992; Buchanan et al. 2000; Duffy et al. 1994), and a wide temperature range (including refrigeration temperature) (HPA 2009). Food contamination is a major way that *L. monocytogenes* infections are acquired (Taormina and Beauchat 2002). As such, the pathogen has been isolated from various ready-to-eat, dairy, and other minimally processed foods in many countries and has caused various documented foodborne disease outbreaks (Piffaretti et al. 1989; Mahmood et al. 2003; Vitas et al. 2004; Aurora et al. 2009; Rahimi et al. 2010; Rivoal et al. 2010). Its virulence and persistence in the environment is further potentiated by its ability to form fimbriae, cellulose, and biofilms (Hood and Zottola 1995; Abu-lail and Camesano 2003; Gulsun et al. 2005; Adetunji 2010). Furthermore, the pathogen is now more resistant to many commercially available antibiotics and sanitizers (Poyart-Salmeron et al. 1992).

Listeriosis is frequently reported in developed countries, but its representation in Africa is still unclear. Research on *L. monocytogenes* in Africa is scarce, so data on listeriosis is limited. However, the prevalence, virulence, and serotypes of *L. monocytogenes* in ready-to-eat foods have been reported in Ethiopia, Uganda, Morocco, Egypt, Nigeria, Botswana, and Lesotho (Molla et al. 2004; Ennaji et al. 2008; Salihu et al. 2008; Morobe et al. 2009, 2012; Abd El Malek et al. 2010; Mugampoza et al. 2011; Abeer et al. 2012; Moshoeshoe and Olivier 2012; Yakubu et al. 2012), but the serotypes and virulence of the circulating strains remain largely unknown. The objectives of this review are to expound the characteristics of *L. monocytogenes* and listeriosis associated with consumption of contaminated diary and ready-to-eat foods in Africa and to briefly discuss the prevalence and persistence of the pathogen in foods and food environments. We also highlight the safety measures that can be applied to limit its contamination, spread, and persistence.

2 Characteristics of *L. monocytogenes*

Listeria are facultative anaerobes that are gram-positive, non-acid-fast, non-spore-forming, acapsular rod-shaped bacteria, and they measure 0.5–2 μm by 0.4–0.5 μm (Walker 2005). The history of *Listeria* is traced to 1926 when Murray, Web, and Swann demonstrated that it was the causative organism of listeriosis (Parihar 2008). In 1940, Pirie suggested the name *L. monocytogenes*. Other species in the genus *Listeria* include *Listeria ivanovii*, *Listeria seeligeri*, *Listeria innocua*, *Listeria*

welshimeri, and *Listeria grayi*. Of these, only *Listeria ivanovii* and *Listeria monocytogenes* are pathogenic.

L. monocytogenes is a facultative intracellular, anaerobic, psychotropic bacterium with low G+C (36–42 %) content (Vazquez-Boland et al. 2001; Monk et al. 2008; Parihar 2008; Sukhadeo and Trinad 2009), and it moves in a distinctive way called "tumbling motility," propelled by its peritrichous flagellum (Ferreira et al. 2003; Adetunji and Adegoke 2008). *L. monocytogenes* has a high motility (Mead et al. 1999) and grows within a wide temperature range (<0–45 °C) (HPA 2009), with an optimum at 30–37 °C (Swaminathan et al. 1995). However, the pathogen can be destroyed when kept at high temperatures (e.g., pasteurization) for short durations (Walker 2005). This microorganism is oxidase negative and catalase positive, it ferments glucose and produces acid without gas, and it survives in vacuum-packaged products at refrigeration temperatures (Duffy et al. 1994). Furthermore, it has low water activity and a low pH (Nolan et al. 1992; Buchanan et al. 1993) and produces a narrow zone of hemolysis on sheep blood agar (Varnam and Evans 1991). The bacterial particles usually occur singly, in short chains, or in diploforms, producing V-shape arrangements (Parihar 2008). Furthermore, it is genetically heterogeneous (Piffaretti et al. 1989; Wiedmann et al. 1997; Kathariou 2002) and displays great biodiversity and serological cross-reactivity with various bacteria strains in other genera (Parihar 2008). *L. monocytogenes* is ubiquitous and can be isolated anywhere in the environment, making it a very dangerous organism. In 2008, the Office International des Epizooties (OIE) documented many molecular and cellular determinants of virulence for this intracellular pathogen, and although there is evidence that polymorphisms influence virulence in some of the different *L. monocytogenes* strains, this heterogeneity cannot be correlated with the organism's ability or inability to produce disease. Therefore, all *L. monocytogenes* strains are considered to be potentially pathogenic.

3 *L. monocytogenes* Infections

L. monocytogenes causes a severe zoonotic illness known as listeriosis, which is associated with morbidity and mortality in humans and livestock (Borucki and Call 2003). Animals usually affected include both large and small ruminants, pigs, rabbits, mice, birds, and fish (Ireton 2006). Listeriosis commonly affects people with compromised immunity (e.g., HIV/AIDS patients), pregnant women, neonates, and elderly people (Farber and Peterkin 1991; Borucki and Call 2003; McLauchlin et al. 2004; Liu 2006; Kuhn et al. 2008), resulting in meningitis, meningoencephalitis, spontaneous peritonitis, abortion, septicemia, arthritis, pelvic infection, or arthritis (Khelef et al. 2006; Sukhadeo and Trinad 2009; Adetunji and Isola 2011a). Infections often have high risks of hospitalization, and human mortality is also high, ranging between 20 % and 30 % (Farber and Peterkin 1991; Rocourt 1996; Mead et al. 1999) and up to 80–99 % in the vulnerable groups (Farber and Peterkin 1991; Gray and Killinger 1966; Rocourt 1996; Sauders

et al. 2003; Chenal-Francisque et al. 2011). Several listeriosis outbreaks have been reported throughout the world, including many multistate outbreaks in the United States. In 2003, mortality from listeriosis was about 500 people from a reported ~2500 illnesses (Mead et al. 1999). However, listeriosis is underreported in Africa (Boukadidda et al. 1994) because food-processing industries are still evolving. In Nigeria, *L. monocytogenes* was isolated from some patients that showed clinical signs of listeriosis, and the infection produced a mortality rate of 27 % (Onyemelukwe et al. 1983). But in Morocco, human listeriosis is uncommon (Benomar et al. 2000). Chintu and Bathirunathan (1975) reported 85 cases of listeriosis in Zambia, while Hohne et al. (1975) reported that the outbreak serotypes (1/2a and 4b) of *L. monocytogenes* were isolated in slaughtered cattle in Togo.

Contamination of ready-to-eat foods—as well as other minimally processed foods—is widely documented as the main source of *Listeria* outbreaks. Consequently, it is a great public health concern. Outbreaks are often linked with the consumption of several contaminated food products, including ready-to-eat dairy and meat products, such as coleslaw, pasteurized milk, milk from lactating ruminants, soft cheeses, raw and pasteurized eggs, poultry meat, cooked meats, cured meats, and smoked salmon (Piffaretti et al. 1989; Mahmood et al. 2003; Vitas et al. 2004; Aurora et al. 2009; Rahimi et al. 2010; Rivoal et al. 2010).

4 Traditional Foods and *L. monocytogenes*: Isolation, Prevalence, and Persistence

4.1 Isolation and Prevalence of L. monocytogenes in Ready-to-Eat Foods in Africa

In Africa, roughly 5.1 % of dairy and ready-to-eat food sample are contaminated with *L. monocytogenes*, as shown in some of the few available prevalence studies. While Africa comprises over 50 countries, there are reports on *L. monocytogenes* incidence and prevalence from ready-to-eat foods from only a few countries (Fig. 1). In North Africa, Egypt has a prevalence of 5 % (Abd El Malek et al. 2010) and Morocco is at 2.4 % (Ennaji et al. 2008). In these two countries, *L. monocytogenes* isolates were obtained from meats, luncheon, and frozen chicken legs using multiplex polymerase chain reaction (PCR). However, in a study by Abeer et al. (2012), *L. monocytogenes* was not detected in camel milk from Egypt. Camel milk contains lysozyme and lactoferrin (El Agamy et al. 1992; Wernery 2003; Al-Majali et al. 2007; Al-Haj and Al-Kanhal 2010), which prevent the pathogen's growth.

Ethiopia and Uganda represent the only countries in East Africa where studies on the prevalence of *L. monocytogenes* were carried out. Using both conventional and molecular methods, *L. monocytogenes* was isolated from ice cream, pork samples, minced beef, fish, and chicken samples, amounting to a prevalence of 5.1 % in Ethiopia (Molla et al. 2004). In Uganda, *L. monocytogenes* contaminated

Fig. 1 Countries in Africa with known prevalence, serotypes, and virulence of circulating *Listeria monocytogenes* strains. Virulence genes: (**A**) *prfA* (Egypt); (**B**) *actA* (Egypt); (**C**) *hly* (Morocco). Circulating serotypes: (**1**) 1/2b, 3b, 7, 4b, 4d, 4e (Morocco); (**2**) 1/2b, 4b, 4e (Ethiopia); (**3**) 1/2b, 3b, 4a, 4b, 4c, 4d, 4e (Botswana). (*asterisk*) Countries with known prevalence (Botswana, Egypt, Ethiopia, Lesotho, Morocco, Nigeria, Uganda). The outline map of Africa was sourced from http://www.worldatlas.com/webimage/countrys/africa/afoutl.htm

3 % and 13 % of locally processed yogurt and bulk raw milk, respectively (Mugampoza et al. 2011). Currently, there is no clear evidence on its prevalence in central Africa. In Southern Africa, recorded prevalences ranged from 2.5 % in Lesotho (Moshoeshoe and Olivier 2012) to 4.3 % in Botswana (Morobe et al. 2009). In this region, the contaminated ready-to-eat foods were unpasteurized bovine milk, cheese, meat, frozen cabbage, and salads. In South Africa, researchers work more on *Listeria ivanovii* (Nyenje et al. 2012a, 2012b, 2012c) rather than *Listeria monocytogenes* so data on this pathogen are limited. Like other parts of the continent, few reports exist regarding the prevalence of *L. monocytogenes* in West Africa. Using agar-based techniques, some studies carried out in northern Nigeria by Yakubu et al. (2012) and Salihu et al. (2008) obtained prevalences of 5.3 % and 25 % in bovine milk and smoked fish, respectively.

4.2 *Virulence and Serotypes of Circulating* Listeria monocytogenes *Strains*

L. monocytogenes strains are diverse (Kathariou 2002), having dissimilar virulence characteristics. Usually, the pathogen's virulence genes inhabit a 9.6-kb chromosomal region (Gouin et al. 1994), where the *prfA* gene regulates these clustered genes on the chromosome (Chakraborty et al. 1992). In every successful infection of a host, *L. monocytogenes* makes use of several virulence factors (Doyle 2001; Vazquez-Boland et al. 2001; Liu 2006). These include internalins (*inlA* and *inlB*), surface protein p104, listeriolysin O (LLO: encoded by *hly*), ActA protein, phospholipases (phosphatidylinositol-specific phospholipase C (PI-PLC, encoded by *plcA*) and a broad-range or phosphatidylcholine-specific phospholipase C (PCPLC, *plcB*)), zinc-dependent metalloprotease, Clp proteases and ATPases, protein p60, and stress response genes (*opuCA*, *lmo1421*, and *bsh*). Only very few of the virulent genes expressed by *L. monocytogenes* have been determined in the existing circulating strains, and they are mostly from North Africa (Fig. 1). Abd El-Malek et al. (2010) and Abeer et al. (2012) determined the presence of *prfA* and *actA* genes in *L. monocytogenes* in some ready-to-eat foods in Egypt; in camel milk, the prevalence of *actA* in *L. monocytogenes* is 2.16 % (Abd El-Malek et al. 2010). Also, Ennaji et al. (2008) confirmed the presence of virulent *hly* genes in some foods sourced from Morocco. However, there are very few additional reports on other virulence factors responsible for *Listeria* pathogenesis in *L. monocytogenes* isolates from other parts of Africa.

Based on the specific O and H surface antigen of *Listeria* species, 12 or more serotypes of *L. monocytogenes* have been typed using serological detection (Liu 2006; Arun 2008). These serotypes include: 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 4e, 4ab, and 7. Serotypes 1/2a, 1/2b, and 4b are the most virulent causing up to 98 % of human listeriosis. This nomenclature has also been used to define three existing *L. monocytogenes* lineages: Lineage I (highly pathogenic with epidemic clones, 1/2b, 3b, 4b, 4d, 4e), Lineage II (medium pathogenic, 1/2a, 1/2c, 3c, 3a), and Lineage III (not very pathogenic, 4a, 4c). Lineages I, II, and III are responsible for most, rare, and small outbreaks, respectively (Wiedmann et al. 1996, 1997; Jacquet et al. 2002; Arun 2008). In ready-to-eat foods in Africa, circulating *L. monocytogenes* strains have been serotyped into 1/2a, 1/2b, 3b, 4b, 4d, 4e, and 7 (Molla et al. 2004; Ennaji et al. 2008; Morobe et al. 2009, 2012), indicating that more of the highly pathogenic lineages with epidemic clones are circulating in Africa. Also, because these identified serotypes are from only three countries (Fig. 1), studies on serological typing *L. monocytogenes* in African foods are needed.

5 Resistance of *Listeria monocytogenes* to Antimicrobials

Antimicrobial susceptibility test have shown that *L. monocytogenes* isolates in Africa were sensitive to a wide range of antibiotics. However, since the first documented report of multidrug-resistant *L. monocytogenes* in France in 1988 (Poyart-Salmeron et al. 1992), several studies in all parts of the world, including Africa, have isolated pathogens that are resistant to one or more antibiotics.

Table 1 Antibiotics commonly resistant to *L. monocytogenes* in Africa

Resistant antibiotics	LM source	Country	Authors
Ampicillin	Raw milk	Nigeria	Yakubu et al. (2012)
	"Kunu"	Nigeria	Nwachukwu et al. (2009)
Penicillin G	Cheese, raw milk, cabbage, salad	Botswana	Morobe et al. (2009)
Sulfamethoxazole/ trimethoprim	Cheese, raw milk, cabbage, salad	Botswana	Morobe et al. (2009)
Chloramphenicol	Cheese, raw milk, cabbage, salad	Botswana	Morobe et al. (2009)
	Meat tables	Nigeria	Adetunji and Isola (2011a, b)
Tetracycline	Cheese, raw milk, cabbage, salad	Botswana	Morobe et al. (2009)
	Meat tables	Nigeria	Adetunji and Isola (2011a, b)
	"Wara" (local cheese)	Nigeria	Adetunji and Adegoke (2008)
Nitrofurantoin	"Wara" (local cheese)	Nigeria	Adetunji and Adegoke (2008)
	Cheese, raw milk, cabbage, salad	Botswana	Morobe et al. (2009)
Novobiocin	Cheese, raw milk, cabbage, salad	Botswana	Morobe et al. (2009)
Streptomycin	Cheese, raw milk, cabbage, salad	Botswana	Morobe et al. (2009)
	Raw milk	Nigeria	Yakubu et al. (2012)
Gentamicin	Raw milk	Botswana	Morobe et al. (2009)
	Raw milk	Nigeria	Yakubu et al. (2012)
Cloxacillin	Meat tables	Nigeria	Adetunji and Isola (2011a, b)
Nalidixic acid	Poultry, red meat, and sausages	Morocco	Ennaji et al. (2008)
Colistin	Poultry, red meat, and sausages	Morocco	Ennaji et al. (2008)
Cephalosporins	Poultry, red meat, and sausages	Morocco	Ennaji et al. (2008)

Antimicrobial resistance is also common in the African food industry. *L. monocytogenes* is confirmed to be resistant to over 13 commonly used antibiotics (Table 1), especially isolates from ready-to-eat foods in Botswana, Morocco, and Nigeria, but *L. monocytogenes* isolates usually have dissimilar resistance patterns. Also, other studies have shown that *L. monocytogenes* strains isolated from various food sources are resistant to tetracycline (Morobe et al. 2009; Adetunji and Adegoke 2008; Adetunji and Isola 2011a, 2011b) more than other commonly used antibiotics, which corroborates findings from other places (Charpentier et al. 1995; Charpentier and Courvalin 1999). One reason for this resistance may be because tetracycline is one of the most commonly used antibiotics in both animal and human therapeutics (Morobe et al. 2009). Of the six classes of tetracycline-resistance genes—*tet*(K), *tet*(L), *tet*(M), *tet*(O), *tet*(P), and *tet*(S)—that confer the reduced susceptibility of bacterial pathogens to tetracycline (Charpentier et al. 1995), *tet*(L) and *tet*(S) are present in *L. monocytogenes* (Poyart-Salmeron et al. 1992; Charpentier and Courvalin 1999). Other commonly resistant antibiotics include ampicillin, gentamicin, nitrofurantoin, and streptomycin.

6 Biofilm, Cellulose, and Fimbriae Virulence Characteristics in *L. monocytogenes*

Factors such as biofilm, cellulose, and fimbriae formation in *L. monocytogenes* are responsible for its virulence and persistence. The level of production of these characteristics has been described to influence virulence in bacterial isolates (Hood and Zottola 1995; Gulsun et al. 2005; Adetunji 2010) and also has major implications in food-processing environments. Fimbria and cellulose formation help pathogens adhere to food contact surfaces (Abu-lail and Camesano 2003; Adetunji 2010). Furthermore, isolates' level of biofilm production influences the virulence characteristics of such isolates (Hood and Zottola 1995; Gulsun et al. 2005). Biofilms are aggregation of microbial cells organized within a glycocalyx (exopolysaccharide material made up of cellulose). Biofilms facilitate resistance of pathogens to antimicrobials, increase access to nutrients, protect bacteria from external assaults, and promote plasmid and gene transfer through quorum sensing (Jefferson 2004). These characteristics enhance persistence of pathogens in food-processing environments. Furthermore, hydrophobicity, nature and type of incubating surface/medium, pH, surface charge, and temperature all affect biofilm formation by pathogenic *Listeria* spp. (Wong 1998; Sinde and Carballo 2000; Donlan 2002). Some studies have investigated biofilm formation by *L. monocytogenes* strains in Nigeria (Adetunji and Adegoke 2008; Adetunji 2010; Adetunji and Isola 2011b; Adetunji and Odetokun 2012), finding that *L. monocytogenes* can form biofilms on cement, glass, wood, and steel food contact surfaces. These biofilms increase with higher incubation temperature and time, but

the degree of biofilm formation varied across the different surfaces. Reports on biofilms from other countries are scarce. Researchers in developing countries should investigate ways of mitigating biofilms in food-processing facilities since *Listeria* can persist for years (Unnerstand et al. 1996).

7 Controlling the Contamination, Spread, and Persistence of *L. monocytogenes*

Success in controlling *L. monocytogenes* contamination of ready-to-eat foods and dairy products depends largely on the level of sanitation and hygiene present during food processing. Various international food safety authorities have recommended that *L. monocytogenes* should not be detected in 25 g of ready-to-eat foods (HPA 2009). If detected at levels greater than 10^2 cfu/g in foods, this indicates problems in various control points during food handling and processing, and it poses significant hazards for the at-risk populations. *L. monocytogenes* contamination usually arises because of poor-quality raw materials, inadequate cooking of foods, cross-contamination, poor cleaning and sanitation, and inappropriate temperature and time controls (HPA 2009).

Since *L. monocytogenes* is ubiquitous, environmental contamination should be avoided. Processing facilities and equipment should always be kept clean, before and after use. Cleaning must be thorough and strategic. Cleaning and sanitation schedules should be carefully formulated and strictly adhered to in every processing facility to achieve an appreciable level of effectiveness. All areas of the facility should be cleaned, including floors, roofs, walls, drains, pipes, etc. During cleaning, detergents and chemical sanitizers sensitive to *L. monocytogenes* should be used. Suggested sanitizers are formulations of iodoform, quarter ammonium compounds, peracetic/peroctanoic acids, and chlorinated solutions. Hot water should also be used during sanitation because it has been shown to be effective in pathogen removal. Processing facilities should be properly designed and suitable food contact surfaces should be carefully selected. Food contact surfaces that have lesser affinity for *L. monocytogenes* adhesion should be used in processing facilities. This will reduce the rate of biofilm formation and forestall dispersal of organisms that facilitate the spread of disease.

Furthermore, clean and dirty operations should be separated. Wastes generated during processing must be treated and properly disposed of. Local processing of ready-to-eat and dairy foods should also be standardized and employ new technologies. Good manufacturing practices are encouraged. *L. monocytogenes* colonization and infection must be treated as a bacterial hazard, and thus, the principles of hazard analysis critical control points (HACCP) must be applied. A good HACCP plan should be designed for all processing lines and the critical control points carefully identified. The HACCP plan should be well monitored to ensure that the system is working. Also, effective pathogen detection procedures must be utilized.

These steps will reduce *L. monocytogenes* colonization, transmission, and cross-contamination in processing facilities. Personnel and workers handling ready-to-eat and dairy foods should be educated and encouraged to observe maximum cleanliness during processing, packaging, transportation, and display. Protective clothing including neat aprons, hand gloves, head caps, face masks, etc. must be worn at all times. Personnel should maintain regular cleanliness. They should not observe any contacts between raw and finished products. Products must be well packaged and any source of possible *L. monocytogenes* contamination must be avoided. Strict operation measures must also be applied when products are displayed for sale. It is imperative that all African countries establish food safety authorities that will set the required microbiological standards for ready-to-eat and dairy products consumed in these countries. Measures to enforce these set standards must also be instituted.

8 Conclusion

L. monocytogenes is a pathogen that is a public health concern in food-processing environments. Its wide environmental distribution pattern, persistence through biofilm formation, and ability to cause illnesses in humans, especially through consumption of contaminated ready-to-eat foods, are of serious concerns. Mostly, ready-to-eat foods including dairy products are poorly processed, making *L. monocytogenes* infections and outbreaks common in Africa, but they are clearly underreported. To improve the microbial quality and control contamination of ready-to-eat foods, especially by *L. monocytogenes*, the processing lines of these foods should be standardized with modern technologies that would limit pathogen contamination, colonization, spread, and persistence in processing plants. Surveillance of *L. monocytogenes* and its associated infections especially in at-risk populations is appropriate. More studies are required to highlight the current prevalence of listeriosis in all African regions and countries. The virulence and serotypes of circulating *L. monocytogenes* in ready-to-eat foods across the continent need to be unraveled, particularly using current molecular isolation and serotyping techniques. This will allow for an important differentiation of all the circulating *L. monocytogenes* strains. Finally, with the increasing resistance of *L. monocytogenes* to most currently used antibiotics, authorities should also consider shifting to the use of natural medical plants as antimicrobials and biopreservatives.

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