This review discusses the status, antimicrobial mechanisms, application, and regulation of natural preservatives in livestock food systems. Conventional preservatives are synthetic chemical substances including nitrates/nitrites, sulfites, sodium benzoate, propyl gallate, and potassium sorbate. The use of artificial preservatives is being reconsidered because of concerns relating to headache, allergies, and cancer. As the demand for biopreservation in food systems has increased, new natural antimicrobial compounds of various origins are being developed, including plant-derived products (polyphenolics, essential oils, plant antimicrobial peptides (pAMPs)), animal-derived products (lysozymes, lactoperoxidase, lactoferrin, ovotransferrin, antimicrobial peptide (AMP), chitosan and others), and microbial metabolites (nisin, natamycin, pullulan, ε-polylysine, organic acid, and others). These natural preservatives act by inhibiting microbial cell walls/membranes, DNA/RNA replication and transcription, protein synthesis, and metabolism. Natural preservatives have been recognized for their safety; however, these substances can influence color, smell, and toxicity in large amounts while being effective as a food preservative. Therefore, to evaluate the safety and toxicity of natural preservatives, various trials including combinations of other substances or different food preservation systems, and capsulation have been performed. Natamycin and nisin are currently the only natural preservatives being regulated, and other natural preservatives will have to be legally regulated before their widespread use.

Keywords: natural preservative, antimicrobial, safety, food application
ally related to the host defense system (Singh et al., 2010; Tiwari et al., 2009). As the demand for biopreservation in food systems has increased, new natural antimicrobial compounds of various origin are being developed, including animal-derived systems (lysozyme, lactoferrin, and magainins), plant-derived products (phytoalexins, herbs, and spices), and microbial metabolites (bacteriocins, hydrogen peroxide, and organic acids) (Lavermicocca et al., 2003). The requirements of natural preservatives are: safety, stability during food processing (pH, heat, pressure, etc.), and antimicrobial efficacy. The representative food pathogens are *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Yersinia enterocolytica*, *Clostridium perfringens*, *Clostridium botulinum*, and *Campylobacter jejuni*. The pathogenic fungi often related to food-borne diseases are toxin-producing *Aspergillus flavus* and *Aspergillus paraciticus* (Prange et al., 2005).

This review summarizes the current knowledge about natural preservatives regarding their antimicrobial effects, antimicrobial mechanism, application, and regulation in food systems.

### Natural Preservatives of Plant Origin

Plant preservatives are composed to polyphenols and phenolics, essential oils, and plant antimicrobial peptides (pAMPs). These substances have evolved to possess antibacterial and antioxidant effect (Dua et al., 2013). Phenolics and polyphenols have various antimicrobial structures: simple phenols (cafeic acid, catechol, eugenol, and epicatechin) and polyphenols (cafeic acid and cinnamic acid), quinones (hypericin), flavones, flavonols, flavonoids, flavonoids (epigallocatechin-3-gallate, catechin, and chrysain), tannins (pentagalloylgucose, procyanidin B-2), coumarins (coumarin, warfarin, and 7-hydroxycoumarin), terpenoids (menthol, artemisin, and capsaicin), and alkaloids (berberine and harmarne) (Table 1) (Cowan, 1999; Hintz et al., 2015). The pAMPs are represented by thionin, plant defensins, lipid transfer proteins (LTPs), myrosinase-binding proteins (MBPs), hevein- and knottin-like peptides, snakins, cyclotides, and peptides from hydrolysates (Hintz et al., 2015).

### Status of plant preservatives

Plant polyphenol extracts have been used as natural

| Class                | Subclass          | Examples                  | References                        |
|----------------------|-------------------|---------------------------|-----------------------------------|
| Phenolics            | Simple phenols    | Catechol, Epicatechin     | Mason and Wasserman, 1987, Cowan, 1999, Hintz et al., 2015 |
| Phenolic acids       | Cinnamic acid     |                           | Cowan, 1999, Hintz et al., 2015   |
| Quinones             | Hypericin         |                           | Cowan, 1999, Hintz et al., 2015   |
| Flavonoids           | Chrysin, Quercetin|                           | Dua et al., 2013, Lee et al., 2011 |
| Flavonols            | Abyssinone        |                           | Cowan, 1999, Lee et al., 2016, Dua et al., 2013 |
| Tannins              | Ellagitannin      |                           | Cowan, 1999, Dua et al., 2013, Lee et al., 2016 |
| Coumarins            | Coumarin, Warfarin|                           | Cowan, 1999, Hintz et al., 2015   |
| Terpenoids, essential oils | Capsaicin, Eugenol, Thymol, Carvacrol | Bajpai et al., 2008, Gutierrez et al., 2008, Helander et al., 1998, Tiwari et al., 1998 |
| Alkaloids            | Berberine, Harmane, Piperine | Cowan, 1999, Garba and Okeniyi, 2012 |
| Lectins and polypeptides | Mannose-specific agglutinin, Fabatin | Cowan, 1999, Cowan, 1999 |
| Polycyclics          | 8S-Heptadec-2(Z),9(Z)-diene-4,6-diyne-1,8-diol | Cowan, 1999 |
| Antimicrobial peptide (pAMP) | Potato defensin, hevein, thionines, snakins, lipid transfer protein etc. | Hintz et al., 2015, Jessen et al., 2006 |
meat preservatives, including extracts from oregano, cranberry, sage, rosemary, grape seed, and others. Polyphenols can act as reducing agents and metal ion chelators in the presence of various hydroxyl radicals.

Oregano and cranberry extracts were evaluated for antimicrobial activity against L. monocytogenes in laboratory media, beef, and fish (Lin et al., 2004). These phenolic-based plant extracts are widely used in food preparation and are classified as Generally Regarded as Safe (GRAS). The effects of neem oil on the meat pathogens Carnobacterium maltaromaticum, Brochothrix thermosphacta, E. coli, and Pseudomonas fluorescens, were investigated as a preservative for fresh retail meat (Del Serrone et al., 2015a, Del Serrone et al., 2015b). Citrus species extracts were investigated as antifungal agents against spoilage fungi including Mucor sp. and Rhizopus sp. (Mohanka and Priyanka, 2014). Ethanol extract of Citrus species showed a higher antifungal effect than water extract did, and the minimum inhibitory concentration of the extract ranged from 6.25 to 25 mg/mL. Inula britannica ethanols extract showed an antimicrobial effect against five B. cereus strains in low fat milk, and the antimicrobial effect depended on terpene and polyphenol compounds (Lee et al., 2012). Brassica juncea extract showed an antiviral effect against influenza virus A/H1N1 in nonfat milk (Lee et al., 2014). Chestnut inner shell extract containing gallic acid and quercetin was shown an antimicrobial effect against C. jejuni in chicken meat at 1 and 2 mg/mL (Lee et al., 2016). Eight different flavonoids [quercetin, kaempferol, apigenin, luteolin, 5,4-dihydroxy-7-methoxyflavone (genkwanin), narigenin, hesperetin and hesperidin] were tested for antimicrobial effects against B. cereus strains (P14 and KCCM 40935) (Lee et al., 2011). Among these flavonoids, only kaempferol and apigenin were inhibitory, and kaempferol showed the greatest antimicrobial effect at 100 μM.

Essential oil or terpenes are secondary metabolites that provide flavor and aroma. The addition of essential oils from marjoram and rosemary was investigated in beef patties (Mohamed and Mansour, 2012). These essential oils were beneficial for antioxidant activity and sensory evaluation.

Plant antimicrobial peptides (pAMPs) were discovered in 1942 as natural defense compounds against pathogens (Hintz et al., 2015). The pAMPs were named as thionins, plant defensins, lipid transfer proteins (LTPs), myrosinase-binding proteins (MBPs), hevein- and knottin-like peptides, snakins, cyclotides, and peptides from hydrolysates. These substances have been isolated from Triticum aestivum (wheat), Impatiens balsamina, Hordeum vulgare (barley), Arabidopsis thaliana, Hevea brasiliensis, Solanum tuberosum (potato), Oldenlandia affinis, etc.

**Antimicrobial mechanisms of plant preservatives**

The antimicrobial mechanisms of phenol compounds depend on their concentration. Phenols affect enzyme activity related to energy production at low concentrations; however, they cause protein denaturation at high concentrations (Fig. 1). These abilities affect microbial cell permeability, thereby interfering with membrane function (material transport, nucleic acid synthesis, and enzyme activity) (Baipai et al., 2008; Fung et al., 1977; Rico-Munoz et al., 1987). The high antibacterial activity of phenolic compounds can be due to alkyl substitution into the phenol nucleus, forming phenoxy radicals, which does not occur in more stable molecules such as the ethers myristicin or anethole (Dorman and Deans, 2000; Gutierrez et al., 2008). Catechol and pyrogalol possess phenolic toxicity to microorganisms through enzyme inhibition by oxidized compounds, possibly by reacting with sulfhydryl groups or through more nonspecific interactions with proteins (Mason and Wasserman, 1987). The antimicrobial targets of quinones may include surface-exposed adhesins, cell wall polypeptide, and membrane-bound enzymes (Cowan, 1999). The antimicrobial activities of isothiocyanates derived from Brassicaceae vegetables, such as cauliflower, broccoli, mustard, and cabbage are related to 1) loss of cell membranes integrity, 2) inhibiting enzyme or regulatory activity by quorum sensing (in Helicobacter pylori, Pseudomonas aeruginosa, Chromobacterium violaceum, etc.), 3) inhibition of respiratory enzymes, 4) induction of heat-shock and oxidative stress, and 5) induction of a stringent response (Dufort et al., 2015). Carvacrol, (β)-carvone, thymol, and trans-cinnamaldehyde decrease the intracellular ATP (adenosine triphosphate) content of E. coli O157:H7 cells (Helander et al., 1998).

Essential oils have multiple cellular targets. Their hydrophobicity results in reactions with lipids on bacterial and fungal cell membranes, increasing membrane permeability and disturbing the original cell structure (Hintz et al., 2015; Pinto et al., 2009). In addition, antiviral effects are achieved by inhibiting viral protein synthesis at multiple stages of viral infection and replication (Wu et al., 2010).

The antimicrobial mechanism of most pAMPs involves cell membranes of targeted organisms and is driven by net positive charge, flexibility, and hydrophobicity to enable interaction with bacterial membranes (Jessen et al., 2006). Their antifungal mechanisms are involved in cell
lysis, interference with fungal cell wall synthesis, permeabilization, binding to ergosterol/cholesterol in the membrane, depolymerization of the actin cytoskeleton, and targeting intracellular organelles such as mitochondria. Antiviral activity is also related to viral adsorption and entry processes.

Natural Preservatives of Animal Origin

There are numerous antimicrobial systems of animal origin related to host defense mechanisms. Preservatives of animal origin include lysozymes, lactoperoxidase, lactoferrin, ovotransferrin, antimicrobial peptide (AMP), chitosan, and others (Table 2).

**Status of animal preservatives**

Lysozyme is obtained from chicken egg whites, and is known as a bacteriolytic enzyme. Lysozyme has been used commercially under the name Inovapure, and can be used against a wide range of food spoilage organisms for extending the shelf life of various food products including raw and processed meats, cheese, and other dairy products (Tiwari *et al.*, 2009).

The lactoperoxidase is a naturally active enzyme in milk with strong antimicrobial effects against both Gram-negative and -positive bacteria (de Wit and van Hooydonk, 1996).

Table 2. Natural preservatives of animal origin

| Examples   | Sources                  | Bacterial target                                      | References                                      |
|------------|--------------------------|-------------------------------------------------------|------------------------------------------------|
| Chitosan   | Crustaceans and arthropods| Antifungal and antimicrobial activity                 | Ben-Shalom *et al.*, 2003; Je and Kim, 2006; Liu *et al.*, 2006 |
| Defensin   | Vertebrates and invertebrates| Bacteria, fungi, and virus                           | Ganz, 2003                                      |
| Dermaseptin S4 | Frog skin          | Bacteria, fungi, and virus                            | Mor and Nicolas, 1994                          |
| Lactoperoxidase | Milk               | Gram-negative and -positive bacteria                  | Russell, 1991; de Wit and van Hooydonk, 1996    |
| Lactoferrin | Milk                    | Gram-negative and -positive bacteria, fungi, and parasites | Al-Nabulsi and Holley, 2005                     |
| Lipids     | Milk, animal            | Gram-negative and -positive bacteria                  | Issacs *et al.*, 1990; Lampe *et al.*, 1998; Wang and Johnson, 1997 |
| Lysozyme   | Chicken egg whites      | Gram-negative and -positive bacteria                  | Tiwari *et al.*, 2009                          |
| Magainin   | African clawed frog     | Gram-negative and -positive bacteria                  | Zasloff *et al.*, 1988                         |
| Ovotranferrin | Egg                   | Gram-negative and -positive bacteria, S. aureus and E. coli | Ibrahim *et al.*, 2000                         |
| Pleurocidin | Skin of winter flounder | Bacteria, fungi, and virus                            | Cole *et al.*, 1997                            |
| PR-39      | Porcine                 | Gram-negative and -positive bacteria                  | Shi *et al.*, 1996                             |
Lactoferrin and ovotranferrin are glycoproteins derived from bovine milk and hen egg respectively, that can bind iron, thereby restricting or preventing bacterial growth. Lactoferrin shows strong antimicrobial effects against various Gram-negative and -positive bacteria, fungi, and parasites in neutral pH and refrigeration temperature (Al-Nabulsi and Holley, 2005). Ovotranferrin peptide fragment OTAP-92 has strong bactericidal activity against both S. aureus and E. coli strains through membrane damage (Ibrahim et al., 2000). However, these transferrin peptides cannot be utilized in food systems because of their high cost.

AMPs are widely distributed in nature and are essential components of nonspecific host defense systems (Park et al., 1997; Tossi et al., 2000). The AMPs produced by animal cells include magainin (Zasloff et al., 1988), MSI-78 (Ge and Yan, 2002), PR-39 (Shi et al., 1996), pleurocidin (Cole et al., 1997), and dermaseptin S4 (Mor and Nicolas, 1994). AMPs are considered a promising solution for antibiotic resistance because of their non-specific molecular targets and fast membrane destruction. Pleurocidin is isolated from the winter flounder (Pleuronectes americanus) is active against Gram-negative and -positive bacteria (Cole et al., 2000). It is stable in heat and salt; however, it is unstable in supraphysiological concentrations to magnesium and calcium. An antimicrobial effect of pleurocidin was reported in foodborne organisms including Vibrio parahemolyticus, L. monocytogenes, E. coli O157: H7, Saccharomyces cerevisiae, and Penicillium expansum (Burrowes et al., 2004). Defensins are widely found in mammalian epithelial cells from chicken, turkey, and others (Brockus et al., 1998). Their antimicrobial spectrum included Gram-negative and -positive bacteria, fungi, and enveloped viruses (Ganz, 2003; Murdock et al., 2007).

Chitosan is a natural biopolymer obtained from the exoskeletons of crustaceans and arthropods, and has been used as an antifungal and antimicrobial agent (Ben-Shalom et al., 2003; Je and Kim, 2006; Liu et al., 2006). Chitooligosaccharides have a bacteriostatic effect on Gram-negative bacteria, E. coli, Vibrio cholera, Shigella dysenteriae, and Bacteroides fragilis (Benhabiles et al., 2012). Chitosan (0.25, 0.5, and 1%) was studied as an antimicrobial ingredient in fresh pork sausage (Bostan and 'Isin Mahan, 2011).

Lipids of animal origin have antimicrobial activity against a wide range of microorganisms. Free fatty acids at mucosal surfaces have been shown to inactivate S. aureus (Bibel et al., 1989). Milk lipids are active against Gram-positive bacteria including S. aureus, C. botulinum, B. subtilis, B. cereus, and L. monocytogenes, and Gram-negative bacteria such as P. aeruginosa, E. coli, and Salmonella enteritidis (Isaacs et al., 1990; Lampe et al., 1998; Wang and Johnson, 1997).

**Antimicrobial mechanisms of animal preservatives**

AMPs, transferrins, and lipids can influence cell membranes and peptide synthesis (Fig. 1) (Brogden, 2005; Zasloff, 2002). AMPs can interact directly with the microbial cell membrane and result in the leaking out of vital cell components (Cole et al., 2000; Hancock, 1997). Lipids mainly inhibit bacterial cell walls or membranes, intracellular replication, or intracellular targets. Lysozymes inhibit bacterial cell membranes by hydrolyzing β-1,4-glycosidic linkages between N-acetylmuramic acid and N-acetylglucosamine in bacterial peptidoglycan.

**Natural Preservatives from Microorganisms**

The preservative of microbial origin include nisin, natamycin, pullulan, ε-polysine, organic acid, and others (Singh et al., 2010) (Table 3).

**Status of microbial preservatives**

Lactic acid bacteria produce antimicrobial compounds

| Table 3. Natural preservatives from microorganisms |
|---------------------------------------------------|
| **Examples** | **Sources** | **Bacterial target** | **References** |
| Bacteriocins | Lactococcus lactis, Lactobacillus acidophilus, Lactobacillus bulgaricus, etc. | Gram positive bacteria | Lee et al., 2013; Anastasiadou et al., 2008; Bhunia et al., 1988 |
| Nisin, diploccocin, acidophilin, bulgaricin, helveticin, lactacin, pediciuin, and plantarin | Streptomyces natalensis | Molds and yeasts | EFSA, 2009 |
| Natamycin | Lactobacillus reuteri | Gram-negative and -positive bacteria, yeasts, and filamentous fungi | Axelsson et al., 1989 |
| Reuterin | | | |
like organic acids, diacetyl, hydrogen peroxide, and proteinaceous bacteriocins (Lee et al., 2013). Bacteriocins are antimicrobial peptides or proteins produced by mainly lactic acid bacteria; these compounds are small and ribosomally synthesized. Most bacteriocins have potential as food preservatives because of their antimicrobial effect against food pathogens. The representative bacteriocins are nisin, diplococcin, acidophilin, bulgaricin, helveticin, lactacin, pediocin, and plantarin. Of these bacteriocins, nisin and pediocin have been used as commercial natural preservatives.

Nisin is a representative bacteriocin produced by various Lactococcus lactis strains, and has activity against food pathogens including Alicyclobacillus spp., L. monocytogenes, Bacillus spp., Micrococcus spp., Clostridium spp., Pediococcus spp., Desulfovomaculun spp., S. aureus, Enterococcus spp., Streptococcus haemolyticus, Lactobacillus spp., Sporolactobacillus spp., and Leuconostoc spp. Nisin is proteinaceous polypeptide that is most stable in acidic conditions. Nisin is soluble in aqueous conditions and is unstable in alkali solutions and heat. It has been used in various food products alone or in combination with other compounds. Nisin is the most widely used bacteriocin approved by the FDA as a food preservative. Dairy and meat products are applied with doses of 50-200 mg/kg. In the USA, nisin is used to inhibit outgrowth of C. botulinum spores and toxin formation in pasteurized processed cheese spread with fruits, vegetables or meats with a limited dose of about 250 ppm in finished products.

Pediocin is GRAS bacteriocin produced by strains of Pediococcus acidilactici (AcH, PA-1, JD, and S) and P. pentosaceus (A, N5p, ST18, and PD1) (Anastasiadou et al., 2008). Most pediocins are stable in heat and a wide range of pH values. Pediocin AcH is effective against both spoilage and pathogenic organisms, including L. monocytogenes, Enterococcus faecalis, S. aureus, and Clostridium perfringens (Bhunia et al., 1988).

Natamycin is an antifungal substance produced by Streptomyces natalensis that is effective against almost all molds and yeasts; however, it has little or no effect on bacteria (EFSA, 2009). Natamycin has been used in dairy, meats, and other foods for antifungal effects, and its use as a surface preservative is regulated (E 235).

Reuterin (β-hydroxypropionaldehyde), an antimicrobial compound produced by Lactobacillus reuteri, is a water-soluble nonproteinaceous metabolite of glycerol (Axelson et al., 1989). Its broad antimicrobial spectrum includes Gram-negative and -positive bacteria, yeasts, and filamentous fungi (Nom and Rombouts, 1992).

**Antimicrobial mechanisms of microbial preservatives**

The antimicrobial mechanism of bacteriocin involves pore formation in the cytoplasmic membrane of target microorganisms (Fig. 1). This leads to cell death by loss of intracellular molecules and a collapse of the proton motive force (Driessen et al., 1995). Bacteriocin originating from Gram-positive bacteria is only effective for Gram-positive bacteria, and is less effective on Gram-negative bacteria due to their selective membrane permeability (Lee et al., 2003). These disadvantages could be compensated for by using other preservatives and preservative methods.

Natamycin has an antimicrobial effect by binding to ergosterol, a cell membrane sterol, in fungal membranes (EFSA, 2009). The structure of natamycin contains a large lactone ring with a rigid lipophilic chain containing conjugated double bonds, and a flexible hydrophilic portion bearing several hydroxyl groups. The hydrophobic groups form a polar pore with ergosterol in the membrane, and this complex affects material passage ($K^+$, $H^+$, amino acids, and other metabolites) (Deacon, 1997).

**Application of Natural Preservatives toward Livestock Food Systems**

Raw meat, meat products, milk, and milk products are major sources of foodborne pathogens, and a variety of methods have been considered to reduce bacterial contaminants. These methods include (a) chemical decontamination with organic acids (Gill and Badoni, 2004; Goncalves et al., 2005; Nissen et al., 2001) and trisodium phosphate (Bashor et al., 2004; Okolocha and Ellerbroek, 2005); (b) physical processes such as irradiation (Badr, 2005; Kim et al., 2004), high pressure processing (Oliveira et al., 2015), steam (Kang et al., 2001a; Kang et al., 2001b; Logue et al., 2005; Stivarius et al., 2002), and UV; (c) natural antimicrobials such as bacteriocins (de Martinez et al., 2002; Gogus et al., 2004) and iron chelating compounds; and (d) combination treatment (Bashor et al., 2004; Koohmaraie et al., 2005).

Challenge studies using meat samples mainly reported efficacy against L. monocytogenes, B. cereus, C. jejuni, and S. aureus (Borman et al., 2014). The efficacy of natural preservatives was tested against commercial formulation (Microgard 100, Microgard 300, nisin, Altak 2002, Perlack 1902) (Lemay et al., 2002). These natural preservatives were investigated in an acidified chicken meat model (pH 5.0). E. coli ATCC 25922 and Brochothrix ther-
mosphacta CRDAV452 were inhibited, however *Lactobacillus alimentarius* BJ33 (FloraCarn L-2) was not inhibited.

The use of fruit byproducts, including rinds of grapefruit, orange, and mandarin with or without γ-irradiation, was applied in raw ground beef (Abd El-khalek and Zahrani, 2013). These substances demonstrated antioxidant and antimicrobial properties on microbial growth, lipid oxidation, and color change of raw ground beef meat. The antimicrobial effects on the survival of *Salmonella typhimurium*, *E. coli* and *B. cereus* were demonstrated.

A combination of plant extracts and MAPs was applied in meat products. Thymol and thymol-MAP were applied in sausage to inhibit *Pseudomonas* spp.; however, the performance is unacceptable respect to sensory acceptability (Mastromatteo et al., 2011). Bay essential oil with MAP (20% CO₂ and 80% N₂) was applied in ground chicken meat, and extend the shelf life without *L. monocytogenes* and *E. coli* (Irkin and Esmer, 2010). Oregano oil was added to fresh chicken breast meat under MAP (Chouliara et al., 2007). At 1%, oregano oil had a very strong taste in the sensory evaluation; however 0.1% oregano oil and MAP extended the shelf life by 5-6 d without strong taste.

Plant preservatives like clove oil showed a synergistic effect with lactic acid and vitamin C for antioxidant and antimicrobial effects (Naveena et al., 2006). Ntzimani et al. (2010) used a mixture of EDTA, lysozymes, rosemary, and oregano oil, and the shelf life of semi-cooked coated chicken fillets was extended under vacuum packaging at 4°C to more than 2 wk.

Nisin was applied with lactoferrin in Turkish-style meatballs. Counts of total aerobic bacteria, coliform, *E. coli*, and other species were decreased by lactoferrin alone and by the mixture of lactoferrin and nisin (Colak et al., 2008). A mixture of lysozyme, nisin, and EDTA effectively inhibited *L. monocytogenes* and meat-borne spoilage bacteria in ostrich patties packaged in air and vacuum (Kim et al., 2002; Mastromatteo et al., 2010).

### Regulation of Natural Preservatives in Livestock Foods

Preservatives permitted in livestock foods are sodium acetate, natamycin, pimamycin, nisin, nitrites (potassium nitrite and sodium nitrite), nitrates (potassium nitrate and sodium nitrate), sorbates (sorbic acid, sodium sorbate, potassium sorbate, and calcium sorbate), and sulphites (sulfur dioxide, sodium sulfite, sodium bisulfite, sodium metabisulfite, potassium metabisulfite, potassium sulfite, and potassium bisulfite) (Food and Drug Administration, 2016).

Natural food preservatives are regulated by maximum permitted levels for food safety and health (Table 4). The only natural preservatives regulated by legislation are natamycin and nisin. Natamycin (E235) is permitted for use in over 150 countries in the surface treatment of hard, semi-hard and semi soft cheeses and dried, cured sausages with a maximum permitted level of 6-40 mg/kg. Nisin (E234) is permitted for use in over 80 countries worldwide, including the United States and European Union, and has been in use as a food preservative for over 50 years (Adams, 2003; EFSA, 2006). The maximum permitted levels in meat, poultry, game products are 5.5-7 mg/kg.

Natural preservatives are considered safer than synthetic preservatives because of their existence in nature and long history of use. However, the use of natural preservatives in food is not powerful enough when considering added amounts in food system. Therefore, effective

| Preservative | Codex general standard for food additives | Maximum permitted levels (mg/kg) |
|-------------|------------------------------------------|---------------------------------|
| Natamycin   | Cheese analogues, processed cheese, ripened cheese, unripened cheese, whey protein cheese | 40 (USA, UK) |
|             | Cured (including salted) and dried non-heat treated processed comminuted meat, poultry, and game products | 20 (USA, Germany) |
|             | Cured (including salted) and dried non-heat treated processed meat, poultry, and game products in whole pieces or cuts | 6 (USA) |
|             | Surface of processed cheeses | 1 mg/dm² (Korea) |
| Nisin       | Heat-treated processed meat, poultry, and game products in whole pieces or cuts | 5.5 (USA) |
|             | Heat-treated processed comminuted meat, poultry, and game products | 6 (Japan) |
|             | Edible casings (e.g., sausage casings) | 5.5 (USA) |
|             | Processed cheeses | 7 (Japan) |
|             | Processed cheeses | 7 (USA) |
|             | Processed cheeses | 250 (Korea) |

ESFA (2006, 2009); GSFA (1995); KFDA (2016).
use levels of conventional and plant extracts/oils against microorganisms are less than 0.1% and 10-20%, respectively (Browne et al., 2012). Therefore, the regulation of these natural preservatives as food additives is necessary regarding their safety, toxicity, and effectiveness.

Conclusion

Chemical preservative have side effects related to the emergence of drug-resistant strains and chronic toxicity. Traditional methods of preservation including refrigeration, pasteurization, and low pH are not completely effective in controlling food pathogens. Therefore, the efficacy of combining natural preservatives with traditional methods has been tested. Combination with other substances or different food preservation systems, coatings, or micro- and nano-capsulation should be tested to assure safety and nontoxicity of natural preservatives. In addition, the use of natural preservatives must be regulated by law for safety, toxicity, and effectiveness.

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