Novel Natural Food Antimicrobials*

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Abstract

Naturally occurring antimicrobial compounds could be applied as food preservatives to protect food quality and extend the shelf life of foods and beverages. These compounds are naturally produced and isolated from various sources, including plants, animals and microorganisms, in which they constitute part of host defense systems. Many naturally occurring compounds, such as nisin, plant essential oils, and natamycin, have been widely studied and are reported to be effective in their potential role as antimicrobial agents against spoilage and pathogenic microorganisms. Although some of these natural antimicrobials are commercially available and applied in food processing, their efficacy, consumer acceptance and regulation are not well defined. This manuscript reviews natural antimicrobial compounds with reference to their applications in food when applied individually or in combination with other hurdles. It also reviews the mechanism of action of selected natural antimicrobials, factors affecting their antimicrobial activities, and future prospects for use of natural antimicrobials in the food industry.

INTRODUCTION

Increased demand for healthy foods has led to many changes in the quality and safety of foods and in the current conformation of food ingredients. Processed foods with minimal food additives and thermal treatment are an increasing trend among consumers. These consumer demands have led to the exploitation of alternative food processing and preservation techniques. The natural antimicrobials are readily available from various natural sources, such as plants, animals, and microorganisms, in which they constitute part of host defense mechanisms against microbial infections (Brul & Coote 1999). Natural antimicrobial compounds extend the shelf life of food by inhibiting the growth of microbial cells or by killing them. The use of natural antimicrobials as food preservatives could help avoid the excessive physical processing of food to ensure microbial safety, which often alters organoleptic properties of food. Natural antimicrobials, such as essential oils and herbs, are traditionally known for their antimicrobial properties and used in different indigenous practices. As many of these compounds are safe to consume, their application in food as natural preservatives could be a preferred option for many food manufacturers. This review summarizes novel natural antimicrobials that could be potentially applied in food systems, their mechanism of action, various factors that affect their antimicrobial activity, and strategies for hurdle concepts combining natural antimicrobials along with various physical treatments to process food to assure their safety. For the ease of discussion, antimicrobials are categorized into three main categories based on their origin: animal, microorganism, and plant sources.

NOVEL NATURAL FOOD ANTIMICROBIALS AND THEIR APPLICATION

Antimicrobials of Animal Origin

Natural defense systems are reported to exist in animal products, such as milk and eggs, that exhibit strong antimicrobial properties due to well-characterized compounds, such as lactoferrin, lactoperoxidase, and lysozyme. Several polypeptides originating from various animal sources, such as chitosan (CH), megainin, pleurocidin, curvacin A, and spheniscin (Cole et al. 1997, Ganzle et al. 1999a, Tikhonov et al. 2006, Tiwari et al. 2009, Zasloff et al. 1988), are reported to exhibit antimicrobial activity. Many of these polypeptides have been studied for their potential application as food preservatives. In this section, some of the most well-characterized antimicrobials of animal origin are described.

Lysozyme. Lysozyme is a bacteriolytic enzyme widely reported for its application as an antimicrobial in food products, and it is nontoxic to humans. Lysozymes from different sources, such as hens' eggs, have been extensively studied for their potential as natural antimicrobials for food applications. Lysozyme has the ability to hydrolyze the β-1,4 linkage between N-acetylmuramic acid and N-acetyl-glucosamine in the peptidoglycan of microbial cell wall (Losso et al. 2000, Naidu 2003). The cell wall of gram-positive bacteria consists of 90% peptidoglycan, which makes it susceptible to the activity of lysozyme. For example, lysozyme isolated from hens' eggs inhibits the growth of *Clostridium tyrobutyricum* spores in cheese (Scott et al. 1987). Lysozyme has been reported as a strong natural preservative in alcoholic beverages, such as unpasteurized beer at pH 4.7 (Makki & Durance 1996). In a recent study, a commercially available lysozyme preparation (ART FRESH 50/50) was effective in controlling *Listeria monocytogenes* growth during the expected shelf life of raw minced tuna and salmon roe products (Takahashi et al. 2011). Similarly, another commercially available lysozyme product, i.e., Inovapure (Neova^{TM} Technologies, Abbotsford, BC, Canada), extended the shelf life of raw and processed meat and milk products (Tiwari et al. 2009). Modified variants of lysozyme can extend the application of lysozyme as a natural antimicrobial. For example, a heat-denatured lysozyme, which does not possess enzymatic activity but does have antimicrobial activity, has been reported to show antimicrobial activity against a variety of bacteria and fungi (During et al. 1999).

Lactoferrin. Lactoferrin, an iron-binding glycoprotein present in milk, is reported to possess antimicrobial activity against a wide range of bacteria and viruses in addition to other activities, such as regulation of immune function, stimulation of intestinal proliferation, and differentiation and facilitation of iron absorption (Lönnerdal 2011). Lactoferrin has been recently approved for application on beef in the United States (USDA-FSIS 2010) and applied as an antimicrobial in a variety of meat products (del Olmo et al. 2009). The antimicrobial functionality of lactoferrin is attributed to its protein structural conformation (Naidu 2003). Lactoferrin binds with specific targets on the cell surface of gram-negative bacteria leading to outer membrane (OM) damage (Tomita et al. 1994a, 1994b). Hence, the antibacterial activity of lactoferrin is dependent on tight binding with the bacterial cell surface. In an in vitro study on bactericidal efficacy of lactoferrin, its amidated and pepsin-digested derivatives, and their combinations, potent antimicrobial activity was observed against *Escherichia coli* O157:H7 (del Olmo et al. 2011).

The functional activity of lactoferrin is dependent on its isolation from milk sources without denaturation or structural alteration of protein conformation of the lactoferrin molecule. The extraction of purified lactoferrin is essential for its biological activities, as association with compounds such as lipopolysaccharide limits its activities (Lönnerdal 2011). The technology to produce an activated lactoferrin (ALF) that retains functional activity against a wide bacterial range has been reported (Naidu 2003). ALF exhibits various antimicrobial activities, including interference with bacterial adhesion/colonization, inhibition of microbial growth/multiplication, detachment of live or dead microbes from biological surfaces, and neutralization of endotoxins, making it ideal for application in food systems. ALF competes with microorganisms for attachment to tissue-matrix components, such as collagens, in foods and detaches matrix-bound bacteria (Hook et al. 1989). Lactoferrin is used in a variety of commercial products, including human infant formulas and sports beverages, and as a dietary supplement with claims of improved immune system function. Activin, the commercial ALF (ALF Ventures, Salt Lake City, Utah), is approved by the USDA's Food Safety and Inspection Service (FSIS) for use in beef carcass rinses. The presence of salt and other physiological factors can inhibit the activity of lactoferricins, hence limiting their universal application.

Similar to lactoferrin, lactoperoxidase is another antimicrobial system that originated from milk and is reported to be effective against gram-negative bacteria (de Wir & van Hooydonk 1996). Edible antimicrobial coatings containing polypeptides, such as lysozyme, peroxides, and lactoferrins, have been prepared for food packaging. These edible coatings can extend the shelf life of food products and make them safer for human consumption, in addition to providing the foods physical protection (Franssen & Krochta 2003).

Ovotransferrin. Ovotransferrin or conalbumin is a glycoprotein of egg white albumin that contributes to antimicrobial defense systems of hens' eggs (Naidu 2003). Ovotransferrin belongs to a group of proteins known as metalloproteinases and has a high affinity for iron, thereby rendering iron unavailable for bacterial metabolism. This subsequently inhibits bacterial growth due to iron deprivation (Valenti et al. 1987). The peptide OTAP-92 is responsible for the bactericidal activity of ovotransferrin (Ibrahim 2000). The purified form of this peptide has strong bactericidal activity against gram-positive and gram-negative bacteria, such as *Staphylococcus aureus* and

E. coli, respectively (Ibrahim 1997, 2000). Purified ovotransferrin is commercially available (Ovoproducts, Neova^{TM} Technologies). The broad antibacterial activity of ovotransferrin has been reported against several foodborne pathogens and spoilage bacteria, including *Pseudomonas* spp., *E. coli*, *Proteus* spp., and *Klebsiella* spp. (Naidu 2003, Valenti et al. 1983). In addition to ovotransferrin, eggshell matrix proteins also have a variable degree of antimicrobial activity against a broad range of spoilage and pathogenic bacteria, including *Pseudomonas aeruginosa*, *Bacillus cereus*, *S. aureus*, *E. coli* and *Salmonella* Enteritidis (Mine et al. 2003). Egg white is widely known for its allergic reactions, which could be a potential detriment to the application of ovotransferrin for food preservation.

Chitosan. CH is a polycationic biopolymer naturally present in the exoskeletons of crustaceans and arthropods. A collective group of all partially and fully deacetylated chitinous compounds is known as CH [poly (β-1, 4)-2-amino-2-deoxy-D-glucopyranose] (Tikhonov et al. 2006). CH has a wide spectrum of antimicrobial activity against bacteria and fungi, and is safe for antimicrobial applications because of its low toxicity against mammalian cells (Franklin & Snow 1981, Kong et al. 2010). CH has become increasingly popular for its applicability in innovative food processing techniques and considered safe as a food additive by countries such as Korea and Japan (KFDA 1995, Kong et al. 2010). Owing to low water solubility, CH is solubilized in organic acids, such as acetic or lactic acid, for use in food processing applications (No et al. 2007). However, the acidic solution may cause hydrolysis and chain depolymerization of CH molecules, thereby diminishing its antimicrobial activity. Modified water-soluble derivatives that constitute a stable CH structure in different solvents have been described. For example, a water-soluble CH derivative known as glucosyloxyethyl acrylated CH has been synthesized using the Michael Addition reaction of CH with glucosyloxyethyl acrylate (Wang et al. 2011). Low-molecular-weight CHs at a pH less than 6.0 can provide effective antimicrobial preservative activities in liquid and solid foods (Friedman & Juneja 2010). CH can be used to improve both the quality and shelf life of food. For example, adding CH to freshly made noodles extended their shelf life by six days at 4°C. Further, when Maillard reaction products prepared from CH and xylose were used, the shelf life was extended to 14 days at 4°C (Huang et al. 2007). Similarly, a CH coating can be used to reduce bacterial contamination of egg contents resulting from *trans*-shell penetration by *S.* Enteritidis and other bacteria, such as *Pseudomonas* sp., *E. coli*, and *L. monocytogenes* (Leleu et al. 2011).

In addition to direct application as a natural food additive or preservative, CH can be used in antimicrobial food packaging as a film-based food preservative. By integrating with polymeric material of high mechanical strength, CH film presents a highly durable packaging material with antimicrobial activity (Moller et al. 2004, Zivanovic et al. 2007). For example, the intrinsic antimicrobial properties of CH were combined with the thermoplastic and film-forming properties of sodium caseinate (SC) to prepare SC-CH film-forming solutions and films that were effective in reducing the native microflora of cheese, salami, and carrots. There was significant reduction (2.0 to 4.5 log CFU g^{-1}) in populations of mesophilic and psychrotrophic bacteria, and yeasts and molds when CH and SC/CH was applied as either an immersant or wrapper (del Rosario Moreira et al. 2011). Further, CH-based edible antimicrobial films can be prepared in combination with other components, such as essential oils, CH-glucomannan-nisin blends, and CH-starch blends (Du et al. 2008), that could be attractive alternates for inedible and nonbiodegradable packaging materials. CH-based films also have antimicrobial activity against a wide spectrum of food pathogens, including *L. monocytogenes* and *E. coli* O157:H7 when adherent to the surface of meat products (Dutta et al. 2009).

Pleurocidin. Pleurocidin is present in myeloid cells and mucosal tissues of many vertebrates and invertebrates, and provides a natural defense system that reduces the mortality of fish infected with pathogenic microorganisms (Jia et al. 2000). Cole et al. (1997) isolated a novel 25-residue linear polypeptide from the skin mucus secretion of the winter flounder (*Pleuronectes americanus*) that possesses antimicrobial activity against a broad range of gram-negative and gram-positive bacteria. Yoshida et al. (2001) reported the significance of structural conformation (α-helicity) that imparts antimicrobial activity to this peptide. On attaching to a bacterial cell, pleurocidin forms pores in its lipid membrane. Pleurocidin has antimicrobial activity against several foodborne bacteria, such as *L. monocytogenes* and *E. coli* O157:H7, and pathogenic fungi (Burrowes et al. 2004, Jung et al. 2007). Pleurocidin is heat-stable, salt-tolerant, and noncytotoxic to human cells; however, the functional activity of pleurocidin is inhibited by divalent ions, such as magnesium and calcium (Cole et al. 2000), which can be a deterrent to its use in food applications. The high cost of chemically synthesizing pleurocidin or isolating it from the winter flounder may be prohibitive for large-scale applications. However, recombinant expression of pleurocidin cDNA has been reported for large-scale production and purification (Burrowes et al. 2005).

Protamine. Protamine is composed of cationic antimicrobial peptides naturally present in salmon and has broad antimicrobial activity against gram-positive bacteria, gram-negative bacteria, and fungi (Uyttendaele & Debevere 1994). The antimicrobial activity of protamine is likely due to its electrostatic affinity to the negatively charged cell envelopes of actively growing bacteria. However, modified protamine, which has a reduced charge, can inhibit the growth of *L. monocytogenes* in milk as well as total bacteria and coliforms in ground beef significantly better than native protamine, indicating that reduced-charge protamine is more inhibitory than protamines in foods with high protein matrices (Potter et al. 2005). Hansen et al. (2001) studied the antimicrobial activity of protamine (clupeine) against a broad range of gram-positive, gram-negative, and spoilage bacteria and determined it to be effective against *Brochothrix thermosphacta* and nonproteolytic *Clostridium botulinum* strains. Alkaline pH increased the level of antimicrobial activity of protamine against *E. coli*, which may be due to an increase in electrostatic affinity for the cell surface of target cells (Hansen & Gill 2000). The nonspecific binding of protamine to negatively charged food particles could be a detriment to its use in food.

Similarly, magainins, peptides isolated from the skin of frogs such as *Xenopus laevis* (Zasloff 1987), possess antimicrobial activity due to its ability to permeabilize microbial cell membranes (Matsuzaki et al. 1998). Expression of a magainin-type antimicrobial peptide gene (MSI-99) in tomatoes enhanced their resistance to *Pseudomonas syringae* pv. *tomato*, a spoilage bacterium (Alan et al. 2004). Such strategies that provide continuous and high expression of antimicrobial peptides in plant tissues could be beneficial in enhancing plant resistance to disease-causing and spoilage organisms. Magainin could also have application as a preservative in different foods (Zasloff 1987). For example, magainin II amide and defensins were inhibitory in vitro to *Bacillus anthracis* Sterne and *B. cereus* ATCC 7004 (Montville et al. 2006).

Defensins. Defensins are cationic peptides that have broad-spectrum antimicrobial activity. For example, a β-defensin from a pituitary cDNA library of a protogynous hermaphroditic orange-spotted grouper (*Epinephelus coioides*) was cloned, and in vitro as well in vivo antibacterial activity was observed against gram-negative bacteria. Further, a recombinant β-defensin construct had antiviral activity in cell culture (Jin et al. 2010). Defensins are naturally found in many animals and have broad-spectrum antimicrobial activities against fungi, viruses, and gram-positive and gram-negative bacteria (Dhople et al. 2006, Jin et al. 2010, Montville et al. 2006).

Lipids. Various lipids of animal origin, such as free fatty acids isolated from mucosal surfaces of animal (Bibel et al. 1989) and milk lipids (Isaacs et al. 1990), possess natural antimicrobial properties against a broad range of bacteria and fungi. For example, caprylic acid, a natural eight-carbon fatty acid present in breast milk and bovine milk that is considered GRAS (generally recognized as safe) by the U.S. Food and Drug Administration (FDA) can inactivate *Enterobacter sakazakii* in reconstituted infant formula (Nair et al. 2004). Similarly, lipids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), originating from fish and shellfish have broad-spectrum antimicrobial activities. The bioconverted extracts of EPA and DHA have antimicrobial activity against a wide spectrum of gram-positive and gram-negative bacteria, including *S.* Enteritidis, *Salmonella* Typhimurium, *E. coli* O157:H7, *L. monocytogenes*, and *S. aureus* (Shin et al. 2007). Although EPA and DHA have similar antibacterial activity against gram-positive bacteria, DHA is more effective against gram-negative bacteria than EPA.

Antimicrobials of Microbial Origin

Many microbial metabolic products act as growth inhibitors against other microorganisms, including spoilage and pathogenic bacteria (Daeschel 1989). Many gram-positive bacteria often produce cationic, amphiphilic, membrane-permeabilizing peptides that are smaller in size and have antimicrobial activity against a wide range of microorganisms. For example, *Lactobacillus* spp. produce one of the groups of antimicrobial peptides known as bacteriocins that can exhibit potent antimicrobial activity. The antimicrobial activities of bacteriocins are due to a heterologous subgroup of ribosomally synthesized cationic peptides (de Vugst & Vandamme 1994a). Various classification schemes have been proposed for bacteriocins (Tiwari et al. 2009). For example, in one such classification, bacteriocins are named after the genus, species, or family of bacteria producing them, such as lantibiotics for the bacteriocins produced by *Lactobacillus* spp. and colicins for bacteriocins from *E. coli* (Riley & Chavan 2006). Other classification schemes for bacteriocins include the mechanism by which the bacteriocin is produced (ribosomal and nonribosomal) and mechanisms by which bacteriocins kill microbes (pore formation, nuclease). One classification scheme divides bacteriocins into I to V classes (Tiwari et al. 2009). The class I bacteriocins consist of small peptides, such as lantibiotics, and other modified bacteriocins, lanthionines, and β-lanthionines (Guder et al. 2000, Jack et al. 1995), such as nisins A and Z, lacticin 481, lactocin S, and lacticin 3147. Nisin is one of the most studied bacteriocins and is produced by many strains of *Lactococcus lactis*. It has been approved by the FDA for use as a food preservative for certain applications. The class II bacteriocins are small heat-stable peptides, which are further divided in to Classes IIa, IIb, and IIc. Class IIa bacteriocins include pediocins, which are well-known food preservatives owing to their antilisterial activities (Nes & Holo 2000). Most of the bacteriocins of interest for food preservation are in class I or II. The class III bacteriocins contain the heat-labile peptides, and those in class IV are complex molecules with lipid and carbohydrate moieties. Bacteriocins are potent antimicrobial peptides that can inhibit pathogenic and food-spoilage bacteria. By themselves, bacteriocins are usually ineffective against gram-negative bacteria because they cannot penetrate the bacterium's OM. It is therefore necessary that bacteriocins are used in combination with other preservation methods (e.g., other antimicrobials and organic acids) to kill gram-negative bacteria. However, cyclic bacteriocins produced by *Carnobacterium maltaromaticum*, such as carnocyclin A (CclA) and carnobacteriocin BM1 (CbnBM1), can inhibit the growth of some gram-negative bacteria, such as *E. coli*, *P. aeruginosa*, and *S.* Typhimurium (Martin-Visscher et al. 2011). Disruption of the OM of some gram-negative bacteria can sensitize them to certain bacteriocins.

Nisin. Nisin is a ribosomally synthesized polycyclic peptide that contains unusual amino acids, including lanthionine, methyllanthionine, didehydroalanine, and didehydroaminobutyric acid (Hansen 1994). Nisin is commercially produced by fermentation of modified milk medium using several strains of nisin-producing *L. lactis* and is an effective antimicrobial against many gram-positive bacteria (Branby-Smith 1992). The antibacterial activity of nisin is attributed to its interaction with phospholipids in the cytoplasmic membrane of bacterial cells resulting in disruption of normal membrane function. It also inhibits spore germination, hence preventing spore outgrowth. Nisin has antimicrobial activity against some gram-negative spoilage and pathogenic bacteria, such as *E. coli*, *P. aeruginosa*, and *S.* Typhimurium (Martin-Visscher et al. 2011). Nisin may be used as a food preservative in some food products, such as meat, juices and other beverages, and cheese, to protect them from foodborne pathogens and extend product shelf life. For example, nisin was applied to various ready-to-eat meat products to suppress *L. monocytogenes* (Mahadeo & Tatini 1994), to Sucuk dough to control *S. aureus* during fermentation and subsequent storage (Hampikyan 2009), and to orange juices to control *Alicyclobacillus acidoterrestris* to extend shelf life (Peña & de Massaguer 2006). In raw and processed meat, nisin can control pathogenic and spoilage organisms, such as *Brochothrix thermosphacta* and *Carnobacterium* sp. in pork tissue (Nattress et al. 2001), *L. monocytogenes* in ready-to-eat turkey ham (Ruiz et al. 2010), lactic acid bacterial spoilage in vacuum-packed bologna-type sausage (Davies et al. 1999), and *L. monocytogenes* in fresh beef (Zhang & Mustapha 1999).

Nisin can be applied in different forms to control pathogens in food. For example, anovesicle-encapsulated nisin in combination with low temperatures was applied to effectively control *L. monocytogenes* in milk (da Silva Malheiros et al. 2010). Similarly, nisin in combination with high pressure homogenization was effective in obtaining a 5-log reduction of *E. coli* in apple and carrot juices (Pathanibul et al. 2009). Results of studies combining nisin with biopolymers to form biodegradable antimicrobial food packaging film have been widely reported. For example, nisin incorporated into biodegradable polylactic acid polymer film effectively controlled foodborne pathogens, including *L. monocytogenes*, *E. coli* O157:H7, and *S.* Enteritidis, in culture media and in liquid foods, such as orange juice and liquid egg white (Jin & Zhang 2008).

Overall, nisin is being applied in countries either alone or in combination with other compounds as a food preservative. However, the ineffectiveness of nisin against gram-negative bacteria and fungi restricts its application as a broad-spectrum lantibiotic.

Reuterin. Some strains of *Lactobacillus reuteri* in the presence of glycerol produce a broad-spectrum antimicrobial substance known as reuterin. Reuterin is effective against many pathogenic and spoilage microorganisms. For example, reuterin has bacteriostatic activity against *L. monocytogenes* and variable bactericidal activities against *S. aureus*, *E. coli* O157:H7, *S.* Choleraesuis, *Y. enterocolitica*, *Aeromonas hydrophila* subsp. *hydrophila*, and *Campylobacter jejuni* (Arqués et al. 2004). Reuterin has been evaluated as a biopreservative in various meat and dairy products, and it was found to be effective in reducing *L. monocytogenes* and *E. coli* O157:H7 populations in inoculated cooked pork (El-Ziney et al. 1999) and *L. monocytogenes* in beef sausage (Kuleaşan & Cakmakçi 2002). Similarly, in another study, reuterin had inhibitory or bactericidal activity to *L. monocytogenes* and *E. coli* O157:H7 in milk and cottage cheese (El-Ziney & Debevere 1998). Reuterin in combination with other natural antimicrobials may be applied as an effective biopreservative at low concentrations. For example, reuterin in combination with nisin and lactoperoxidase effectively inactivated *L. monocytogenes* and *S. aureus* in cuajada, a semisolid dairy product (Arqués et al. 2008). Overall, reuterin could be an ideal candidate for antimicrobial application to foods because it is active over a wide range of pH values and resistant to a variety of proteolytic and lipolytic enzymes commonly present in foods.

Pediocin-like bacteriocins and cystibiotics. The pediocin-like bacteriocins (36-48 residues) are produced by many lactic acid bacteria, which share a 40% to 60% amino acid sequence similarity and have a well-conserved hydrophilic N terminus. Pediocin-producing strains mainly include *Pediococcus acidilactici*, which is commonly associated with fermentations of vegetables and meat-based products, *Pediococcus pentosaceus*, which is associated mainly with fermentation processes in the brewing industry and as starter cultures in sausage fermentations, and *Pediococcus damnosus* (Papagianni & Anastasiadou 2009). The electrostatic interactions of the cationic patch of the N-terminal β-sheet-like region of pediocin AcH/PA-1 govern the binding of the pediocin and its fragments to phospholipids vesicles on microbial cells (Chen et al. 1997). Pediocins are thermostable peptides that are antimicrobial compounds over a wide range of pH values. Pediocins have random coil structures in water and assume a defined conformation only in hydrophobic environments or in solvents.

Pediocins are one of the most extensively studied and well-characterized class IIa bacteriocins that are included as GRAS additives in certain food applications. Pure or mixed cultures of pediocin-producing strains may be used as protective cultures against food spoilage and pathogenic bacteria as well as to improve food quality and sensory attributes. Pediocins, such as Alta 2341®, are commercially available for application as biopreservatives in foods. Purified pediocin PA-1/AcH has antimicrobial activity against various pathogenic and spoilage bacteria (Kalchayanand et al. 1992, Ray & Daeschel 1992, Ray & Miller 2000). The antibacterial potency and spectrum of activity of pediocin against gram-positive bacteria vary considerably. These variations in antimicrobial activities could be attributed to differences in their structural conformation and affinity to targets on the cell surface (Ennahar et al. 2000). In addition to pediocins, other cystibiotics, such as sakacin-A, sakacin-P, and enterocin-A, also have antimicrobial properties against a variety of bacterial targets (Eijsink et al. 1998). Cystibiotics bind to anionic molecules on the microbial cell wall and form ion conductance pores in the cytoplasmic membrane. Cell lysis can occur because of prolonged exposure of microbial cells to certain cystibiotics after the induction of autolytic enzymes within the cell wall.

Bactericidal potency and the activity spectrum against gram-positive and gram-negative bacteria can be enhanced by the synergistic effect of using bacteriocins, such as pediocin PA-1/AcH in combination with nisin A, lysozyme, organic acids, sodium dodecyl sulfate or ethylenediaminetetraacetic acid (EDTA) (Hanlin et al. 1993, Kalchayanand et al. 1992, Ray & Miller 2000). Additional strategies using bacteriocins can be applied to control the growth of spoilage and pathogenic bacteria. For example, the bacteria-producing bacteriocins can be used as starter cultures in fermentation processes to simultaneously generate antimicrobial compounds to control undesirable microorganisms. For instance, pediocin-producing cultures used for the fermentation of sausages and cheddar cheese can control the growth of *L. monocytogenes* (Buyong et al. 1998). Similarly, cystibiotic-producing bacteria added in food products before packaging controls the growth of undesirable microorganisms in foods during storage. For example, pediocin PA-1/AcH-producing strains applied to frankfurters, fresh chicken, and Munster cheese control the growth of *Listeria* spp. during storage (Degnan et al. 1992).

In addition to well-established biopreservative and antimicrobial activities, the costly production and instability of several pediocin-like bacteriocins due to methionine residues, whose sulfur atom may be oxidized, hinder their routine use. However, the methionine could be replaced with another hydrophobic residue to develop more structurally stable pediocins that retain their antimicrobial activity (Papagianni & Anastasiadou 2009).

Natamycin. Natamycin is an antifungal compound produced by *Streptomyces natalensis*. Owing to the amphiphilic nature of its molecule, natamycin has less solubility in water than bacteriocins.

The dry powder of natamycin is stable for a long period of time without substantial loss of activity. Natamycin has several characteristics that make it an ideal broad-spectrum antifungal biopreservative for foods and beverages: It is safe for consumption, is effective at low concentrations, has no effect on quality of foods, and has prolonged antimicrobial activity specifically on food surfaces, such as cheese and sausages (Stark 2003). Natamycin binds irreversibly to the cell membrane of fungi because of its high affinity for ergesterol. This causes membrane hyperpermeability leading to rapid leakage of essential ions and peptides and ultimately cell lysis (Teerlink et al. 1980). As bacterial membranes do not contain sterol, natamycin is not effective against bacteria. This makes natamycin a suitable antimicrobial during bacterial ripening and fermentation processes for cheese and sausage formation. The dissolved fractions of natamycin do not deeply penetrate into food products (Daamen & van den Berg 1985). Natamycin crystals remain on the surface of food products, making it suitable for the surface treatment of cheese and sausage without inhibiting the inside microflora that give texture and flavor to these food products (Morris & Castberg 1980). For the surface treatment of cheese, natamycin is added to the aqueous polymer dispersion (plastic emulsion) for coating the cheese rind, or it can be applied by dipping or spraying (Stark 2003). Natamycin has been used as an antifungal compound during the processing of various kinds of cheese, such as cheddar (Luck & Cheesman 1978) and blue cheese (Morris & Castberg 1980). Natamycin is widely applied as an antifungal agent in the dipping or spraying of sausage during its normal aging and storage (Holley 1981). Natamycin can be applied as an antifungal dip or spray to prevent fungal growth on fruits, such as strawberries, cranberries, and raspberries, and extend the shelf life of berries by several days (Ayres & Denisen 1958). In a recent study, natamycin was used to successfully inhibit the growth of yeast and molds during natural black olive fermentation (Hondrodimou et al. 2011).

Natamycin is suitable for use in beverage products before their packaging, as it can control the mold growth, remains stable in beverages during prolonged storage at refrigeration temperature, is effective at low concentrations, and is not affected by a wide range of pH values (Shirk & Clark 1963). Hence, it can be an effective antifungal for alkaline and acidic beverages.

Antimicrobials of Plant Origin

Plant parts, such as herbs and spices, which are historically used in foods to add flavors and fragrances, are well known for their antimicrobial activities (Nychas & Skandamis 2003). The products derived from plant parts, specifically essential oils, contain active ingredients that may act as antimicrobial compounds against bacteria, yeast, and molds. Although most essential oils from herbs and spices are classified as GRAS substances, their use as preservatives in foods is limited because of flavor considerations (Kabara 1991). The dose needed for effective antimicrobial properties activity in foods often exceeds their organoleptic acceptance level. The major groups of principal components that make essential oils effective antimicrobials include saponins, flavonoids, carvacrol, thymol, citral, eugenol, linalool, terpenes, and their precursors. Essential oils are derived from various plant parts, such as leaves of basil and tea plants, bulbs of garlic and onion, clove buds, seeds of parsley, and fruits, rhizomes, and other plant parts (Nychas & Skandamis 2003). Plant parts rich in essential oil content can have stronger antimicrobial activity. For example, spices with a large concentration of eugenol and cinnamon bark with large concentrations of cinnamic aldehyde have strong antimicrobial activity (Davidson & Naidu 2000). A variety of phytophenols are present in plants, such as thymol in thyme; vanillin in vanilla, cinnamic acid in brassica oil seeds; vanillin, benzoic acid, coumarin, and other phytophenols in spices; sesamol in sesame oil; cinnamic acid and eugenol in cinnamon; eugenol in cloves; and terpene in sage and rosemary (Davidson & Naidu 2000). Essential oils have a broad range of antimicrobial activity against a variety of bacteria and fungi (Tiwari et al. 2009). For example, essential oils from basil retard the growth of *Pseudomonas fluorescens* and *Aeromonas hydrophila* (Wan et al. 1998). Similarly, *Salmonella*, *B. cereus*, *E. coli*, and *Enterobacter aerogenes* are inhibited by the essential oils of marjoram and basil (Gutierrez et al. 2008). Mint (*Mentha piperita*) essential oil inhibits the growth of *S.* Enteritidis and *L. monocytogenes* in pure cultures (Tassou et al. 1995). However, there is variability in the ability of mint essential oil to inhibit the growth of these pathogens in foods, such as taramasalata.

Thymol and carvacrol have antimicrobial activity against a broad range of foodborne pathogens, such as *L. monocytogenes*, *S.* Typhimurium, and *Vibrio parahaemolyticus* (Karapinar & Aktug 1986, Ting & Diebel 1992). Similarly, cinnamon essential oil, which is rich in cinnamic aldehyde, exhibits antimicrobial activity against a broad spectrum of bacteria, such as *C. jejuni*, *L. monocytogenes*, and *S.* Enteritidis (Smith-Palmer et al. 1998). Clove essential oil is rich in eugenol (Deans et al. 1995, Smith-Palmer et al. 1998). Eugenol inhibits *L. monocytogenes* in cooked beef (Hao et al. 1998), *A. hydrophila* in vacuum-packaged and air-packed cooked pork (Stecchini et al. 1993), and *Aspergillus* and *Penicillium* on Sabouraud dextrose agar (Mansour et al. 1996). Tea-tree oil also has potent antimicrobial activity owing to various active components, such as terpinen-4-ol, α-terpinene, γ-terpinene, α-pinene, and α-terpineol (Davidson & Naidu 2000). It is also a broad-spectrum antibacterial agent, likely because of its ability to disrupt microbial cytoplasmic membranes (Cox et al. 1998).

Saponins are naturally occurring glycosides in many plants, such as *Solanum* and *Allium* spp., oats, soya, clover, and a variety of herbs and seeds. Saponins interact with sterols and fatty acids on microbial membranes (Davidson & Naidu 2000). Antimicrobial activities of saponin have activity against a wide range of microorganisms, such as *P. fluorescens*, *E. coli*, and *Salmonella* Typhi (Davidson & Naidu 2000, Oleszek et al. 1999). In a recent study, quillaja saponin–rich extracts had antibacterial activity against *S. aureus* (Hassan et al. 2010). Saponins also have significant antifungal activity that depends on the structural features of saponin, specifically its aglycone structure. Saponins have a wide range of antifungal activity against fungal species, such as *Aspergillus* spp. (Jadhav et al. 1981). Two plant sources of saponin, *Quillaja saponaria* and *Yucca schidigera*, are regarded as GRAS and allowed for use in food and beverages as per FDA guidelines. Although saponins are mainly used as emulsifiers and flavoring agents in food and beverages, they have potential for use as natural antimicrobials and preservatives for foods.

Flavonoids are bioactive compounds that are found in the form of pigments in plant parts, such as fruits and flowers. They include groups of yellow-colored compounds with a flavone moiety, less-colored flavanones, colorless flavon-3-ols, and other flavonoids, such as red and blue anthocyanidins. The antimicrobial activity of flavonoids is largely due to their ability to penetrate biological membranes (Davidson & Naidu 2000). They are antimicrobial to a variety of bacteria, such as *Pseudomonas* spp. and *S. aureus* (Locher et al. 1995). Similarly, water-soluble extract from pine needles of *Cedrus deodara*, which is rich in phenolic compounds and flavonoids, has a broad spectrum of antimicrobial activity against gram-positive and gram-negative bacteria (Zeng et al. 2011). Flavonoids also have potent antifungal activity against a variety of fungi, including *Aspergillus* spp. and *Penicillium* spp. (Kramer et al. 1984).

Many of the antimicrobial components of essential oils are naturally produced because of the enzymatic activity of plants on inactive precursors inside plants to boost the plant defense system during adverse conditions, including stress (Sofos et al. 1998). For example, glucosinolates in mustard and horseradish are converted into a variety of isothiocyanates by the enzyme myrosinase. Allyl isothiocyanates have strong antimicrobial properties (Delaquis & Mazza 1995). The antimicrobial activity of essential oils depends on the structural conformation of the active components and their concentration.

Edible coatings containing essential oils can be prepared for food packaging. These coatings slowly diffuse antimicrobial activity inside the food packages, hence maintaining for a longer duration the preservative concentration. For example, thyme oil and *trans*-cinnamaldehyde added to soy and whey protein coating over shrimp extended the shelf life of the shrimp by 12 days at 4°C under aerobic conditions (Quattara et al. 2000).

ANTIMICROBIAL ACTIVITY OF NATURAL ANTIMICROBIALS

Mechanisms of Action of Selected Natural Antimicrobials

Natural compounds of animal, plant, and microbial origins have different mechanisms of action for their antimicrobial activities. Various mechanisms and theories have been proposed for the antimicrobial activities of natural antimicrobials. The mechanism of action of many natural antimicrobials is not well defined, hence only the mechanism of action of selected well-studied antimicrobial compounds is described in this section, including metal-binding peptides, such as lactoferrin, hydrolytic proteins, such as lysozyme, bacteriocins, such as nisin and pediocin, and a natural antifungal agent, such as natamycin.

The metal-binding proteins of animal origin, such as milk lactoferrin and egg phosvitin, deprive the target microorganism of essential multivalent metals, such as iron (Baron et al. 1999). The unavailability of iron leads to disruption of essential growth and survival mechanisms in bacteria. The iron-chelating capability of lactoferrin in the presence of anions, such as bicarbonate, leads to nutritional deprivation and consequently inhibition of microbial growth. This was evident from a study in which lactoferrin markedly inhibited the growth of *B. cereus* until the effect was reversed by $FeCl_3$ supplementation (Sato et al. 1999). The carbohydrate moiety in lactoferrin variants blocks the adhesion of enteric pathogens disrupting their in vivo colonization (Izhar et al. 1982). Similarly, basic residues near the N terminus of lactoferrin provide antimicrobial activity by binding to the surface components of microbial cells (Tomita et al. 1994a, 1994b).

Hydrolytic proteins, such as lysozymes and chitinases, degrade the key structural components of the cell wall of bacteria and fungi (Fuglsang et al. 1995). The amino acid constituents, such as Asp52 and Glu35, in lysozyme promote catalysis by inducing steric stress in the substrates on microbial cells (Imoto 1996). The cell wall of gram-positive bacteria, which are made up of approximately 90% peptidoglycan, is more susceptible to lysozyme than gram-negative bacteria because lysozyme catalyzes the degradation of the 1,4 β-D-linkage between N-acetylhexosamines in the peptidoglycan layer of the microbial cell wall. Cell death is a consequence of a cascade of events, including osmotic uptake of excessive water followed by expansion and eventual rupture of the cytoplasmic membrane. Another mechanism for antimicrobial activity of lysozyme is related to membrane disruption, leading to induction of cellular autolysis due to intake of autolytic enzymes. Lysozyme activity may also produce porin channels, a specific class of major protein in planar lipid membrane through which lysozyme enters the outer membrane en route to the periplasm (Ohno & Morrison 1989). These activities of lysozyme vary depending on the target microbe and molecular conformation of the lysozyme.

Bacteriocins, such as nisin, act on vegetative bacterial cells by a process of binding, insertion, aggregation, and pore formation (Giffard et al. 1997). Nisin binds to anionic phospholipids on the microbial membrane by electrostatic interactions and inserts into the microbial membrane through its hydrophobic patches (Breukink et al. 1997). The pores that are formed lead to the leakage of small molecules, such as potassium ions, metabolites, such as amino acids and ATP, and other solutes (Giffard et al. 1997). This may further lead to energy deprivation and vital functional losses related to cellular metabolism and biosynthesis (Ruhr & Sahl 1985). Nisin can also interfere with cell wall synthesis by inhibition of N-acetylglucosamine synthesis (Henning et al. 1986a, 1986b; Linnett & Strominger 1973) and induction of cellular autolysis (Ruhr & Sahl 1985) of vegetable bacterial cells. Nisin also prevents the postgermination swelling and subsequent outgrowth of spores, thereby exhibiting activity against endospores (de Vuyst & Vandamme 1994a,b).

The adsorption of another bacteriocin known as pediocin PA-a/AcH on the bacteria cell wall leads to impairment of cytoplasmic membrane function and leakage of essential intracellular small molecules, resulting in cell death (Bhunia et al. 1991). Targets for the attachment of pediocin to bacterial cells include lipoteichoic and teichoic acid, which are absent in gram-negative bacteria. Cell membrane damage leads to the loss of membrane potential and protons, thereby inhibiting glucose transport and resulting in cell death (Christensen & Hutkins 1992). The inhibition of bacterial growth by pediocin PA-1/AcH-induced cell lysis has also been reported in *L. monocytogenes* (Pucci et al. 1988).

Reuterin is considered an analog of D-ribose, which is capable of inhibiting DNA synthesis by inhibiting the ribonucleotide reductase activity required for the production of deoxyribonucleotides (Talarico & Dobrogosz 1989). The inhibition of ribonucleotide reductase activity could explain the broad spectrum antimicrobial activity of reuterin. Similarly, the antifungal compound natamycin binds irreversibly on the cell membrane of fungi because of its high affinity for ergesterol. This causes membrane hyperpermeability leading to rapid leakage of essential ions and peptides and cell lysis (Teerlink et al. 1980).

When applied in food matrices, several factors, such as physical and chemical properties of food, could affect the antimicrobial activity of natural antimicrobials; some of the most-studied factors are discussed in the next section.

Factors Affecting Antimicrobial Activity of Natural Antimicrobials

The activity of natural antimicrobial depends on a variety of factors that regulate structure-function properties of antimicrobial agents. The activity of an antimicrobial system in food depends on the complexity and/or biochemical composition of the food in which the antimicrobials are applied. The physical properties of food, such as pH, temperature, and water activity, could affect the potency of antimicrobials. Ionic conditions or pH can interfere with the ionic charge of antimicrobials and affect their interaction with microbial targets (Naidu 2003). For example, the bactericidal activity of cationic antimicrobials, such as lactoferrin, is higher in acidic food environments (Salamah & al-Obaidi 1995). This may be due to enhanced antimicrobial access to the cytoplasmic membrane components in an acidic environment. Similarly, the efficacy of essential oils in foods depends on several factors, such as their concentration, pH of the food, food composition, storage temperature, and the nature and behavior of the microorganism (Tassou et al. 1995). For example, the antibacterial activity of oregano essential oil against *S.* Enteritidis in homemade taramasalata is affected by factors such as pH of food, storage temperature, and concentration of essential oils (Koutsoumanis et al. 1999).

Food substances, such as proteins, proteases, lipids, salts, and metal ions, can interfere in the interaction of antimicrobials with their target pathogen either by reacting to the antimicrobials directly or by interacting with the cellular target(s) for antimicrobials (Touch et al. 2009). Proteases present in food can digest antimicrobial peptides, thereby affecting their antimicrobial properties. For example, lactoferrin is degraded by cell-associated proteases binding with receptors on *Porphyromonas gingivalis* (de Lillo et al. 1996). The proteases, which are often abundantly present in foods, can degrade the activity of antimicrobials, such as bacteriocins and antimicrobial peptides (Aasen et al. 2003). Similarly, excessive iron in food components can inactivate lactoferrin by saturating its iron-binding moiety (Rogers & Synge 1978). Food components, such as certain lipids and proteins, can nonspecifically bind to bacteriocins, affecting the hydrophobic properties needed for binding to bacterial targets. Thiol compounds, such as glutathione, that are abundantly present in animal tissue and plants can inhibit the activity of the lactoperoxidase system and nisin either by binding to them directly or by scavenging their oxidation products (Heuvelink et al. 1999).

Many traditionally used food preservatives can degrade natural antimicrobials when they are applied in food treated with these preservatives. For example, sodium metabisulfite can degrade nisin (Delves-Broughton et al. 1996). The antimicrobial activity of nisin is inhibited in the presence of certain proteases, strong anionic detergents, divalent metal ions and oxidizers (Naidu 2003). Various fats present in foods can affect the antimicrobial efficacy of essential oils in foods. For example, the antimicrobial activity of essential oils of bay, clove, cinnamon, and thyme is either significantly or totally lost against *L. monocytogenes* in the presence of low- or full-fat soft cheese (Smith-Palmer et al. 2001).

Factors such as packaging material and packaging conditions can also affect the antimicrobial activity of essential oils in solid foods. For example, the effect of aerobic, modified atmosphere packaging (MAP) (40% CO_2/30% O_2/30% N_2) and vacuum packaging (VP) on the growth/survival of *L. monocytogenes* on sterile and naturally contaminated beef meat fillets was studied in relation to film permeability and oregano essential oil. The addition of 0.8% (v/w) oregano essential oil resulted in an initial reduction of most of the bacterial population in all gaseous environments, whereas limited growth was observed in aerobic packaging, and survival or death of *L. monocytogenes* was observed in MAP/VP packaging, regardless of film permeability (Tsigarida et al. 2000). The antimicrobial application methods, such as dipping, spray, and antimicrobial coatings, can also affect the antimicrobial activity of natural antimicrobials.

Many compounds can inhibit the activity of natural antimicrobials. Irreversible inactivation of lactoperoxidases is reported in the presence of excess H_2O_2 (Jenzer & Kohler 1986). Similarly, fluoride ions (F^-) inactivate lactoperoxidase (Hannuksela et al. 1994). The activity of antimicrobial lipids, such as lactolipids, is inhibited in the presence of proteins, such as albumin (Shibasaki & Kato 1978), and surface-active agents, such as cholesterol (Ammon 1985). The negatively charged proteins in foods, such as casein and bovine serum albumin, and lipids, such as milk fats and fatty acids, can interfere with the electrostatic and hydrophobic interactions needed for the antimicrobial activity of peptides, lysozyme, and bacteriocins on target microbes (Hughey & Johnson 1987). Metal cations, such as Na^+, Fe^{2+}, and Ca^{2+}, that are commonly present in foods can bind with the anionic constituents of the microbial cell surface, such as phospholipids and lipopolysaccharides. This in turn could lead to neutralization of negative charges of targets on the cell surface and tighten the membrane lipids, thereby interfering with the activity of antimicrobials that target these cell-surface components (Ganzle et al. 1999b). Conversely, divalent cations in food can compete to replace the divalent cations on cell surfaces that are removed by antimicrobials or bind with the antimicrobials by competing with their cationic cell-surface targets (Branen & Davidson 2004). The bactericidal activity of lactoferrin is influenced by many factors, such as the presence of metal salts (such as $CaCl_2$ and $MgCl_2$), pH, and environmental bacterial growth conditions (Bortner et al. 1986).

The consistency and texture of the food matrix can also contribute to the efficacy of antimicrobials. If an antimicrobial cannot mix homogeneously with the food matrix because of its substance heterogeneity, then uniform mixing of the antimicrobial in food could be a challenge, which can affect its activity. For example, owing to its hydrophobic nature, nisin may preferentially associate with the fatty components of food, thereby rendering it unavailable for antimicrobial activity (Jung et al. 1992). The food consistency, such as liquid or solid state, can affect the diffusion of antimicrobials in food, and antimicrobials can therefore have variable antimicrobial activity.

Some factors that affect diffusion include pH, salt concentration, nitrite, fat contents, proteolytic activity, sugar content, and salt concentration. Nisin is more active in homogeneous liquid substrates than heterogeneous solid foods because of its diffusibility (Delves-Broughton et al. 1992).

The metabolic state and growth phase of microbial cells can affect their susceptibility to antimicrobials. The induction of heat shock/acid shock proteins during certain food processing conditions can make the bacterial outer membrane resistant to antimicrobials (Naidu 2003). Overall, a variety of factors related to food components and target cells as well as structural-functional stability of antimicrobials, food processing conditions, composition of foods, and background microbial populations can affect the overall activity of natural antimicrobials.

COMBINATION OF ANTIMICROBIALS WITH OTHER HURDLES

Combining natural antimicrobials with novel preservation techniques (other hurdles), such as pulse electric field, high hydrostatic pressure, and thermal treatments, can achieve more effective antimicrobial activity for enhanced food preservation and safety. The synergetic effect from the application of a natural antimicrobial along with physical treatments can decrease the intensity of the physical treatment, including pressure, temperature, and total time, needed to achieve the degree of cellular damage needed to kill the cells. For example, when applied in the presence of nisin, a lower level of high pressure was effective in killing a high level of pathogenic bacteria than the level of high pressure needed without nisin (Ray 1995). Similarly, the killing activity of ultra high pressure was significantly improved against *E. coli* and *Listeria innocua* when applied in combination with nisin (Ponce et al. 1998). Furthermore, combining pressurization with bacteriocins increased the rates of inactivation of *L. monocytogenes*, *E. coli* O157:H7, and *S.* Typhimurium (Kalchayanand et al. 1998). Applying the combination of hydrostatic pressure with cystibiotics can limit the growth of bacteria during storage of packaged food. For example, vacuum-packaged, artificially contaminated processed meat products treated with pediocin PA-1/AcH and subjected to hydrostatic pressure significantly reduced several pathogenic and spoilage bacterial populations (Ray et al. 2001).

Reduced quantities of natural antimicrobials can be used in combination with physical treatments to achieve the desired level of antimicrobial activity. In this way, food quality and organoleptic properties of food can be preserved. For example, the sensitivity of *E. coli* cells to nisin is increased when the nisin treatment is preceded with a pressure treatment that disrupts their cell membrane (Hauben et al. 1996).

The combined application of an antimicrobial in the presence of other antimicrobials can also provide an enhanced synergistic antimicrobial. For example, *Listeria monocytogenes* populations in ostrich meat patties were reduced to below acceptable criteria when a combination of lysozyme, nisin, and EDTA were added to the meat (Mastromatteo et al. 2010). Similarly, the antimicrobial activity of lactoferrin in combination with EDTA and sodium bicarbonate reduced the populations of *E. coli* O157:H7 and selected meat starter culture (Al-Nabulsi & Holley 2007). The antimicrobial activity of nisin can also be enhanced by combination with other compounds. For example, nisin when combined with lysozyme (Monticello 1989) or pediocin (Hanlin et al. 1993) had enhanced antimicrobial activity. Combining nisin with organic acids, such as lactic acid or citric acid, provided enhanced antimicrobial activity against thermally stressed *Bacillus* spores (Oscroft et al. 1990). Combining several other antimicrobial hurdles, such as heat treatment, low temperature, low pH, and low water activity, can also enhance the antimicrobial activity of nisin. For example, nisin is more effective against psychrotrophic *B. cereus* in beef gravy at 8°C than at 15°C (Beuchat et al. 1997).

The antimicrobial activity of natural antimicrobials can also be used in conjunction with irradiation such as gamma irradiation, electron beams, and X-rays, to achieve the desired level of bacterial inactivation at much lower doses without affecting the flavor of the food. For example, combining irradiation with a mixture of rosemary and thyme extract in chicken extended its microbiological shelf life at 4°C by seven to eight times compared with the untreated chicken (Mahrour et al. 1998). Similarly, a combined treatment of a protein-based coating containing 0.9% thyme oil and 1.8% *trans*-cinnamaldehyde, and 3 kGy irradiation extended the shelf life of shrimp by 20 to 21 days (Quattara & Mafu 2003). Several studies have reported the application of bacteriocins in combination with irradiation to substantially reduce the populations of spoilage and pathogenic microbes in foods; e.g., nisin in combination with gamma irradiation to eliminate *L. monocytogenes* on raw meat (Mohamed et al. 2011) and pediocin with postpackaging irradiation for control of *L. monocytogenes* on frankfurters (Chen et al. 2004).

FUTURE CONSIDERATIONS

Natural antimicrobials are gaining interest among food technologists for their use as alternatives to physical- and chemical-based antimicrobial treatments. However, there are many constraints in the application of natural antimicrobials in foods that require further research on their antimicrobial efficacy, consumer acceptability, and cost. Some of the major research issues that need to be addressed include development of microbial resistance to natural antimicrobials, homogeneously mixing of some antimicrobial compounds in food matrices, large-scale production of these compounds from their natural sources without losing their functional activity, and the approval of their use by regulatory agencies. Antimicrobial resistance could develop if the antimicrobials applied are not applied at their effective doses for the target microbial population. Injured or stressed cells could recover in the presence of suboptimal doses of antimicrobials by resistance adaptive mechanisms. Some studies have revealed that if not applied at the proper stage of food processing, the antimicrobial compounds can provide heat/thermal resistance to microbes. For example, the addition of lysozyme and other lytic enzymes prior to heating food can provide heat resistance to spores of nonproteolytic *C. botulinum* (Ueno et al. 1996). The greatest challenge that remains is consumer acceptability of various natural antimicrobials, such as herbs and essential oils. When applied at the concentrations needed to achieve the desired level of antimicrobial potency, these antimicrobials can adversely affect the organoleptic properties of food beyond consumer acceptance. Many of the natural antimicrobials are categorized as GRAS for specific food applications, but their use in other commercial applications requires regulatory approval. Natural antimicrobials can provide a tremendous opportunity for advancing the field of food preservation and safety; however, additional research is needed to optimize their applications.

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DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Contents

Errata

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