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Natural antimicrobial/antioxidant agents in meat and poultry products as well as fruits and vegetables: A review

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ABSTRACT

Synthetic preservatives are widely used by the food industry to control the growth of spoilage and pathogenic microorganisms and to inhibit the process of lipid oxidation extending the shelf-life, quality and safety of food products. However, consumer's preference for natural food additives and concern regarding the safety of synthetic preservatives prompted the food industry to look for natural alternatives. Natural antimicrobials, including plant extracts and their essential oils, enzymes, peptides, bacteriocins, bacteriophages, and fermented ingredients have all been shown to have the potential for use as alternatives to chemical antimicrobials. Some spices, herbs and other plant extracts were also reported to be strong antioxidants. The antimicrobial/antioxidant activities of some plant extracts and/or their essential oils are mainly due to the presence of some major bioactive compounds, including phenolic acids, terpenes, aldehydes, and flavonoids. The proposed mechanisms of action of these natural preservatives are reported. An overview of the research done on the direct incorporation of natural preservatives agents into meat and poultry products as well as fruit and vegetables to extend their shelf-life is presented. The development of edible packaging materials containing natural preservatives is growing and their applications in selected food products are also presented in this review.

KEYWORDS

Natural antimicrobials; natural antioxidants; bioactive compounds; shelf-life; bioactive packaging; food applications

Introduction

Bacterial growth and lipid oxidation are the main factors that determine food quality loss and shelf-life reduction (Fernandez-Lopez et al., 2005; Shahidi and Zhong, 2010; Tajkarimi et al., 2010). Chemical additives are commonly used in food products to inhibit the process of lipid oxidation and microbial growth and to extend their shelf-life. However, there is a growing concern among the consumers about health-related issues associated with the use of synthetic antimicrobial/antioxidant agents (Fernandez-Lopez et al., 2005; Shahidi and Zhong, 2010; Brewer, 2011; Ahmad et al., 2015). In addition, consumers are more and more asking for minimally processed, preservative-free food products with longer shelf-life (Fernandez-Lopez et al., 2005). Furthermore, consumers prefer products with clean label containing food ingredients/additives that are natural, with familiar names and that are perceived to be good for health (Brewer, 2011). In recent years, there also have been concerns about food safety due to an increasing occurrence of foodborne illness outbreaks caused by pathogenic microorganisms (Tajkarimi et al., 2010). In order to satisfy consumer's demands and restore its confidence in the safety of food products, the food industry was motivated to look for natural alternatives that exhibit strong antimicrobial and/or antioxidant properties (Fernandez-Lopez et al., 2005; Ahmad et al., 2015). Plant extracts and their essential oils, enzymes, peptides, chitosan, bacteriocins, bacteriophages, fermented ingredients, and ozone can all be used as potential alternatives to synthetic

antimicrobial agents to improve the shelf-life and the safety of food products (Tiwari et al., 2009; Elsser-Gravesen and Elsser-Gravesen, 2014; Glowacz et al., 2015; Irkin and Esmer, 2015). Vitamins (ascorbic acid and α -tocopherol), herbs (rosemary, oregano, marjoram, sage, basil, etc.), spices (Cinnamon, clove, nutmeg, ginger, black pepper, garlic, etc.) and plant extracts (tea, grape seed, cranberry, blueberry, strawberry, etc.) also contain antioxidant components and can be used as natural antioxidant agents to inhibit lipid oxidation in food products (Brewer, 2011; Ahmad et al., 2015). Some natural antioxidants/antimicrobials were found not only to be able to extend the shelf-life of food products but also to be beneficial as preventative medicine against various human diseases (Ahmad et al., 2015). Natural preservatives can be either directly added to foods or incorporated in packaging systems. The latter possesses many advantages (Irkin and Esmer, 2015). This literature review will provide an overview on the natural antioxidant and antimicrobial agents that can be used in food products, their reported bioactive compounds, their proposed mechanisms of action as well as their applications to extend the shelf-life of meat and poultry products as well as fruit and vegetables.

Natural antimicrobial agents

Natural antimicrobial agents, including plant extracts, their essential oils and their pure bioactive compounds, enzymes, peptides, chitosan, bacteriocins, bacteriophages, and fermented

Table 1. Examples of potential natural antimicrobial agents for use in the food industry (Tiwari et al., 2009; Elsser-Gravesen and Elsser-Gravesen, 2014; Irkin and Esmer, 2015).

Classification	Antimicrobial agents
Pure bioactive compounds	Allyl isothiocyanate, cinnamaldehyde, eugenol, thymol, carvacrol, citral.
Essential oils	Thyme, oregano, pimento, clove, citron, lemon verbena, lemon balm, cypress leaf
Plant extracts	Grape seed, green tea, pomegranate peel/rind, acerola, pine bark, bearberry, cinnamon bark, rosemary, garlic, oregano, sansho, ginger, sage.
Polysaccharide	Chitosan
Peptides	Lactoferrin
Enzymes	Lysozyme, glucose oxidase, lactoperoxidase system
Bacteriocins	Nisin, pediocin, subtilin, lactacin
Bacteriophages	Listex™P100, Ecoshield™, Salmofresh™
Fermented ingredients	Microgard™, Prolong II™

ingredients are presented in Table 1 (Tiwari et al., 2009; Elsser-Gravesen and Elsser-Gravesen, 2014; Irkin and Esmer, 2015).

Antimicrobial agents of plant origin and their bioactive compounds

Plant extracts and essential oils of plant origin can be used as potential alternatives to synthetic preservatives to improve the shelf-life and the safety of food products (Tajkarimi et al., 2010). The strong antimicrobial effects of some plants materials are mainly due to the presence of some major bioactive compounds, including phenolics, terpenes, aliphatic alcohols, aldehydes, acids, and isoflavonoids (Tiwari et al., 2009). These bioactive compounds are commonly found in the essential oil fraction of leaves (rosemary, sage, basil, oregano, thyme and marjoram), bulbs (garlic and onions), fruits (cardamom and pepper), flowers or buds (clove), and seeds (caraway, fennel, nutmeg) (Tiwari et al., 2009).

Essential oils of plants

Plant essential oils tend to be more effective towards Gram-positive bacteria rather than Gram-negative bacteria. The resistance of Gram-negative bacteria to essential oils may be due to the presence of a lipopolysaccharide outer membrane surrounding the cell wall (Tongnuanchan and Benjakul, 2014). Nevertheless, the nonphenolic compounds of essential oils of oregano, clove, cinnamon, garlic, coriander, rosemary, parsley, lemongrass, purple, and bronze muscadine seeds as well as sage have been reported to be effective on both types of bacteria (Tiwari et al., 2009; Tajkarimi et al., 2010). The plant essential oils usually contain a mixture of several bioactive compounds (Tiwari et al., 2009; Tajkarimi et al., 2010) and their antimicrobial efficacy is generally depended on the chemical structure and concentration of those bioactive compounds (Tiwari et al., 2009). Based on the chemical analysis of various essential oils, it was found that the major constituents of many of them were carvacrol, thymol, eugenol, and citral (Tiwari et al., 2009; Irkin and Esmer, 2015). The plant essential oils that have been

reported to exhibit strong antimicrobial effects include oregano, clove, cinnamon, garlic, coriander, rosemary, parsley, lemongrass, and sage (Tajkarimi et al., 2010). Hence, oils with high levels of eugenol (allspice, clove bud and leaf, bay, and cinnamon leaf), *Trans*-cinnamaldehyde (cinnamon bark, cinnamon Chinese cassia oil and cinnamon oleoresin) and citral (lemon myrtle, *Litsea cubeba*, and lime) have been reported to exhibit strong antimicrobial effects (Tiwari et al., 2009; Dussault et al., 2014). The antimicrobial activity of oregano, thyme and savory is due partly to the presence of volatile oils, such as carvacrol, ρ -cumene, γ -terpinene, and thymol (Tiwari et al., 2009). Dussault et al. (2014) and Emiroglu et al. (2010) reported that the antimicrobial activity of thyme and oregano essential oil is mainly due to the presence of the phenolic compounds carvacrol and thymol. In addition, Tongnuanchan and Benjakul (2014) reported that oregano and thyme essential oils contain 60% to 74% and 45% of carvacrol, respectively as the major component. This latter exhibits a broad spectrum of antimicrobial activity against the majority of Gram-positive and Gram-negative bacteria. It has been reported that the presence of a hydroxyl group in the structure of phenolic compounds is responsible for the antimicrobial activity and its relative position is critical for the effectiveness of these natural components. These findings could explain the higher antimicrobial potency of carvacrol when compared to other plant phenolics (Tongnuanchan and Benjakul, 2014). The antimicrobial activity of sage is due to the terpene thujone (Tiwari et al., 2009). The essential oil of rosemary exhibits an antimicrobial effect against both Gram negative (*Escherichia coli*, *Klebsiella pneumoniae*) and Gram positive (*Bacillus subtilis*, *Staphylococcus aureus*) bacteria (Tongnuanchan and Benjakul, 2014). Its antimicrobial activity is mainly due to a group of terpenes, mainly borneol, camphor, 1,8-cineole, α -pinene, camphone, verbenone, and bornyl acetate (Tiwari et al., 2009). Allyl and related isothiocyanates are responsible for the antimicrobial activity of mustard, whereas allicin is responsible for that of garlic and onion (Holley and Patel, 2005). However, spices such as ginger, black pepper, red pepper, chili powder, cumin, and curry powder have been reported to have lower antimicrobial properties (Tajkarimi et al., 2010). Dussault et al. (2014) investigated the antimicrobial activities in vitro of 67 essential oils, oleoresins and pure compounds against six food pathogens (*E. coli* O157:H7, *S. aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella enterica* serovar *typhimurium*, and *Pseudomonas aeruginosa*). These authors reported that allyl isothiocyanate, cinnamon oleoresin as well as cinnamon Chinese cassia, oregano and red thyme essential oils were the ones found to exhibit strong antimicrobial activities against all investigated food pathogens. The addition of oregano and cinnamon cassia essential oils to ham at a concentration of 500 ppm resulted in growth rate reduction of mixed cultures of *L. monocytogenes* by 19% and 10%, respectively (Dussault et al., 2014). Burt (2004) reported that the concentration of essential oils required to exhibit strong antimicrobial effect is around 0.5–20 $\mu\text{L/g}$ in foods and around 0.1–10 $\mu\text{L/mL}$ in solutions for washing fruit and vegetables. Low pH,

temperature, and oxygen levels are physical conditions that can improve the action of essential oils (Burt, 2004).

Plant extracts

Grape seed and green tea extracts. Grape seed is rich in monomeric phenolic compounds, including catechin, epicatechin, and epicatechin-3-O-gallate, as well as in dimeric, trimeric, and tetrameric procyanidins, whereas green tea leaves contain high amounts of epicatechin, epicatechin gallate, epigallocatechin, teaflavin gallate, teaflavin monogallate A and B, and teaflavin digallate (Banon et al., 2007). Grape seed and green tea extracts have been reported to delay microbial growth in low sulfite raw beef patties (Banon et al., 2007). In addition, cooked pork meatballs containing grape seed and green tea extracts had lower microbial counts than samples containing sodium ascorbate. However, the presence of these extracts caused the formation of brown shades in cooked pork meatballs (Price et al., 2013). When compared to the control samples, grape seed extract at a level of 1.0% effectively decreased the numbers of *E. coli* O157:H7 and *S. typhimurium* and delayed the growth of *L. monocytogenes* and *Aeromonas hydrophila* in cooked beef (Ahn et al., 2007).

Cranberry extracts. The antimicrobial activity of cranberry is associated with its high content of phenolic compounds, which include low molecular weight phenolic acids, condensed tannins, proanthocyanidins and flavonoids such as anthocyanins (Côté et al., 2011b; Caillet et al., 2012a). In particular, proanthocyanidins consisting mainly of epicatechin tetramers and pentamers with at least one A-type linkage have been suggested to play a key role in the antimicrobial effect of cranberries against pathogenic bacteria (Côté et al., 2011b). Côté et al. (2011b) investigated the antimicrobial activity of three cranberry extracts and cranberry juice against seven bacterial strains (*Enterococcus faecium* resistant to vancomycin (ERV), *E. coli* O157:H7 EDL 933, *E. coli* ATCC 25922, *L. monocytogenes* HPB 2812, *P. aeruginosa* ATCC 15442, *Salmonella typhimurium* SL1344, and *S. aureus* ATCC 29213). The cranberry extract 1 was mainly composed of water-soluble phenolics, whereas cranberry extract 2 and 3 mainly contained apolar phenolics (flavonols, flavan-3-ols, and proanthocyanidins) and anthocyanins, respectively. Among the investigated extracts, the cranberry extract 1 was found to be the most effective one against the targeted bacterial strains, with ERV and to a lesser degree *P. aeruginosa*, *S. aureus*, and *E. coli* ATCC 25922 being the most sensitive ones. *L. monocytogenes* and ERV were completely inactivated after 30 min of exposure to pure neutralized cranberry juice (Côté et al., 2011b). The juice process was shown to have a general enhancing effect on the antibacterial properties of cranberry extracts 1 and 3 but a negative effect on the antibacterial properties of cranberry extract 2 rich in apolar phenolics (Côté et al., 2011c). Caillet et al. (2012a) studied the antimicrobial effect of thirty HPLC fractions of different polarities obtained from two cranberry juices and three extracts isolated from frozen cranberries and pomace against seven bacterial strains. The three extracts and bacterial strains studied were the same as those reported by Côté et al. (2011b). All pathogens were at least very sensitive to 7 fractions with

minimum inhibitory concentrations (MICs) below 2 µg phenol/mL and five fractions with MICs below 10 µg phenol/mL. Moreover Caillet et al. (2012a) reported that four fractions rich in apolar phenolics were very effective against all bacterial strains with MICs below 10 µg phenol/mL and 25 fractions completely inhibited microbial growth with MICs below 100 mg phenol/mL. Sagdica et al. (2006) showed that cranberry fruit extract at a concentration of 15% was able to inhibit completely the growth of *A. hydrophila*, *B. cereus*, *Enterobacter aerogenes*, *E. coli*, *K. pneumoniae*, *Proteus vulgaris*, *P. aeruginosa*, *S. typhimurium*, *S. aureus*, and *Yersinia enterocolitica* using the agar diffusion method. Puupponen-Pimiä et al. (2001) reported that berry extracts inhibited the growth of Gram-negative bacteria but not gram-positive bacteria, with cloudberry, raspberry and strawberry extracts showing strong inhibition against *Salmonella*.

Mechanisms of antimicrobial action

The exact mechanism of antimicrobial action of essential oils of plant extracts is yet to be elucidated (Holley and Patel, 2005; Tiwari et al., 2009). Nevertheless, plant substances can affect microbial cells by a number of proposed antimicrobial mechanisms, including attacking the phospholipid bilayer of the cell membrane, disrupting enzyme systems, compromising the genetic material of bacteria and oxidizing unsaturated fatty acids resulting in the formation of fatty acid hydroperoxides (Tajkarimi et al., 2010). It is known that the antimicrobial effects of aromatic and phenolic compounds are exerted at the cytoplasmic membrane by changing its structure and function (Holley and Patel, 2005). The outer membranes of both *E. coli* and *S. typhimurium* disintegrated upon exposure to carvacrol and thymol (Holley and Patel, 2005; Fisher and Phillips, 2008), whereas major thickening and disruption of cell wall along with increased roughness and lack of cytoplasm was observed in *L. monocytogenes* upon exposure to thyme essential oil (Fisher and Phillips, 2008). Similar findings were reported for *E. coli* O157:H7 and *L. monocytogenes* in the presence of oregano and cinnamon, respectively (Fisher and Phillips, 2008). The antimicrobial activity of nonphenolic isothiocyanates is thought to be due to the inactivation of extracellular enzymes by means of disulfite bonds cleavage (Holley and Patel, 2005). Terpenes were reported to be capable of disrupting and penetrating the lipid structure of the cell wall of bacteria and causing eventual cell death (Fisher and Phillips, 2008). Carvacrol is capable of disintegrating the outer membrane of Gram-negative bacteria releasing lipopolysaccharides and enhancing the permeability of the cytoplasmic membrane to ATP. The antimicrobial activity of carvacrol against Gram-positive bacteria is due to its interaction with the membranes of bacteria altering the permeability for cations such as H⁺ and K⁺ (Tongnuanchan and Benjakul, 2014).

Antimicrobial agents of animal origin and their antimicrobial mechanism of action

Enzymes

Lysozyme. Lysozyme is a single peptide enzyme that is naturally produced by humans and animals. Its antimicrobial

activity is due to its ability to hydrolyze the beta 1,4-glucosidic linkages between N-acetylmuramic acid and N-acetylglucosamine found in peptidoglycan. As the cell wall of Gram-positive bacteria is composed of 90% of peptidoglycan, Gram-positive bacteria are very sensitive to lysozyme (Barbiroli et al., 2012). Hence, lysozyme is capable of damaging the structural integrity of the cell wall leading to the lysis of bacterial cells. The activity of lysozyme against the cellular structure of bacteria along with its natural aspect makes it of great interest for use as an antimicrobial agent in food products (Irkin and Esmer, 2015). Lysozyme, on the other hand, is ineffective against Gram-negative bacteria. The resistance of Gram-negative bacteria to lysozyme is due to the lipopolysaccharide layer surrounding their outer membrane preventing lysozyme from accessing to the peptidoglycan layer. Nevertheless, lysozyme can be effective against Gram-negative bacteria in the presence of membrane destabilizing agents, such as detergents and chelators (Ünalán et al., 2011; Barbiroli et al., 2012; Bayarri et al., 2014). The combination of lysozyme with ethylenediaminetetraacetic acid (EDTA) has been reported by Ünalán et al. (2011) to increase the sensitivity of Gram-negative bacteria to lysozyme. EDTA not only can destabilize the protective lipopolysaccharide layer of Gram-negative bacteria but can also act as a chelating agent in food products preventing lipid oxidation catalyzed by metals (Ünalán et al., 2011). In addition to its antimicrobial activity, lysozyme exhibits high stability over a wide range of temperature and pH allowing its use in antimicrobial edible films (Bayarri et al., 2014). Inovapure is a commercially available lysozyme that has been reported in model studies to be effective under certain conditions, either alone or in the presence of synergistic compounds, against pathogens like *L. monocytogenes*, *Clostridium botulinum*, *Campylobacter jejuni*, *Pseudomonas* spp., and *Salmonella enteritidis* as well as against spoilage microorganisms such as *Clostridium thermosaccharolyticum*, *Bacillus stearothermophilus*, and *Clostridium tyrobutyricum*. It can be used to extend the shelf life of food products, including raw and processed meats, cheese and other dairy products (Tiwari et al., 2009). Lysozyme from egg-white is currently approved by Health Canada for use in hard cheeses to prevent the late gas blowing caused by the growth of *Clostridium tyrobutyricum* (Tiwari et al., 2009).

Lactoperoxidase system. Lactoperoxidase system is a natural antimicrobial system secreted in various mammalian glands such as milk, saliva, and tears (Min and Krochta, 2005). The lactoperoxidase system is composed of lactoperoxidase, thiocyanate, and hydrogen peroxide (H_2O_2). Lactoperoxidase catalyzes the oxidation of thiocyanate ion using H_2O_2 . The resulting products, which are hypothiocyanite and hypothiocyanous acid, exhibit an inhibitory effect on microorganisms by the oxidation of sulfhydryl groups in their enzyme systems and proteins (Min and Krochta, 2005; Campos et al., 2010). This system has been reported to exhibit an antimicrobial activity against Gram-positive and Gram-negative bacteria as well as against a variety of fungal species (Campos et al., 2010). Min and Krochta (2005) reported that the lactoperoxidase system at a concentration of $\geq 0.1\%$ (w/w) inhibited *Penicillium commune* in 1% peptone water and in potato dextrose broth. In addition, the incorporation of this system into whey protein

isolate films also resulted in the inhibition of the growth of *P. commune* (Min and Krochta, 2005). Min et al. (2005) also reported that the lactoperoxidase system-whey protein isolate films inhibited completely *Salmonella enterica* and *E. coli* O157:H7 ($4 \log CFU/cm^2$) that were inoculated either onto the agar before placing the film disc or on the top of the film disc. The main considerations for the use of the lactoperoxidase system in packaging films is (1) cost and (2) the fact that the antimicrobial action of the lactoperoxidase system is depended on thiocyanate and H_2O_2 , which are found in milk but not in many other food products (Appendinia and Hotchkissb, 2002; Joerger, 2007). Toxicological concerns may also arise if H_2O_2 levels exceed government regulations in food products (Appendinia and Hotchkissb, 2002).

Antimicrobial peptides

Antimicrobial peptides are widely found in nature. They are used as essential components of nonspecific host defense systems in many if not all life forms (Tiwari et al., 2009). Lactoferrin, an 80 kDa iron-binding glycoprotein, is a natural component of milk (Barbiroli et al., 2012). Tiwari et al. (2009) reported that lactoferrin exhibits an antimicrobial activity against a wide range of Gram-positive bacteria, Gram-negative bacteria, fungi, and parasites. The antimicrobial activity of lactoferrin against *Salmonella* and *E. coli* has also been reported in the literature (Min et al., 2005). Jenssen and Hancock (2009) explained that the antibacterial activity of lactoferrin is due to two different and unrelated mechanisms: (1) Inhibition of bacterial growth by sequestering iron from bacterial pathogens, and (2) The ability of large cationic patches present on the lactoferrin surface to facilitate direct interaction with the anionic Lipid A, a component of the lipopolysaccharide of Gram-negative bacteria, modifying the permeability of the outer membrane and resulting in the release of lipopolysaccharide. Lactoferrin has been already used in an antimicrobial spray to treat beef carcasses (Barbiroli et al., 2012). It is currently approved for application on beef in the United States (Tiwari et al., 2009). Other antimicrobial peptides include defensins, magainin, and pleurodicin (Tiwari et al., 2009).

Chitosan

Chitosan is one of the few natural cationic polysaccharides. It is a linear polysaccharide consisting of (1,4)-linked 2-amino-deoxy- β -D-glucan and is a deacetylated derivative of chitin. The latter is the second most abundant polysaccharide in nature after cellulose (Dutta et al., 2009). Sources of chitosan include: shrimp's shell, fungi, yeast, protozoa, and green microalgae (Irkin and Esmer, 2015). It has been reported to be effective against Gram-positive and Gram negative bacteria as well as yeasts and molds (Joerger, 2007). Chitosan is more soluble and exhibits a better antimicrobial activity than chitin, which is due to the presence of a positive charge on the C2 of the glucosamine monomer below pH 6. The mechanism of the antimicrobial action of chitin, chitosan, and their derivatives is not fully understood. Nevertheless, several mechanisms have been proposed. The positively charged amino group of chitosan can interact with negatively charged microbial cell membranes resulting in the leakage of proteinaceous and other intracellular constituents of the microorganisms. Chitosan can also act as a

chelating agent inhibiting the production of toxins and microbial growth by selectively binding trace metals. It has also been reported to possess the ability to activate several defense processes in the host tissue as well as to inhibit various enzymes. In addition, chitosan can penetrate to the nuclei of microorganisms and interfere with the synthesis of proteins and mRNA (Dutta et al., 2009). The antimicrobial mechanism of action of chitosan has been reported to be different in Gram-positive and in Gram-negative bacteria. The antimicrobial activity against Gram-negative *E. coli* increased with decreasing the molecular weight of chitosan, whereas the opposite effect was found on the Gram-positive bacteria *S. aureus* (Zheng and Zhu, 2003). These authors suggested that the chitosan of higher molecular weight forms a polymer membrane on the surface of *S. aureus* cell inhibiting nutrients from entering the cell, whereas the chitosan of lower molecular weight entered the cell of *E. coli* through pervasion (Zheng and Zhu, 2003).

Antimicrobial agents of microbial origin and their antimicrobial mechanism(s) of action

Glucose oxidase

Glucose oxidase, naturally produced by molds, including *Aspergillus niger* and *Penicillium* spp., is an oxido-reductase that catalyzes the oxidation of D-glucose to H₂O₂ and D-glucono- δ -lactone. The latter reacts with water to form D-gluconic acid. Glucose oxidase can also be naturally produced from insects (Wong et al., 2008). While the antimicrobial activity of glucose oxidase is mainly due to the cytotoxicity of H₂O₂ formed, the lowering of pH due the formation of D-gluconic acid may also have an effect on the growth of microorganisms (Fuglsang et al., 1995; Joerger, 2007). The main considerations for the use of glucose oxidase in packaging films is its cost as well as its dependence on the glucose as substrate, which is not present in many foods in sufficient concentrations (Joerger, 2007). In addition, hydrogen peroxide amounts may exceed the permitted US Food and Drug Administration (FDA) levels in food products and may pose toxicological concerns (Appendin and Hotchkiss, 2002). The long-term exposure of foods to H₂O₂ can also promote lipid oxidation leading to their rancidity (Fuglsang et al., 1995). Nevertheless, H₂O₂ can be removed from food products by using as second enzyme called catalase, which converts it to water and oxygen (Bankar et al., 2009). Glucose oxidase in liquid and solid form is currently available in bulk for use as an additive in the food industry. It has been reported that the food grade glucose oxidase preparation is composed of mixture of glucose oxidase and catalase as these two enzymes are naturally found together in the mycelium cell wall (Wong et al., 2008). The microbial glucose oxidase is currently approved by Health Canada for the removal of oxygen from the top of bottled beverages before they are sealed to maintain their taste and flavor. It is also approved by Health Canada for use as a food additive in bread, flour, whole wheat flour, unstandardized bakery products as well as liquid whole, yolk and white egg, which are destined for drying. D-Gluconic acid, the catalytic product of glucose oxidase, is found safe for human consumption and WHO has not specified any acceptable daily limit. Hence, glucose oxidase can be used as a potential natural antimicrobial/antioxidant agent in food products to

replace chemical additives satisfying consumers demand (Wong et al., 2008).

Nisin

Nisin is an antimicrobial peptide, produced by fermentation of a modified milk medium with certain strains of the lactic acid bacterium *Lactococcus lactis* (Tiwari et al., 2009). Nisin is used in more than 48 countries and has Food and Drug Administration and Health Canada approval for use as an antimicrobial preservative in food products. Nisin has been reported to be effective in a number of food systems against a wide range of Gram-positive bacteria such as *L. monocytogenes* and *S. aureus* (Deegan et al., 2006; Campos et al., 2010). It can also be effective against Gram-negative bacteria when combined with membrane destabilizing agents such as EDTA (Joerger, 2007; Campos et al., 2010). The antimicrobial mechanism of action of nisin involves its interaction with the phospholipids in the cytoplasmic membrane of bacteria causing disruption of the membrane function and inhibiting the swelling process of germination preventing hence the outgrowth of spores (Tiwari et al., 2009). Nisin is effectively used by the cheese industry against heat-resistant organisms such as *Bacillus* and *Clostridium* (Deegan et al., 2006; Tiwari et al., 2009). Nisin has been incorporated alone or in a combination with other antimicrobial agents into food products. Under modified atmosphere packaging (MAP) conditions, the shelf-life of fresh chicken meat using 500 IU/g nisin and 50 mM EDTA was extended by 13–14 days when compared to the control samples (Economou et al., 2009). Nisin has also been used in edible films made of tapioca starch, whey protein, sodium caseinate, soy protein, methylcellulose, hydroxypropylmethylcellulose, corn zein, and glucomannan (Campos et al., 2010). The small molecular size of nisin permits the production of films that release the peptide when it gets into contact with food or liquid (Joerger, 2007).

Bacteriophages

Bacteriophages are viruses that are capable of invading bacterial cells. Lytic bacteriophages disrupt bacterial metabolism leading to the death of the bacteria. The use of bacteriophages in food for the biocontrol of pathogens is very promising as these viruses have been proven to be harmless to mammalian cells. They are also easy to handle and exhibit high and specific antimicrobial activity. Bacteriophages are suitable to be used (1) for decontamination of carcasses and other raw products such as fruits and vegetables, and (2) as natural preservatives to extend the shelf-life of various food products. In order to minimize cost, bacteriophages can be used in combination with other preservation methods (Garcia et al., 2008). In 2006, the bacteriophages ListexTMP100 and LMP-102 were approved by the FDA for use in selected foods to control the contamination of *L. monocytogenes*. In 2010, Health Canada issued a letter of no objection for the use of ListexTMP100 as a processing aid against *L. monocytogenes* in deli meat and poultry products, cold-smoked fish, vegetable prepared dishes, and some dairy products (Chibeu et al., 2013). ListexTMP100 is based on the virulent phage P100. The complete eradication of *L. monocytogenes* can occur in the presence of this bacteriophage and it will depend on the

phage dose, the chemical composition of the food and its specific matrix. Chibeu et al. (2013) reported that *L. monocytogenes* population was significantly lower in Listex™P100 treated-cooked turkey and roast beef samples than in the untreated control samples throughout the 28 days of incubation period at 4°C. LMP-102 is composed of six bacteriophages that were isolated from the environment and was developed to be used as an additive for ready to eat foods (Garcia et al., 2008). In 2014, Health Canada approved the use of Ecoshield™ as a processing aid in red meat to control the growth of *E. coli* O157:H7. Salmofresh™ was also approved by Health Canada in 2014 to be used as a processing aid in all food and ready to eat food products to control the growth of *Salmonella*.

Fermented ingredients

Fermented ingredients can be produced from a variety of raw materials (milk, sugar or plant-derived material) using food-grade microorganisms, such as lactic acid bacteria and propionic acid bacteria. They may be composed of organic acids (lactic, acetic or propionic acid), diacetyl, bacteriocins as well as other sensory metabolites, which will be depended on the properties of strain(s) used for the fermentation. There is limited information in the literature on the use of fermented ingredients as potential antimicrobial agents in food products; however these ingredients are currently commercially available on the market (Elsser-Gravesen and Elsser-Gravesen, 2014). Microgard™ is a commercially available milk product that is fermented by specific dairy organisms. Microgard™ was found to be effective in inhibiting Gram-negative bacteria, including *Pseudomonas*, *Salmonella*, and *Yersinia* when incorporated into agar media at a concentration of 1%. Nevertheless, it was ineffective against the Gram-positive *B. cereus*, *S. aureus*, and *L. monocytogenes* (Al-Zoreky et al., 1991). The addition of 1% Microgard™ to hamburgers resulted in some initial reduction of *E. coli* O157:H7 and in a bacteriostatic effect against *L. monocytogenes* during refrigerated storage (Elsser-Gravesen and Elsser-Gravesen, 2014). The Microgard™ products have been reported to be used in a wide range of food products, including cottage cheese, yogurt, sour cream, dairy desserts, sauces, dressings, pasta, baked goods, and prepared meals (Elsser-Gravesen and Elsser-Gravesen, 2014). Kim et al. (2005) used an antimicrobial edible film made from soybean meal that has been fermented with *B. subtilis* to coat different types of food products (Surimi, jerked beef and mashed sausage media). The antimicrobial edible film exhibited a high inhibitory effect on the growth of all the investigated bacteria (*E. coli*, *S. aureus*, *S. tiphimurium*, and *L. monocytogenes*) (Kim et al., 2005). Fermented dextrose is a concentrated dextrose broth that has been fermented by a bacterium that belongs to the *Propionibacterium* genus (Dussault et al., 2012). It is commercially sold by BSA under the name of Prolong II™ as an antimicrobial solution with applications in fresh and cooked meat and poultry products as well as in ready to eat and bakery products. Dussault et al. (2012) showed that a concentrated fermented dextrose (FD) was able to extend the shelf life of fresh pork sausages from 5 days to up to 13 days. At day 13, mesophilic bacteria were 2 logCFU/g less in raw pork sausages containing FD than in control samples. When combined, FD and low dose

γ-irradiation (1.5 KGy) were shown to act in synergy to reduce the growth of the total bacterial flora in fresh pork sausages (Dussault et al., 2012).

Use of ozone as a natural antimicrobial agent

Due to its potential oxidizing capacity, ozone is a strong antimicrobial agent (Guzel-Seydim et al., 2004). The fresh produce industry has been using ozone as an antimicrobial agent for few years (Guzel-Seydim et al., 2004; Glowacz et al., 2015). Ozone in gaseous and aqueous phases is approved by U.S. FDA to be used as an antimicrobial agent in food, including meat and poultry (Glowacz et al., 2015). Ozone is approved by Health Canada to be used as an antimicrobial agent in spring or mineral water during the bottling process to inhibit the growth of harmful microorganisms. By breaking down into oxygen, ozone is also effective in removing objectionable odors and flavors improving taste and other qualities. Ozone is also approved by Health Canada to be used as a maturing agent in cider and wine. In contrast to other sanitizers, ozone does not leave any residues on the surface of the produce due to its rapid decomposition making it safe for use in the food industry (Guzel-Seydim et al., 2004; Glowacz et al., 2015). The bactericidal effects of ozone on a wide variety of Gram-Positive and Gram-negative bacteria as well as spores and vegetative cells have been reported in the literature (Guzel-Seydim et al., 2004; Glowacz et al., 2015). Prior to storage, the treatment of apples, carrots, celery, lettuce, peppers, spinach, and strawberries with ozonated water resulted in the reduction of their microbial counts. Furthermore, the microbial counts of blueberries, carrots, papaya, peppers, spinach and tomatoes were reported to be reduced when exposed to gaseous ozone. Foodborne pathogens, including *E. coli*, *Listeria* spp., and *Shigella* spp. were also reduced on fresh produce when treated with ozone (Glowacz et al., 2015). In addition to microbial reduction and removal of pesticide residues, Glowacz et al. (2015) indicated that ozone treatment can also reduce the weight loss, improve the texture maintenance and visual quality as well as enhance the nutritional content of the fresh produce when it is used at the right dose. Although chlorinated agents are used worldwide in the food industry to disinfect water, wastewater, and to sanitize food processing plant equipment, these agents possess many disadvantages. Chlorinated agents can combine with many organic compounds leading to the formation of toxic by-products, which can have adverse effects on the population health and the environment (Guzel-Seydim et al., 2004). Ozone can be used as an effective alternative sanitizer to chlorine in the food industry. Fresh 24-h bacterial cultures of *Pseudomonas fluorescens* (ATCC 948), *Pseudomonas fragi* (ATCC 4973), *Pseudomonas putida* (ATCC 795), *Enterobacter aerogenes* (ATCC 35028), *Enterobacter cloacae* (ATCC 35030), and *Bacillus licheniformis* (ATCC 14580) were exposed to ozone (0.6 ppm for 1 min and 10 min), chlorine (100 ppm for 2 min) or heat (77 ± 1°C for 5 min). While 1 min-ozone treatment was ineffective against the investigated spoilage bacteria, 10 min-ozone treatment exhibited the highest level of bacterial population reduction, with a mean reduction over the species of 7.3 logs units, following by heat (5.4 log reduction) and chlorine (3.07 log reduction) (Dosti et al., 2005). These authors also showed that, when

compared to the control, ozone and chlorine both significantly reduced the biofilm bacteria that was adhered to the sterile stainless steel metal coupons after their incubation in ultra-high temperature sterile milk inoculated with *P. fluorescens*, *P. fragi*, or *P. putida* for 24–72 h. Two major mechanisms were identified to describe the antimicrobial potency of ozone against microorganisms: (1) Oxidization of sulfhydryl groups and amino acids of enzymes, peptides and proteins to shorter peptides by ozone; (2) Oxidization of polyunsaturated fatty acids to acid peroxides by ozone resulting in cell disruption and subsequent leakage of cellular contents (Guzel-Seydim et al., 2004).

Natural antioxidant agents

Lipid oxidation, which occurs during storage, processing and heat treatment, is one of the major causes of quality deterioration of food products (Shahidi and Zhong, 2010). Antioxidant agents are compounds that play a major role in delaying/preventing autoxidation by inhibiting the formation of free radicals or by interrupting propagation of the free radical by one or more of several mechanisms. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG), are effectively used in foods to prevent autooxidation. However, recent studies have shown that synthetic antioxidants, such as BHA and BHT may exhibit weak carcinogenic effects in some animals at high levels (Shahidi and Zhong, 2010; Brewer, 2011). These findings together with consumer preference for natural food additives have motivated the food industry to seek natural alternatives. Natural antioxidants can not only extend the shelf-life of food products but also be beneficial as preventative medicine against various human diseases (Ahmad et al., 2015).

Antioxidant agents of plant origin and their bioactive compounds

Vitamins (ascorbic acid and α -tocopherol), herbs (rosemary, oregano, marjoram, sage, basil, etc.), spices (Cinnamon, clove, nutmeg, ginger, black pepper, garlic, etc.), and plant extracts (tea, grape seed, cranberry, blueberry, strawberry, etc.) have been reported to contain antioxidant components (Brewer, 2011; Ahmad et al., 2015). The antioxidant activities of liquid extracts from 22 selected culinary herbs and spices (ginger, cinnamon, clove, bay, sage, rosemary, thyme, savory, oregano, sweet basil, parsley, coriander, tarragon, sansho, allspice, cumin, black and white peppercorns, nutmeg, caraway, dill, and fennel) were assessed on homogenized samples of porcine meat. Methyl alcohol extracts of all the investigated herbs and spices exhibited significant suppression of lipid oxidation when added to pork meat homogenates. Nevertheless, among the investigated herbs and spices, the extracts of sansho, sage, and ginger had the highest level of inhibition on lipid oxidation, which was 85%, 82%, and 75%, respectively (Tanabe et al., 2002). The antioxidative bioactive components of herbs and spices can be concentrated as extracts, essential oils or resins (Brewer, 2011). The antioxidant activity of essential oils is depended on the extraction method and type of solvents used (Tongnuanchan and Benjakul, 2014). The antioxidant activity

of plant extracts is mainly due to presence of phenolic acids (gallic, protocatechuic, caffeic, and rosmarinic acids), phenolic diterpenes (carnosol, carnosic acid, rosmanol, and rosmadial), flavonoids (quercetin, catechin, naringenin, and kaempferol), and volatile oils (eugenol, carvacrol, thymol, and menthol) as bioactive compounds. Plant pigments such as anthocyanins and anthocyanidins have been also reported to exhibit antioxidant activities. Catechins, epicatechins, phenolic acids, proanthocyanidins, and resveratrol are bioactive compounds that contribute to the antioxidant activity of tea and grape seed extracts (Brewer, 2011).

Herb extracts

Rosemary. The antioxidant activity of rosemary is mainly due to the presence of phenolic diterpenes (carnosic, carnosol, rosmanol, rosmadial, 1,2-methoxycarnosic acid, epi-, and iso-rosmanol) and phenolic acids, mainly rosmarinic and caffeic acids (Brewer, 2011). Dorman et al. (2003) investigated the antioxidant properties of the de-odourized aqueous extracts of oregano, rosemary, sage and thyme belonging to the Lamiaceae family. Rosemary extract exhibited the highest total phenolic content of 185 mg gallic acid equivalents (GAE)/g. These authors reported that the antioxidant characteristics of these herb extracts is not entirely related to their total phenolic contents but appear to be strongly dependent on rosmarinic acid, the major phenolic component present in these extracts. Different varieties of rosemary grown in different regions and under different conditions may differ in their content of phenolic compounds. It has been reported the antioxidant activity of carnosic acid is \geq to that of synthetic antioxidants. Higher oxidative stability was exhibited in chicken frankfurters in the presence of two commercially available oil-soluble rosemary extracts, VivOX4 and VivOX20 containing 4% and 20% (w/w) of carnosic acid, respectively at all the investigated storage temperatures (Rižnar et al., 2006). Cooked turkey products containing water-soluble rosemary extracts exhibited lower thiobarbituric acid reactive substances (TBARS) and hexanal values and better color change protection than the control samples (Yu et al., 2002). All natural extracts, including oleoresin rosemary retarded the formation of TBARS and lowered the hexanal content in cooked ground beef throughout the storage period (Ahn et al., 2007).

Oregano and sage. The total phenolic content of oregano was reported to be 149 mg GAE/g (Dorman et al., 2003). Oregano extracts contain high concentrations of phenolic acids, mainly rosmarinic acid as well as phenolic carboxylic acids and glycosides that exhibit both an antioxidant activity and are effective superoxide anion radical scavengers (Brewer, 2011). Among the investigated herbs and spices (bay leaves, rosemary, sage, marjoram, oregano, cinnamon, parsley, sweet basil, and mint), Muchuweti et al. (2007) reported that oregano had the highest total phenolic compound concentration and exhibited one of the highest antioxidant activities (58.28%). Fasseas et al. (2008) reported that oregano essential oil is composed of about 20 compounds with the most abundant ones being thymol (60.9%), *p*-cumene (10.5%), γ -terpinene (7.6%), and carvacrol (5.8%). 37 substances are found in sage essential oil with

eucalyptol (49.4%), camphor (8.5%), and α -pinene (5.4%) being the most abundant ones (Fasseas et al., 2008). After 6 days of storage, hexanal levels were 56- and 12-times lower in precooked chicken meatballs containing oregano and sage, respectively than in control samples (Marques Pino et al., 2013). Porcine and bovine ground meat stored at 4°C, in the raw and cooked state, during 12 days of storage exhibited reduced oxidation in the presence of oregano and sage essential oils (3% w/w) (Fasseas et al., 2008). Among the 22 investigated herbs and spices, sage extract was found to exhibit one of the highest levels of inhibition (82%) on lipid oxidation when added to pork meat homogenates (Tanabe et al., 2002).

Marjoram. The essential oil of marjoram contains a significant amount of rosmarinic acid and carnosol. It is also rich in terpinen-4-ol, cis-sabinene hydrate, *p*-cumene, and γ -terpinene (Brewer, 2011). Among the investigated herbs and spices (bay leaves, rosemary, sage, marjoram, oregano, cinnamon, parsley, sweet basil and mint), Muchuweti et al. (2007) reported that marjoram contained the highest proportion of simple phenolic compounds (95.57%) and exhibited along with cinnamon the highest radical scavenging activity of 91.3%.

Basil and thyme. The major aroma constituents of basil were 3,7-dimethyl-1,6-octadien-3-ol (linalool; 3.94 mg/g), 1-methoxy-4-(2-propenyl) benzene (estragole; 2.03 mg/g), methyl cinnamate (1.28 mg/g), 4-allyl-2-methoxyphenol (eugenol; 0.896 mg/g), and 1,8-cineole (0.288 mg/g) (Lee et al., 2005). Lee et al. (2005) reported that 2-isopropyl-5-methylphenol (thymol; 8.55 mg/g), 4-isopropyl-2-methylphenol (carvacrol; 0.681 mg/g), linalool (0.471 mg/g), α -terpineol (0.291 mg/g), and 1,8-cineole (0.245 mg/g) were the major aroma constituents of thyme. Among the investigated aroma compounds, eugenol, thymol, carvacrol, and 4-allylphenol were reported to exhibit strong antioxidant activities, which were found to be comparable to those of BHT and α -tocopherol (Lee et al., 2005). Brewer (2011) reported that purple basil extracts possessed a higher total phenolic acid content and a higher antioxidant activity than green basil extracts.

Spice extracts

Cinnamon. Among the investigated herbs and spices (bay leaves, rosemary, sage, marjoram, oregano, cinnamon, parsley, sweet basil, and mint), Muchuweti et al. (2007) reported that cinnamon contained the highest polyphenolic compound concentration. These authors also showed that cinnamon exhibited the highest antioxidant (61.8%) and highest radical scavenging (92.0%) activities among the investigated herbs and spices. Eugenol and cinnamaldehyde are the major components identified in cinnamon leaf oil and cinnamon bark oleoresin, respectively. Vanillic, caffeic, gallic, photochatechuic, *p*-hydroxybenzoic, *p*-coumaric, and ferulic acids as well as *p*-hydroxybenzaldehyde are also antioxidant compounds that were reported in cinnamon (Brewer, 2011). The cinnamon deodorized aqueous extract (CinDAE) was reported to contain a total phenolic and flavonoid content of 315.3 \pm 35.4 mg GAE/g and 99.3 \pm 9.6 mg rutin equivalents (RE)/g, respectively (Chan et al., 2014). When compared to the control samples, Chan

et al. (2014) reported that cooked chicken balls containing CinDAE had increased induction period and redness, whereas their peroxide and TBARS values were significantly lower throughout the storage period at 8°C. In addition, CinDAE did not affect negatively the sensory acceptability of the food product and its antioxidant activity was found to be comparable to that of ascorbic acid, BHA, and BHT (Chan et al., 2014).

Garlic and onions. Garlic and shallots have been reported to contain two main classes of antioxidants, which are the flavonoids (flavones and quercetins) and the sulfur-containing compounds (allyl-cysteine, diallyl sulfide, and allyl trisulfide) (Gorinstein et al., 2008; Brewer, 2011). Besides its antimicrobial effect, garlic has been shown to exhibit antioxidant activity in vitro and in vivo. XXXXSallam et al. (2004) indicated that garlic-rich organosulfur compounds and their precursors (allicin, diallyl sulfide and diallyl trisulfide) are thought to play a key role in its antioxidant potential. Allicin is a major component of the thiosulfates in garlic and is responsible for its characteristic odor. When garlic is crushed, allicin is the product of the conversion of alliin by alliinase. The capacity of allicin to scavenge the peroxy radical and to act as antioxidant was reported by Okada et al. (2005). These authors suggested that the combination of the allyl group ($-\text{CH}_2\text{CH}=\text{CH}_2$) and the $-\text{S}(\text{O})\text{S}-$ group is necessary for the antioxidant action of thiosulfates in the garlic extract. In addition, the $-\text{S}(\text{O})\text{S}-\text{CH}_2\text{CH}=\text{CH}_2$ combination was found to make a much larger contribution to the antioxidant activity of the thiosulfates than $\text{CH}_2 = \text{CH}-\text{CH}_2-\text{S}(\text{O})\text{S}-$ one (Okada et al., 2005). Gorinstein et al. (2008) reported that garlic contained almost double the amount of *trans*-hydroxycinnamic acids (caffeic, *p*-coumaric, ferulic and sinapic acids) than white and red onions. However, the highest amount of quercetin was found in red onions (Gorinstein et al., 2008). The higher radical scavenging activities of onion extracts over than of garlic extracts were shown to be due primarily to their higher total phenolic contents (Nuutila et al., 2003). Among the investigated ingredients (fresh garlic, garlic powder, BHA and garlic oil), fresh garlic was found to be the most effective one in controlling lipid oxidation in raw chicken sausages during storage at 3°C followed by garlic powder (Sallam et al., 2004). The addition of garlic extracts to pork patties resulted in decreased TBARS values, pH and redness (Park and Chin, 2010).

Other spices. Fresh and dried ginger were reported to contain relatively high amounts of the volatile oils camphene, *p*-cineole, α -terpineol, zingiberene and pentadecanoic acid, whereas, the major components of cumin were cuminal, γ -terpinene and pinocarveol. Cumin essential oil was better in reducing Fe^{3+} ions than dried and fresh ginger (El-Ghorab et al., 2010). Ginger extract was shown to exhibit an antioxidant activity that is almost equal to that of BHA and BHT (Brewer, 2011). Kikuzaki and Nakatani (1993) indicated that 12 out of the 5 gingerol-related compounds and 8 diarylheptanoids isolated from ginger rhizomes exhibited higher antioxidative activity than α -tocopherol. These authors suggested that this antioxidant activity is probably dependent upon side chain structures and substitution patterns on the benzene ring. Among the 22 investigated herbs and spices, ginger extract was found to exhibit one of the

highest levels of inhibition (75%) on lipid oxidation when added to pork meat homogenates (Tanabe et al., 2002). Turmeric is well known for its medicinal value in traditional Indian systems of medicine and has been commonly used as a spice throughout Asia for centuries. It was also shown to possess antioxidant properties (Jagannath et al., 2006). Ground turmeric is composed mainly of curcumin, dimethoxycurcumin, bis-dimethoxycurcumin and 2,5-xyleneol. The free radical scavenging ability of turmeric oil was reported to be comparable to that of vitamin E and BHT. α - and β -turmerone, curlone as well as α -terpineol are the major components of turmeric oil that are responsible for this antioxidant activity (Brewer, 2011). Carrots with edible coating made of a miscible blend of casein and turmeric exhibited satisfactory color and carotenoid content for 10 days as opposed to 3 days in uncoated carrots (Jagannath et al., 2006). Other spices with antioxidant activities include black pepper, nutmeg and clove (Brewer, 2011).

Tea and fruit extracts

Tea and grape seed extracts. Green, black and oolong are the 3 main types of tea, which differ in their processing procedures. Among these types, green tea extract contains the highest total phenolic content of which 94% are flavonoids. On the other hand, oolong tea contains around 18% total phenolics and 4.4% flavonoids. In black tea, teaflavins and thearubigins are the predominant components (Brewer, 2011). Abdullin et al. (2001) reported that the strong antioxidant activity of tea is mainly due to the presence of naturally-occurring flavonoids, tannins and some vitamins. Grape seed extracts are rich in catechin and epicatechin and their contents of total phenols are depended on the variety of grape, on environmental and climate conditions, soil type, degree of ripeness and on the extraction procedure and solvents used (Brewer, 2011). Grape seed and green tea extracts delayed redness loss and lipid oxidation in low sulphite raw beef patties when compared to the control samples. They also retarded the onset of rancid flavors in cooked patties (Banon et al., 2007). These extracts were also found to be more effective than sodium ascorbate at preventing lipid oxidation in cooked pork meatballs (Price et al., 2013). Grape seed extract retained the redness in cooked beef during 9 days of refrigerated storage. It also delayed the formation of TBARS by 92% after 9 days of storage and significantly reduced the hexanal content throughout the storage period (Ahn et al., 2007). Lower lipid oxidation values were reported in raw pork burgers containing red grape pomace extract than in the control samples (Garrido et al., 2011). Pork patties containing 0.3% of sea buckthorn extract and 0.1% of grape seed extract exhibited acceptable physico-chemical oxidative stability for 35 days under aerobic and modified atmosphere packaging conditions at refrigerated temperature (Kumar et al., 2015). Grape antioxidant dietary fiber (GADF) inhibited lipid oxidation in raw and cooked chicken hamburgers during the 13 days of storage, which may be due to the presence in GADF of a number of oligomer procyanidins, including catechin and epicatechin (Sáyago-Ayerdi et al., 2009). Fresh-cut lettuce treated with green tea extract (0.25 g/100 mL) exhibited higher ascorbic and carotenoid contents than those treated with chlorine (Martín-Diana et al., 2008).

Cranberry extracts. Among other fruits, cranberries have been shown to exhibit one of the highest antioxidant properties. Caillet et al. (2011) investigated the antioxidant activities of cranberry juice and three extracts obtained from frozen cranberries at pH 2.5 and 7. The cranberry extract 1 was mainly composed of water-soluble phenolics, whereas cranberry extract 2 and 3 mainly contained apolar phenolics (flavonols, flavan-3-ols and proanthocyanidins) and anthocyanins, respectively. Among the tested samples, cranberry extract 1 exhibited the highest free radical-scavenging (68.2 mmol Trolox equivalent (TE)/mg phenol) and antioxidant (13.4 mmol TE phenol) activities. Phenol polarity, pH of the medium and the processing of the juice were all found to influence the antioxidant activities of the investigated samples (Caillet et al., 2011; Côté et al., 2011a). Caillet et al. (2012b) investigated the antioxidant and antiradical activities of fractions of different polarities obtained from two cranberry juices (clarified juice and juice concentrate) and three extracts isolated from frozen cranberries and pomace containing anthocyanins, water-soluble and apolar phenolic compounds, respectively. Among the samples tested, the intermediate polarity fraction rich in apolar phenolics of fruit exhibited the highest antiradical capacity, whereas the most hydrophobic fractions of the anthocyanin-rich extract from fruit and pomace were found to be the most effective at inhibiting lipid oxidation. In addition, the phenol polarity and the industrial processing of cranberry juice were found to influence the antioxidant and antiradical activities of the investigated fractions (Caillet et al., 2012b).

Pomegranate extracts. The peel and rind of pomegranate have been reported to contain good amounts of tannins, anthocyanins and flavonoids. Commercial pomegranate juice was reported to have an antioxidant activity that is three times higher than that of green tea and red wine (Ahmad et al., 2015). After 15 days of storage at 4°C, the TBARS values were significantly lower from 1.272 in control cooked chicken patties to 0.896, 0.763, and 0.203 mg malonaldehyde per kg samples in patties containing BHT, pomegranate juice and pomegranate rind powder extract, respectively (Naveena et al., 2008b). Naveena et al. (2008b) indicated that the addition of pomegranate juice and pomegranate rind powder extract at a level of 10 mg equivalent phenolics/100 g meat would be sufficient to prevent lipid oxidation in chicken patties for a period of time than can be longer than the most commonly used synthetic additives such as BHT. Naveena et al. (2008a) also showed that the pomegranate rind powder extract was able to inhibit lipid oxidation in cooked chicken patties to a greater extent than vitamin C. Kanatt et al. (2010) also reported that pomegranate peel extract was effective in controlling lipid oxidation in chicken chilly and chicken lollipop.

Other fruit extracts. Several studies have reported the antioxidant potentials of fruit extracts (Caillet et al., 2011). Among 92 phenolic extracts from edible and nonedible plant materials (berries, fruits, vegetables, herbs, cereals, tree materials, plant sprouts and seeds), Kähkönen et al. (1999) showed that berries contained relatively high total phenol contents (12.4–50.8 mg/g GAE) and exhibited high antioxidant activities. The formation of methyl linoleate-conjugated diene hydroperoxides was

shown to be inhibited over 90% by crowberry, rowanberry, cloudberry, cranberry, whortleberry, aronia, gooseberry, bilberry, and cowberry extracts when used at levels of 500 ppm. Raspberry and black current extracts were less effective with inhibitions of 88% and 83%, respectively. The presence of bearberry extract in raw pork patties significantly decreased lipid oxidation on day 9 and 12 of storage under MAP conditions when compared to the controls (Carpenter et al., 2007). The addition of bearberry extract at 80 and 1000 $\mu\text{g/g}$ concentrations was also shown to significantly decrease lipid oxidation in cooked pork patties stored under MAP conditions without affecting their sensory properties (Carpenter et al., 2007). The addition of acerola fruit extract extended the shelf-life of beef patties stored under MAP conditions by 3 days by improving their color and lipid stability (Realini et al., 2015). Citrus fruits have also been reported to possess antioxidant activities (Ahmad et al., 2015). Citrus fruits tend to be abundant in flavonoids and more specifically the glycosylated flavanones and polymethoxyflavones. Citrus waste water (5–10%) obtained as a co-product during the extraction of dietary fiber was shown to reduce the residual nitrite levels and degree of lipid oxidation in bologna sausage samples after 24 h of storage (Viuda-Martos et al., 2009). After 12 days of storage, Swedish-style meatballs containing orange and lemon extracts exhibited lower TBARS values than control samples (Fernandez-Lopez et al., 2005).

Mechanism of action of antioxidants

Antioxidants are compounds that play a major role in delaying/preventing autoxidation by inhibiting the formation of free radicals or by interrupting the propagation of the free radical by one or more of the following mechanisms: (1) Scavenging species that initiate peroxidation, (2) Chelating metal ions, (3) Quenching $\cdot\text{O}_2$ preventing the formation of peroxides, (4) Breaking the autooxidative chain reaction, (5) Decreasing localized oxygen concentrations, and/or (6) Stimulating the antioxidative enzyme activities. The most effective antioxidants are the ones who are capable of interrupting the free radical chain reaction. They usually contain one or more aromatic rings (often phenolic) with one or more $-\text{OH}$ groups and are capable of donating H^\bullet to the free radicals produced during oxidation becoming a radical themselves (Dorman et al., 2003; Yoo et al., 2008; Brewer, 2011). Phenolic acids act as antioxidants by trapping free radicals. On the other hand, flavonoids have the ability to scavenge free radicals and chelate metals thus slowing the process of autoxidation via two mechanisms (Brewer, 2011).

Addition of natural antimicrobial/antioxidants directly to food products

Chemical additives are commonly used in food products to inhibit the process of lipid oxidation and microbial growth and to extend their shelf-life (Fernandez-Lopez et al., 2005). However, the potential health risks associated with these synthetic chemicals along with consumer preference for natural food additives have motivated the food industry to seek natural alternatives (Ahmad et al., 2015). Natural antimicrobial/antioxidant agents can be directly added to food products. Nevertheless, the direct addition of plant-based essential oils to food products as preservatives can be limited by their flavor aspect

especially when used at high concentrations (Dussault et al., 2014). Some applications for the use of natural antimicrobials/antioxidants for shelf-life extension of meat, poultry as well as fruit and vegetable products are presented in Tables 2, 3 and 4, respectively.

Incorporation of natural antimicrobial/antioxidant agents into packaging systems

Due to their poor biodegradability and nonrenewability, the high demand for synthetic packaging materials is causing many environmental problems. Over the last decade, these environmental issues have become more important for both the consumer and the food industry, which had lead to the extensive research on biopolymer-based packaging systems (Irkin and Esmer, 2015). The use of edible films is not only to replace synthetic packaging films but also to provide opportunities for new product development. Edible films are capable of controlling the mass transfer between food and the environment as well as between various food product components, thus extending shelf-life and quality (Khwaldia et al., 2004). Edible films can be used as a wrapping material on food products in order to reduce surface contamination. The incorporation of natural antimicrobial/antioxidant agents into edible films can provide additional protection against pathogenic and spoilage microorganisms that are known to contaminate food surfaces as well as meet growing consumer desires for safe, natural food products (Ravishankar et al., 2012). Bioactive packaging is a novel technique used to preserve various types of foods by releasing antimicrobial/antioxidant agents, which have been incorporated into the edible packaging material during its preparation. The release of these agents can be controlled over an extended period of time in order to maintain or prolong the quality as well as shelf-life of food products, without the need for direct addition of these additives into the food product. The edible packaging material can be made of proteins, polysaccharides, lipids, etc. (u Nisa et al., 2015).

Advantages of antimicrobial/antioxidant packaging system

Antimicrobial/antioxidant films can possess many advantages over the direct addition of preservatives into food products to extend their shelf-life, quality and safety (Irkin and Esmer, 2015). By incorporating the preservatives into the packaging material, only the necessary amount of antimicrobial/antioxidant agent is used, limiting the levels of preservatives that come in contact with the food product. Furthermore, the incorporation of antimicrobial/antioxidant agents into films would prevent them from leaching into the food matrix and interacting with other food compounds, including lipids and proteins, which could lead to some loss in their activity. Moreover, by the controlled release of these agents from the packaging material to the surface of the food product, antimicrobial/antioxidant films would not only allow initial inhibition of undesirable microorganisms but also residual activity over time during the transport and storage of food products for distribution, which could make them much more effective. Last but not least, the



Table 2 Natural ingredients used for shelf-life extension of meat products

Natural ingredients	Meat products	Major bioactive ingredients	Antimicrobial tests	Oxidative stability	Experimental findings	References
Acerola fruit extract-MAP ^a (80%O ₂ /20%CO ₂)	Beef patties	Vitamin C/Phenolic acids/Anthocyanins	TVC ^b	TBARS ^c Color measurement	Shelf-life extension by at least 3 days by improving color and lipid stability. The addition of acerola fruit extract had no effect on microbial count of beef patties.	(Realini et al., 2015)
Red sweet cayenne-MAP ^a Hot cayenne-MAP ^a Black pepper-MAP ^a White pepper-MAP ^a (80%O ₂ /20%CO ₂)	Fresh pork sausages	Carotenoids Capsacnoids/Carotenoids β -Caryophyllene/Limonene/ β -Pinene/ Sabinene Capsacnoids/Caratenoids.	TVC ^b	TBARS ^c Color measurement	Black pepper was the best suited for shelf-life extension of product (Delayed discoloration and off-odor formation as well as small change in color).	(Martinez et al., 2006)
Grape seed extract Green tea extract	Low sulphite beef patties	Flavonoids (Catechins/Proanthocyanidins) Flavonoids (Catechins)	TVC ^b Coliform counts	TBARS ^c Color measurement Metmyoglobin percentage	Grape seed and green tea extracts delayed microbial spoilage, redness loss and lipid oxidation, extending the shelf-life of low sulphite raw beef patties by 3 days. They also delayed the onset of rancid flavors in cooked patties. No anomalous sensory traits were caused by either extract. The compounds improved the preservative effect of sulphite on beef patties.	(Banon et al., 2007)
Grape seed extract Green tea extract	Cooked pork meatballs	Flavonoids (Catechins/Proanthocyanidins) Flavonoids (Catechins)	TVC ^b Coliform counts Molds/Yeasts	TBARS ^c Volatile compounds Cholesterol oxidation Color stability	Green tea and grape seed extracts were more effective than sodium ascorbate at preventing lipid oxidation and decreasing microbial growth. The addition of extracts provided brown shades.	(Price et al., 2013)
Grape seed extract Oregano extract Oleoresin rosemary	Raw beef and pork patties	Flavonoids (Catechins/Proanthocyanidins) Carvacrol/Thymol Phenolic diterpenes/Phenolic acids		TBARS ^c Color stability	The antioxidants had minimal effect on lipid oxidation in comparison to the control which could be due to concentration used.	(Rojas and Brewer, 2008)
Vitamin E/Rosemary extract/Irradiation	Overwrapped minced meat	Phenolic diterpenes (Carsonic acid) Phenolic acids (Rosmarinic and caffeic acids)		TBARS ^c Color stability Metmyoglobin percentage	Incorporation of antioxidants resulted in better retention of color. Metmyoglobin values were decreased in irradiated samples during the 8 days of storage. Antioxidant treatments were effective at inhibiting lipid oxidation even at the high irradiation dose of 4 kGy.	(Formanek et al., 2003)

Grape pomace Extract	Raw pork burgers	Anthocyanins		TVC ^b Psychrophile count Total coliform count	TBARS ^c Color measurement	A significant decrease in TBARS ^c values in the presence of grape pomace extract. The addition of grape pomace extract did not have an effect on the spoilage microorganisms of pork burgers.	(Garrido et al., 2011)
Sesamol Olive leaf extract Lutein Ellagic acid	Raw and cooked pork sausages	Carotenoid Phenolic compound		TVC ^b	TBARS ^c Color stability	The addition of natural ingredients had no effect on TVC ^b . Antioxidant potency was in the order: sesame oil > ellagic acid > olive leaf extract > lutein in raw and cooked pork sausages.	(Hayes et al., 2011)
Tea Catechins	Cooked beef and pork patties	Catechins		TBARS ^c	TBARS ^c	Tea catechin levels of 300 mg/kg (Tang et al., 2001) inhibited the pro-oxidation caused by NaCl and controlled the lipid oxidation for all cooked muscle patties.	
Grape seed extract Oleoresin rosemary beef Pine bark extract	Cooked ground	Flavonoids (Catechins/Proanthocyanidins) Phenolic diterpenes/Phenolic acids Proanthocyanidins (Procyanidins)		Escherichia coli O157:H7 Salmonella typhimurium Listeria monocytogenes Aeromonas hydrophila	TBARS ^c Color measurement Hexanal content	Pine bark and grape seed extracts reduced the number of E. coli and S. typhimurium and retarded the growth of L. monocytogenes and A. hydrophila. Pine bark and grape seed extracts also maintained the redness in cooked ground beef during storage. All natural extracts retarded formation of TBARS ^c and lowered the hexanal content throughout the storage period.	(Ahn et al., 2007)
Thymol	Fresh minced beef patties	Thymol		Enterobacteriaceae Pseudomonas spp. Lactic acid bacteria Brochothrix thermosphacta Colliforms count Total psychrotrophic count		Thymol significantly reduced the coliform and Enterobacteriaceae counts. Thymol in combination with MAP had a synergistic effect effectively slowing down the growth of all the investigated microorganisms.	(Del Nobile et al., 2009)
Tea polyphenol	Pork sausages	Polyphenol		TVC ^b Lactic acid bacteria	TBARS ^c Color measurement	Samples with tea polyphenol exhibited lower changes in TVC, TBARS ^c and sensory characteristics than control samples.	(Wenjiao et al., 2014)

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Table 2 (Continued)

Natural ingredients	Meat products	Major bioactive ingredients	Antimicrobial tests	Oxidative stability	Experimental findings	References
SBTE ^d Grape seed extract	Pork patties	Flavonols/catechins/Phenolic acids Flavonoids (Catechins/Proanthocyanidins)	TVCh ^b Coliform count Yeasts/Molds Staphylococcus spp. Psychrophilic count	Peroxide value TBARS ^c Free fatty acids Color measurement	Pork patties containing 0.3% SBTE ^d + 0.1% grape seed extract possessed acceptable physico-chemical oxidative stability, sensory and microbiological quality for 35 days under aerobic and MAP ^a packaging conditions at refrigerated temperature. The addition of garlic extracts to pork patties decreased pH, redness and TBARS ^c values as well as number of TVCh ^b and Enterobacteriaceae.	(Kumar et al., 2015)
Garlic extract	Pork patties	Flavonoids Sulphur-containing compounds	TVCh ^b Enterobacteriaceae	TBARS ^c Color measurement	Grape seed and bearberry extracts significantly decreased lipid oxidation in raw pork patties on day 9 and 12 of storage under MAP conditions when compared to the controls. They also significantly reduced lipid oxidation in cooked pork patties stored under MAP conditions without affecting their sensory properties. Grape seed and bearberry extracts had no impact on microbial population of pork patties when compared to controls.	(Park and Chin, 2010)
Grape seed extract Bearberry extract	Raw and cooked pork patties	Flavonoids (Catechins/Proanthocyanidins) Anthocyanins	Mesophilic count	TBARS ^c Color measurement	Grape seed and bearberry extracts significantly decreased lipid oxidation in raw pork patties on day 9 and 12 of storage under MAP conditions when compared to the controls. They also significantly reduced lipid oxidation in cooked pork patties stored under MAP conditions without affecting their sensory properties. Grape seed and bearberry extracts had no impact on microbial population of pork patties when compared to controls.	(Carpenter et al., 2007)
Thyme oil Oregano oil Citrus waste water	Bologna samples	Thymol/Carvacrol/ <i>p</i> -cumene/ <i>γ</i> -terpinene Carvacrol/Thymol/ <i>p</i> -cumene/ <i>γ</i> -terpinene Flavonoids		TBARS ^c Radical scavenging capacity	Citrus waste water (5-10%) obtained as co-product during the extraction of dietary fiber and oregano or thyme essential oils (0.02%) reduced the residual nitrite levels and the degree of lipid oxidation in bologna sausages samples.	(Viuda-Martos et al., 2009)

aMAP: Modified atmosphere packaging.

bTVC: Total viable count.

cTBARS: Thiobarbituric acid reactive substances.

dSBTE: Sea buckthorn extract.

Table 3 Natural ingredients used for shelf-life extension of poultry products

Natural ingredients	Poultry products	Major bioactive ingredients	Antimicrobial tests	Oxidative stability	Experimental findings	References
CinDAE ^a	Cooked chicken meatballs	Eugenol ^b Cinnamaldehyde		PVC ^c TBARS ^d	PVC ^c and TBARS ^d values < control. Comparable to ascorbic acid/BHTe ^e /BHAF ^f .	(Chan et al., 2014)
Garlic	Raw chicken Sausages	Flavonoids Sulphur-containing compounds	TVCh ^h	PVC ^c TBARS ^d	Antioxidant activity FGg ^g > GPo ^g > BHAF ^f > GOG ^g . Antimicrobial activity FGg ^g > GPg ^g > GOG ^g > BHAF ^f . Shelf-life extension up to 21 days using FGg ^g /GPg ^g .	(Sallam et al., 2004)
GADFI ⁱ	Raw and cooked chicken hamburgers	Flavonoids (Catechins)	TVCh ^h Pseudomonas spp. Brochothrix thermosphacta LABm ^m Enterobacteriaceae Listeria monocytogenes	ABTS ^j , TBARS ^d	GADFI ⁱ inhibited lipid oxidation during 13 days of storage.	(Sáyago-Ayerdi et al., 2009)
Nisin-EDTAK ^k	Fresh chicken meat				Shelf-life extension by 13-14 days, using 500 IU/g nisin and 50 mM EDTAK ^k under MAP ^l conditions (65%CO ₂ /30%N ₂ /15%O ₂).	(Economou et al., 2009)
Listex™ P100	Cooked Turkey	Phage P100			Initial reduction of <i>L. Monocytogenes</i> numbers. To be used in combination with other antimicrobials to enhance the shelf-life of RTE-foods.	(Chibeu et al., 2013)
LMP-102	RTE-Poultry products	6 phages	170 strains of <i>L. Monocytogenes</i>		Application by spraying on RTE-poultry products	(García et al., 2008)
Oregano Sage	Precooked chicken meatballs	Cavacrol/Thymol Thujone		Hexanal levels by GC/MSn ⁿ	After 6 days of storage, hexanal levels were 56- and 12-times lower in samples containing oregano and sage, respectively than in control samples.	(Marques Pino et al., 2013)
Oregano oil-MAPI	Fresh chicken breast	Cavacrol/Thymol/ <i>ρ</i> -cumene/ <i>γ</i> -terpinene	TVCh ^h Pseudomonas spp. Brochothrix thermosphacta LABm Enterobacteriaceae, Yeasts	TBARS ^d	Shelf-life extension by more than 20 days using MAPI ^l (30%CO ₂ /70%N ₂ or 70%CO ₂ /30%N ₂)-1% oregano oil. Microbial populations were the lowest using MAPI ^l -oregano oil combination. TBARSd values were low in all treatments.	(Chouliara et al., 2007)
PPEo ^o	Chicken chilly Chicken lollipop	Flavonoids	Total bacterial count Coliform count Staphylococcal count	TBARS ^d	Using 0.1 and 0.5% PPEo ^o , shelf-life extension of chicken chilly and chicken lollipop by 13 days, respectively. Lower TBARS values in the presence of PPEo ^o .	(Kanatt et al., 2010)
VivOX4 VivOX20	Chicken frankfurters	4% (w/w) Carnosic acid 20% (w/w) Carnosic acid	APCp ^p	Rancimat method	Higher oxidative stability than control. Lower APCp ^p than control at 4 and 12°C.	(Riznar et al., 2006)
Dry honey	Turkey slices		TVCh ^h	TBARS ^d Oxidative stability instrument	Product with 15% dry honey showed little to no bacterial growth during 11 weeks storage. TBARS ^d values were the lowest in products containing 15% honey.	(Antony et al., 2006)

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Table 3 (Continued)

Natural ingredients	Poultry products	Major bioactive ingredients	Antimicrobial tests	Oxidative stability	Experimental findings	References
Rosemary extract	Cooked turkey products	Phenolic diterpenes (Carnosic acid) Phenolic acids (Rosmarinic and caffeic acids)	TBARS ^d		Lowest TBARS ^d and hexanal values obtained using 500 ppm of rosemary extract.	(Yu et al., 2002)
Tea Catechins	Cooked chicken duck and ostrich patties	Catechins	TBARS ^c		Tea catechin levels of 300 mg/kg minced muscle significantly inhibited the pro-oxidation caused by NaCl and controlled the lipid oxidation for all cooked muscle patties.	(Tang et al., 2001)
Pomegranate juice Req ^q	Cooked chicken patties	Tannins, anthocyanins, flavonoids	TBARS ^c		After 15 days of storage at 4°C, the TBARS values were significantly lower from 1.272 in control cooked chicken patties to 0.896, 0.763 and 0.203 mg malonaldehyde per kg samples in patties containing BHT, pomegranate juice and rind pomegranate powder extract, respectively.	(Naveena et al., 2008a)
Req ^q	Cooked chicken patties	Tannins, anthocyanins, flavonoids	TBARS ^c		Req ^q inhibited lipid oxidation in cooked chicken patties to a greater extent than vitamin C.	(Naveena et al., 2008a)

aCinDAE : Cinnamon bark deodorised aqueous extract.

bBrewer (2011).

cPV: Peroxide value assay.

dTBARS: Thiobarbituric acid reactive substances.

eBHT: Butylated hydroxytoluene.

fBHA: Butylated hydroxyanisole.

gFG: Fresh garlic; GP: garlic powder; GO: garlic oil.

hTVC: Total viable count.

iGADF: Grape antioxidant dietary fiber.

jABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay.

kEDTA: Ethylene diamine tetra acetate.

lMAP: modified atmosphere packaging.

mLAB: Lactic acid bacteria counts.

nGC/MS: Gas chromatography/Mass spectrometry.

oPPE: Pomegranate peel extract.

pAPC: Aerobic plate count.

qRE: pomegranate rind powder extract.

Table 4 Natural ingredients used for shelf-life extension of vegetable and fruit products

Natural ingredients	Vegetable products	Major bioactive ingredients	Antimicrobial tests	Oxidative stability	Experimental findings	References
Green tea extract	Fresh-cut lettuce	Flavonoids (Catechins)		Ascorbic acid content Carotenoid content	Green tea extract (0.25 g/100) better preserved the ascorbic acid and carotenoid contents in the samples than Chlorine.	(Martín-Diana et al., 2008)
Lemon verbena oil Lemon balm oil Cypress leaf oil	Grated carrots	Geraniol/Neral/1,8-Cineole Citronellal/ Geraniol/Neral/ β -Carophyllene α -Pinene/ d-3-Carene/ β -pinene/Limonene	<i>Listeria innocua</i> <i>Staphylococcus aureus</i> <i>Escherichia coli</i>		In the presence of oils, <i>L. innocua</i> and <i>S. aureus</i> were not detected after 8 days of storage in plastic bags. The presence of oils reduced <i>E. coli</i> population in samples stored in plastic bags after 8 days of storage. Reduction of <i>S. enterica</i> below detection level using 1% Carvacrol at day 0.	(Romeo et al., 2010)
Carvacrol Cinnamaldehyde	Celery	Carvacrol Cinnamaldehyde	<i>Salmonella enterica</i>		Reduction of <i>S. enterica</i> by 1 log using 1% cinnamaldehyde at day 0.	(Ravishankar et al., 2010)
Citron essential oil Citral	fruit-based salad	Citral Citral	<i>Salmonella enteritidis</i> E4 <i>Listeria monocytogenes</i> <i>Escherichia coli</i> 555 Yeasts Lactic acid bacteria (LAB)		Both citral and citron essential oil extended the shelf-life of ready-to-eat fruit salads. Citron essential oil did not induce any undesirable color/structure change of fruits in comparison to citral. Citron essential oil was more effective against the Gram- positive bacteria <i>L. monocytogenes</i> .	(Belletti et al., 2008)
Thymol Eugenol (Under MAP ^a)	Table grapes	Thymol Eugenol	Mesophilic aerobic bacteria Yeasts/molds		Reduction of yeasts/molds counts (1.7–2.4 log cfu/g) and mesophilic aerobic bacteria (2.2–2.4 log cfu/g) counts were observed.	(Valero et al., 2006)
MJb ^b AITCc ^c Tea tree oil	Raspberries	MJb ^b AITCc ^c		Antioxidant enzyme measurement ORACd ^d assay	Raspberry extract after MJb ^b treatment had the highest antioxidant capacity and the highest activity in all antioxidant enzymes among the tested treatments. AITCc ^c showed the best result for decay inhibition, which is due to its antimicrobial properties.	(Chanjirakul et al., 2006)

aMAP: Modified atmosphere packaging.

bMJ: Methyl jasmonate.

cAITC: Allyl isothiocyanate.

dORAC: Oxygen radical absorbance capacity.

industrial production process can be greatly simplified with the use of antimicrobial/antioxidant films (Irkin and Esmer, 2015).

Methods of incorporation

The incorporation of preservative agents into edible films can be done through three different methods: (1) Their incorporation directly into edible films by blending them with the biopolymer material before manufacturing; (2) Coating preservative agents onto polymer surfaces; and (3) Their immobilization by chemical grafting. There are two types of antimicrobial packaging systems and the difference is depended on the type of preservative used and its interactions with the packaging and the food matrix (Irkin and Esmer, 2015). Migrating antimicrobial packaging systems are films that contain a preservative that will migrate to the surface of the food product, whereas the nonmigrating antimicrobial packaging systems contain a preservative that is immobilized onto the packaging system. In the latter case, the antimicrobial packaging system becomes effective against the growth of undesirable microorganisms when there is a direct contact between the packaging material and the food (Kuorwel et al., 2011).

Type of materials used for the development of edible films/coatings

Based on the literature (Joerger, 2007; Kuorwel et al., 2011; Irkin and Esmer, 2015), polysaccharides and proteins are the most commonly investigated materials for the development of edible films/coatings. Edible films can contain antimicrobial agents, such as bacteriocins, enzymes, chitosan, plant extracts, essential oils or their components (Irkin and Esmer, 2015). Some applications of natural ingredients and biopolymer coating/packaging materials in meat, poultry and fruits and vegetables are found in Tables 5, 6 and 7, respectively. Edible antimicrobial films tend to exhibit high moisture sensitivity, poor water barrier and poor mechanical properties when compared to the synthetic polymers. Therefore, plasticizers are commonly mixed with biopolymers to improve processing, increase their film flexibility and lower the glass transition temperature. The list of commonly used plasticizers in combination with biopolymers include glycerol, sorbitol, mannitol, fructose, mannose and poly(ethylene glycol). Biopolymers can also be modified physically, mechanically and/or chemically to improve their physico-mechanical properties (Kuorwel et al., 2011).

Polysaccharides and derivatives

Polysaccharides-based edible films possess low gas permeability allowing for shelf-life extension of food products without creating anaerobic conditions. While they also have adequate film-forming properties, they tend to be sensitive to moisture due to the presence of hydrophilic groups in their structure (Kuorwel et al., 2011). Examples of polysaccharides that have the potential to be used as a packaging material for the development of edible films/coatings include starch, alginate, cellulose, chitosan, pectin, gums, and carageenan (Nur Hanani et al., 2014).

Starch. Among the polysaccharide-based polymers, the starch-based ones are the most abundant and cost-effective ones (Cha and Chinnan, 2004). Sources of starch include cereal grains, potatoes, tapioca and arrowroot. Starch is composed of amylose and amylopectin molecules present at different molecular ratios (Kuorwel et al., 2011). Starch-based edible films have superior gas barrier properties but inferior mechanical properties to synthetic films. Nevertheless, when a plasticizer like water is added to a starch-based film, its native granular structure and hydrogen bonding are broken and the film exhibit thermoplastic behavior (Cha and Chinnan, 2004; Kuorwel et al., 2011). Amylose is the molecule responsible for the film-forming capacity of starches. Hence, a high amylose starch polymer can form strong and flexible films that are highly impermeable to oxygen and carbon dioxide (Cha and Chinnan, 2004; Kuorwel et al., 2011). Plantic[®], EverCorn[™], and Bio-P[™] are all commercially available starch-based packaging materials that were developed respectively by Plantic Technologies (Melbourne, Australia), Novamont (Italy) and Bioenvelope (Japan) to package various type of food products (Kuorwel et al., 2011). Antimicrobial starch-based edible films have been reported to exhibit an inhibitory activity against various microorganisms, including *Brochothrix thermosphaceta* B2, *L. monocytogenes*, *E. coli* O157:H7, *S. aureus*, *Lactobacillus plantarum*, *S. enteritidis*, and *S. typhimurium* (Kuorwel et al., 2011). Starch-based edible films containing antioxidants delayed lipid oxidation in raw beef samples (u Nisa et al., 2015). The coating of carrots with yam starch containing 1.5% chitosan inhibited the total coliform and lactic acid bacteria growth throughout the storage period of 15 days. In addition, there was a reduction in yeast/mold, mesophilic and psychrotropic counts in the coated carrots during the storage period of 15 days (Durango et al., 2006).

Chitosan. Chitosan can perform a dual role as a film matrix and as an antimicrobial agent (Joerger, 2007). Chitosan exhibit a good film property. It is also biodegradable, biocompatible and nontoxic. Pure chitosan films reduced *L. monocytogenes* inoculated on bologna slices by 2 logs, whereas films with 1% and 2% oregano essential oil reduced *L. monocytogenes* by 3.6–4 logs and *E. coli* by 3 logs in bologna slices (Zivanovic et al., 2005). The dipping of fresh chicken breast with pomegranate juice followed by its coating with chitosan containing 2% Zataria multiflora essential oil extended its shelf-life by 15 days (Bazargani-Gilani et al., 2015). The coating of strawberries with chitosan reduced microbial load. In addition, the antimicrobial effect of chitosan was maintained on the strawberries during the 12 days of storage (Devlieghere et al., 2004).

Cellulose and cellulose derivatives. Cellulose is a linear natural polymer composed of anhydroglucose. It is the most abundant natural polymer on earth. It is highly crystalline, fibrous and insoluble. Many water soluble composite coatings are prepared commercially from cellulose, such as carboxymethylcellulose. Methylcellulose and hydroxypropylcellulose are cellulose derivatives that form strong and flexible water soluble films (Cha and Chinnan, 2004). Papers containing carboxymethyl cellulose and lysozyme or lysozyme-lactoferrin reduced the microbiota of the veal capaccio sample by almost 1 log cycle when compared to the control (Barbiroli et al., 2012). Matthews et al.

Table 5 Some applications of natural ingredients and biopolymer coating/packaging materials in meat products

Natural ingredients	Meat products	Type of edible films/coating	Antimicrobial tests	Oxidative stability	Experimental findings	References
Carvacrol Cinnamaldehyde	Ham/Bologna inoculated with <i>Listeria monocytogenes</i>	Apple-based edible film Carrot-based edible film Hibiscus-based edible film	<i>L. monocytogenes</i>		Carvacrol films showed better antimicrobial activity than cinnamaldehyde films. Apple films containing 3% carvacrol reduced microbial population on ham 1 to 2 logs CFU/g more than carrot or hibiscus films on day 0. Films were more effective on ham than bologna. Carvacrol films resulted in greater microbial reductions than cinnamaldehyde films at all tested concentrations. The reduction of <i>L. monocytogenes</i> on ham at 23 °C was greater than at 4 °C.	(Ravishankar et al., 2012)
Carvacrol Cinnamaldehyde	Ham inoculated with <i>L. monocytogenes</i>	Apple-based edible film	<i>L. monocytogenes</i>		Carvacrol films resulted in greater microbial reductions than cinnamaldehyde films at all tested concentrations.	(Ravishankar et al., 2009)
Mix spice	Buffalo meat patties	Sodium alginate coating	TVCa ^a Psychrophilic bacterial count Yeast/mold counts Staphylococcus spp. <i>L. monocytogenes</i> <i>Escherichia coli</i> O157:H7	TBARSb ^b Tyrosine value	Alginate coating significantly decreased TBARSb ^b and tyrosine values, TVCa ^a , psychrophilic bacterial count, staphylococcus spp. count as well as yeast and mold count.	(Chidanandaiah et al., 2009)
Oregano essential oil	Bologna slices	Chitosan edible film			Pure chitosan films reduced <i>L. monocytogenes</i> by 2 logs whereas films with 1 and 2% oregano essential oil reduced <i>L. monocytogenes</i> by 3.6 to 4 logs and <i>E. coli</i> by 3 logs.	(Zivanovic et al., 2005)
Green tea extract	Raw beef samples	Starch-based edible film		TBARSb ^b Metmyoglobin percentage	Starch-based edible films containing antioxidants decreased TBARSb ^b and metmyoglobin values. Starch-based edible films containing BHTc ^c were more effective than those containing green tea extract in delaying lipid oxidation. Green tea extract was more efficient in inhibiting oxymyoglobin oxidation than BHTc ^c and better retained the red color of the raw beef samples.	(u Nisa et al., 2015)
Lysozyme-Lactoferrin	Veal Capaccio	Paper containing Carboxymethyl cellulose	TVCa ^a		Papers containing lysozyme or lysozyme-Lactoferrin reduced the microbiota in the meat sample by almost 1 log cycle when compared to the control.	(Barbiroli et al., 2012)
Nisin	Fresh beef cubes	Cellulose coating of pre-made barrier film pouch	<i>L. monocytogenes</i>		Significant reduction of <i>L. monocytogenes</i> after 36 days of storage at 4°C with the use of pre-made barrier film pouch with interior cellulose coating containing nisin.	(Matthews et al., 2010)
Oregano oil Thyme oil Mixture of both	Ground beef patties	Soy edible film (SPEF)	TVCa ^a LABd ^d Staphylococcus spp. Total coliform count Pseudomonas spp.		SPEF edible films containing 5% (v/w) essential oils reduced the coliform and Pseudomonas spp. counts.	(Emiroglu et al., 2010)
Pomegranate peel Extract	Ground beef	Sodium caseinate film	TVCa ^a Staphylococcus aureus		SPEF edible films had no effect on TVCa ^a , LABd ^d and Staphylococcus spp. Edible films containing the natural antimicrobial agent decreased the TVCa ^a and <i>S. aureus</i> count as compared to control samples. The antimicrobial activity of pomegranate peel extract was more effective on Gram-positive bacteria rather than Gram-negative bacteria.	(Ennam-Djomeh et al., 2015)

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Table 5 (Continued)

Natural ingredients	Meat products	Type of edible films/coating	Antimicrobial tests	Oxidative stability	Experimental findings	References
Oregano oil	Fresh beef	Sorbitol-plasticized whey protein isolate film	TVCa ^a Pseudomonas LABd ^d		The maximum specific growth rate (μ_{max}) of total flora (TVCa ^a) and Pseudomonas were significantly reduced by a factor of 2 in the presence of antimicrobial films, while growth of LABd ^d was completely inhibited.	(Zinoviadou et al., 2009)
Oregano oil Pimento oil	Beef muscle slices	Milk-protein based edible film (calcium caseinate-whey protein isolate)	<i>Escherichia coli</i> O157:H7 Pseudomonas spp.	TBARSb ^b	Pimento edible films exhibited higher antioxidant activity than oregano edible films. Oregano edible films were more effective against <i>E. coli</i> O157:H7 and Pseudomonas spp. than pimento edible films. 0.95 and 1.12 log reduction of Pseudomonas spp. and <i>E. coli</i> population, respectively in films containing oregano oil as compared to control after 7 days of storage at 4°C.	(Oussalah et al., 2004)
Lysozyme-Na ₂ EDTA	Ground beef patties	Zein edible film	TVCa ^a Total coliform counts	TBARSb ^b Color measurement	The TVC of beef patties significantly decreased in the presence of edible films containing lysozyme and Na ₂ EDTA after 5 And 7 days of storage. No effect on the total coliform counts after 7 days of storage. Redness indices of patties coated with zein films were significantly lower than those of uncoated control patties during storage. Significant decrease in oxidation due the presence of Na ₂ EDTA.	(Ünal et al., 2011)

aTVC: Total viable count.

bTBARS: Thiobarbituric acid reactive substances.

cBHT: Butylated hydroxytoluene.

dLAB: Lactic acid bacteria.

Table 6 Some applications of natural ingredients and biopolymer coating/packaging materials in poultry products

Natural ingredients	Vegetable products	Type of edible films/coating	Antimicrobial tests	Oxidative stability	Experimental findings	References
Cinnamaldehyde Carvacrol	Chicken breast (inoculated with various strains of <i>Campylobacter jejuni</i>)	Apple-based edible film	<i>C. jejuni</i> strains		Films with cinnamaldehyde were more effective than carvacrol films. Reductions at 23 °C of microbial population were greater than those at 4 °C. Films \geq 1.5% cinnamaldehyde reduced population of all strains to below detection at 72 h and 23 °C.	(Mild et al., 2011)
Cinnamaldehyde Carvacrol	Chicken breast (inoculated with <i>Salmonella enterica</i> or <i>Escherichia coli</i> O157:H7)	Apple-based edible film	<i>S. enterica</i> <i>E. coli</i> O157:H7		At 23 °C, films with 3% antimicrobials showed the highest reductions (4.3 to 6.8 log CFU/g) for both <i>S. enterica</i> and <i>E. coli</i> O157:H7. At 4 °C, carvacrol films exhibited greater activity than cinnamaldehyde. Films with 3% carvacrol reduced the bacterial population by about 3 logs.	(Ravishankar et al., 2009)
PJa ^a Ze ^e	Fresh chicken breast	Chitosan edible coating (CH)	TVC ^b LAB ^f Enterobacteriaceae Psychrotrophic bacteria Yeasts-molds	PVC ^c -TBARS ^d Total carbonyl content	Shelf-life extension by 10-15 days using PJa ^a , PJa ^a -CH, PJa ^a -CH-Ze ^e 1% and PJa ^a -CH-Ze ^e 2%. Best microbial reduction obtained using PJa ^a -CH-Ze 2%. Lowest PVC ^c and TBARS ^d values were obtained using PJa ^a -CH-Ze ^e 2%. Lowest carbonyl content was attained using PJa ^a -CH-Ze ^e 2%.	(Bazargani-Gilani et al., 2015)
Oregano oil Clove oil	Chicken breast	Whey protein isolate edible coating	Total aerobic mesophilic bacteria Total aerobic psychrotrophic bacteria <i>Pseudomonas</i> spp. Enterobacteriaceae Lactic acid bacteria		Coatings with 20 g/kg of oregano oil extended the shelf-life of chicken breast by 7 days. Oregano edible coatings were more effective on the 5 microbial populations than clove edible coatings, with <i>Pseudomonas</i> spp. being the most resistant.	(Fernández-Pan et al., 2014)
Nisin	Ready to eat chicken (inoculated with <i>L. monocytogenes</i>)	Zein edible coating	<i>L. monocytogenes</i>		The most effective storage temperature to suppress the growth of <i>L. monocytogenes</i> using zein coating containing nisin was found to be 4 °C.	(Janes et al., 2002)

aPJ: Pomegranate juice.
bTVC: Total viable count.
cPY: Peroxide value.
dTARS: Thiobarbituric acid reactive substances.
eZ: Zataria multiflora essential oil.
fLAB: Lactic acid bacteria.

Table 7 Some applications of natural ingredients and biopolymer coating/packaging materials in vegetable and fruit products

Natural ingredients	Vegetable products	Type of edible films/coating	Antimicrobial tests	Oxidative stability	Experimental findings	References
	Cut carrots Cut potatoes Cut onions Cut apples	Sour whey powder (SWP) coating Soy protein isolate coating Calcium caseinate coating		Color measurement Oxidative browning Moisture loss	4.8-fold reduction in browning of cut potatoes coated with SWP. Reduction of moisture loss in cut apples, potatoes and carrots by 18.1, 40% and 59%, respectively over control with the use of SWP. The coated cut apples and potatoes better maintained their fresh appearance. No treatment effect on cut onions. SWP was found to be the most effective one among the investigated coating materials.	(Shon and Haque, 2007)
Turmeric	Carrots	Casein edible coating	Total coliform count TVCa ^a Yeasts/molds	Carotenoid content Color measurement	Coated carrots exhibited satisfactory color, carotenoid content, texture retention and antimicrobial properties for 10 days, as opposed to 3 days in uncoated carrots.	(Jagannath et al., 2006)
Trans-cinnamaldehyde	Fresh cut cantaloupe	Chitosan edible coating Pectin edible coating	TVCa ^a Psychrotrophic count Yeast/mold count	Color measurement	A multilayer edible coating composed of 2 g/100g of encapsulated trans-cinnamaldehyde, 2 g/100g chitosan and 1 g/100g pectin extended the shelf-life of fresh cantaloupe by up to 5 days in comparison to uncoated samples.	(Martín et al., 2014)
Lemon oil	Strawberries	Chitosan (1% w/w) edible coating	<i>Botrytis cinerea</i>		Adding lemon essential oil to chitosan edible coating enhanced the antifungal activity of chitosan against <i>B. cinerea</i> .	(Perdones et al., 2012)
	Strawberries	Chitosan edible coating	Aerobic psychrotrophs Lactic acid bacteria Yeast/mold count		Microbial load was lower in all the chitosan dipped samples. The antimicrobial effect of chitosan was maintained on the Strawberries during the 12 days of storage.	(Devlieghere et al., 2004)
	Carrots	Yam starch – chitosan edible coating	Total coliform count Lactic acid bacteria Yeasts/molds Mesophilic aerobes Psychrotrophs		Yam starch +1.5% chitosan edible coating inhibited the total coliform and lactic acid bacteria growth throughout the storage period of 15 days. Reduction in yeast/mold, mesophilic and psychrotrophic counts in the coated carrots during the storage period of 15 days.	(Durango et al., 2006)

aTVC: Total viable count.

(2010) reported a significant reduction of *L. monocytogenes* in fresh beef cubes after 36 days of storage at 4°C with use of a pre-made barrier film pouch with interior cellulose containing nisin.

Alginate. Alginates are the salts of alginic acid, a linear copolymer of D-mannuronic and L-guluronic acid monomers. They are extracted from brown seaweeds of the *Phaeophyceae*. The alginate film formation is due to the ability of alginates to react with divalent and trivalent cations. Calcium ions are the most effective cations and are commonly used as the gelling agents (Cha and Chinnan, 2004). Cha et al. (2002) reported that Nalginatate film containing ethylene diamine tetraacetic acid, nisin, and lysozyme exhibited the highest inhibitory effect against all the investigated Gram-positive and Gram-negative microorganisms. The alginate coating of buffalo meat patties significantly decreased TBARS and tyrosine values, as well as total viable, psychrophilic bacterial, *Staphylococcus* spp., yeast, and mold counts (Chidanandaiah et al., 2009).

Proteins

Proteins are polymers containing more than 100 amino acid residues as their monomeric units (Kuorwel et al., 2011). They must be denatured by heat, acid, alkali and/or solvent in order to form the more extended structures, which are required for film formation. The films produced are made up of chain-to-chain interactions (hydrogen, ionic, hydrophobic and covalent bonding) and these interactions are critical in forming a continuous three-dimensional network resulting into an effective cohesive film. The inter-action is highly depended on the degree of chain extension and the nature and sequence of amino acid residues (Nur Hanani et al., 2014). Protein-based edible films possess poorer water resistance and lower mechanical strength than synthetic films. Nevertheless, proteins exhibit generally a superior capacity to form films with better mechanical and barrier properties than polysaccharides. Examples of proteins that have the potential to be used as a packaging material for the development of edible films/coating include whey protein, soya protein, corn zein, and/or their derivatives (Nur Hanani et al., 2014).

Milk proteins. Casein. Casein is the major protein component of milk. Casein consists of three principal components (α , β , and κ -casein), which together make up the colloidal micelles in milk. The properties of casein are partly due to its amino acid composition. Casein exhibits better emulsifying properties than whey protein due to its higher content in proline. Caseins are also soluble and capable of forming films with resistance to thermal denaturation and/or coagulation. As a result, casein-based films can remain stable over a wide range of pH, temperature, and salt concentrations (Khwaldia et al., 2004). Casein-based edible films are transparent exhibiting high oxygen barrier properties but also high water vapor permeability. Edible sodium caseinate films containing pomegranate peel extract as an antimicrobial agent decreased the total viable and *S. aureus* counts in ground beef in comparison to the control samples (Emam-Djomeh et al., 2015). Carrots with edible coating made of a miscible blend of casein and turmeric exhibited satisfactory color, carotenoid content, texture retention, and

antimicrobial properties for 10 days, as opposed to 3 days in uncoated carrots (Jagannath et al., 2006).

Whey protein. After casein precipitation at pH 4.6, the protein that remains soluble is called whey protein (Khwaldia et al., 2004). Zinoviadou et al. (2009) reported that the maximum specific growth rate (μ_{max}) of total flora and *Pseudomonas* were significantly reduced by a factor 2 in the presence of sorbitol-plasticized whey protein isolate films containing oregano oil, while the growth of lactic acid bacteria was completely inhibited. Edible films composed of calcium caseinate and whey protein isolate in a ratio of 1:1 (w/w) containing oregano oil resulted in a 0.95 and 1.12 log reduction of *Pseudomonas* spp. and *E. coli* population, respectively in beef muscle slices after 7 days of storage at 4°C, when compared to samples without films (Oussalah et al., 2004). Edible coating made of whey protein isolate with 20 g/kg oregano essential oil extended the shelf-life of chicken breasts by 7 days (Fernández-Pan et al., 2014).

Zein. Zein is a prolamine protein extracted from corn gluten. It is insoluble in water and has a generally recognized as safe status for use in food products. Zein edible coatings are only soluble in organic solvents and form hard and glossy coatings. Due to the good oxygen and lipid barrier properties of zein films, zein is currently being used to coat candy, dried fruits and nut meats (Janes et al., 2002). Zein propylene glycol coating with nisin and calcium propionate, zein ethanol coating with nisin and calcium propionate as well as zein ethanol coating with nisin suppressed the growth of *L. monocytogenes* inoculated at a concentration of 2.7 log CFU/g counts onto ready to eat chicken during 24 days of storage at 4°C. At a higher initial inoculation level of 6.8 CFU/g counts, these films suppressed the growth of *L. monocytogenes* by 4.5–5 log CFU/g after 16 days of storage at 4°C (Janes et al., 2002). The total viable counts of beef patties significantly decreased in the presence of zein edible films containing lysozyme and Na₂EDTA after 5 and 7 days of storage. In addition, the redness indices and oxidation of patties with zein films were significantly lower than those of control samples during storage (Ünalán et al., 2011).

Other proteins. Emiroglu et al. (2010) reported that soy protein edible films containing 5% (v/w) oregano oil, thyme oil or a mixture of both reduced the coliform and *Pseudomonas* spp. counts in ground beef patties but had no effect on total viable count, lactic acid bacteria and *Staphylococcus* spp. Apple-based films containing 3% carvacrol reduced microbial population on ham 1 to 2 logs CFU/g more than carrot and hibiscus-based edible films. These edible films were more effective on ham than bologna (Ravishankar et al., 2012). Ravishankar et al. (2009) reported that apple-based films containing carvacrol resulted in greater microbial reductions in ham inoculated with *L. monocytogenes* than apple-based films containing cinnamaldehyde at all tested concentrations. In addition, the microbial reduction on ham was greater at 23°C than at 4°C. However, apple-based films containing cinnamaldehyde were found to be more effective on chicken breast inoculated with various strains of *Campylobacter jejuni* than apple-based films containing

carvacrol. The reduction of these strains was greater at 23°C than at 4°C. Moreover, apple-based films containing $\geq 1.5\%$ cinnamaldehyde were able to reduce the growth of various strains of *C. jejuni* below the detection level at 72 h and 23°C (Mild et al., 2011).

Conclusions

This review has shown that natural antimicrobials/antioxidants have the potential to replace chemical additives in meat and poultry products as well as fruits and vegetables to extend their shelf-life as well as safety and quality. The strong antimicrobial/antioxidant activities of some plant extracts and essential oils are mainly due to the presence of phenolic compounds, including terpenes and flavonoids. The use of pure bioactive compounds of plants as preservatives was also found to be effective. Enzymes were shown to be promising natural antimicrobials due to their ability to produce antimicrobial compounds or due to their ability to disintegrate the outer membrane of some bacteria; however, their applications in food products have to be further investigated. Bacteriophages are currently approved for use as processing aids to control the growth of specific pathogens rather than to extend the shelf-life of food products. Although there is limited information in the literature on the use of fermented ingredients as potential antimicrobial agents in food products, these ingredients are currently commercially available on the market. Ozone can be used as an effective antimicrobial agent in food products as well as an effective alternative sanitizer to chlorine in the food industry. The development of edible films/coatings containing natural antimicrobials/antioxidants is growing due their biodegradability and ability to extend the shelf-life, safety and quality of food products. However, further research is needed in order to improve the properties of edible films. There is great scope of further exploration of these natural antimicrobials/antioxidants to determine their synergy and allow their more effective use in food products. Moreover further studies are needed in order to determine the best method of incorporation of these natural additives into food.

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