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Review

Current trends in natural preservatives for fresh sausage products

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Natural preservatives from bacteria, plants and animals currently in use in fresh sausage manufacture were investigated. Bacteriocins and organic acids from bacterial origins showed good antimicrobial activities against pathogens. Plant-derived antimicrobials could increase the shelf-life of fresh sausages and in some cases also decrease lipid oxidation and decrease colour loss. Chitosan was the only animal-derived antimicrobial investigated and also increased shelf life of fresh sausages. It was evident that the natural antimicrobials would perform even better in combination with other natural antimicrobials, or lowered levels of synthetic antimicrobials or other hurdles such as specific packaging material.

Introduction

Sausages are products manufactured from fresh comminuted meats from different meat species, such as pork, beef, chicken, fish and buffalo (Raju, Shamasundar, & Udupa, 2003; Sachindra, Sakhare, Yashoda, & Rao, 2005; Sallam, Ishioroshi, & Samejima, 2004). The comminuted meats are then modified by various processing technologies and stuffed in a casing to yield specific sensory and storage characteristics (Savic, 1985). Preservatives are commonly used to enhance their quality, shelf life and safety (Sultana *et al.*, 2014).

Fresh sausages are highly perishable products since it is manufactured from fresh ground meat, is favourable for microbial growth of spoilage and pathogenic organisms, has a high fat content favourable for lipid oxidation, is stored in oxygen semi-permeable packaging and is kept at refrigeration temperatures. These products, therefore, need to be preserved to maintain the quality of the products. The antimicrobial and/or antioxidant preservatives currently employed in these products are chemicals, e.g. sulphur dioxide (SO_2) as antimicrobial and colour preservative (Romans, William, Carloson, Greaser, & Jones, 2001) and/or synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate (Kim, Cho, & Han, 2013).

The synthetic preservatives have many advantages but have come under the scrutiny of consumers. These preservatives include nitrates, benzoates, sulfites, sorbates, formaldehyde and others pay possess life-threatening side effects (Sultana *et al.*, 2014). Gunnison, Jacobsen, and Schwartz (1987) have stated that the use of sulphite as a preservative can trigger different allergic reactions in sulphite hypersensitive consumers. Symptoms such as asthma, urticaria, abdominal pains, nausea, diarrhoea, seizures and anaphylactic shock resulting in death have been recorded. Antioxidants such as BHT and BHA are associated with possible carcinogenic effects although their use has been restricted (Kim *et al.*, 2013). These health dangers have resulted in the need and demand by consumers for using natural preservatives (Bañón, Díaz, Rodríguez, Garrido, & Price, 2007).

The research on natural preservatives included investigations of the antimicrobial and antioxidant characteristics of compounds derived from microbial, plant and animal sources (Dillon & Board, 1994). Plant derived preservatives (grape, rosemary extract, etc.) and animal derived preservatives (e.g., chitosan from fish) have been shown to have antioxidant and antimicrobial properties (Bañón *et al.*, 2007). Some plant extract products, e.g. rosemary, even have health benefits such as liver protective and anti-tumour activities (Balentine, Crandall, O'Bryan, Duong, & Pohlman, 2006).

The aims of this review were to give a definition and classification of sausages and fresh sausages and illustrate the factors influencing the quality of fresh sausages. The chemical antimicrobials and antioxidants traditionally used for fresh sausage preservation will be mentioned. Natural antimicrobials and/or antioxidants from microbial-, plant- and animal-derived compounds and its use in fresh

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sausages will then be discussed in terms of mode of action, safety and applications.

Classification of sausage products

Because sausage manufacturing has been practiced before recorded history, it is not certain how and when the first sausage was produced (Savic, 1985). The word "sausage", however, is derived from *saussiche* (Old Norman French), *salsīcia* (Late Latin) or from *salsus* (Latin) which means "salted" (Harper, 2001–2014). According to Rust (1987) the preparation of sausages began with a simple process of salting and drying meats which aided in the preservation of the sausages. Flavourings and spices were added to improve the flavour of the product. The product was then stuffed in a container, namely the gastrointestinal tract of animals, to make the product more convenient to eat.

Sausages are defined as comminuted seasoned meats, stuffed into casings, and may be smoked, cured, fermented and heated. A wide variety of sausages products can be produced by altering the meat formulation, processing temperature, types of casings and the particle size of the casings (Savic, 1985). A classification of sausages based on characteristics, is given in Table 1. This review will, however, focus only on fresh sausages.

Fresh sausages

According to the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA-FSIS, 2014), fresh sausages are a coarse or finely comminuted (reduced to minute particles) meat food product prepared from one or more kinds of meat, or meat and meat byproducts (e.g., heart, kidney, liver). They may contain not more than 3% water of the total ingredients in the product. Fresh sausages are usually seasoned, frequently cured, and may contain binders and extenders (e.g., wheat flour, nonfat dry milk). They must be refrigerated and thoroughly cooked before eating. Although plenty of varieties of fresh sausages do exist, the content of the most known varieties is illustrated in Table 2.

Factors influencing the quality of fresh sausages

The quality of fresh sausages is of major importance since the shelf-life of the products depends on this aspect. Spoilage of food involves a complex process and excessive amounts of food may be lost, which results in high economic losses or even pose health hazards (Al-Sheddy, Al-Dagal, & Bazaraa, 1999; Liu, Yang, & Li, 2006).

Spoilage of fresh sausages may result in changes in the sensory (colour, odour, flavour, texture) characteristics of the product which may be unacceptable for consumers. These changes may be brought about by proteolysis, lipolysis and lipid oxidation in the absence of micro-organisms. Microbial growth is, however, by far the most important factor causing spoilage of fresh products (Zhou, Xu, & Liu, 2010).

Microbial composition

Meat in general is an ideal growth medium for a wide range of microorganisms (Mathenjwa, Hugo, Bothma, & Hugo, 2012; Russo, Ercolini, Mauriello, & Villani, 2006; Zhang, Kong, Xiong, & Sun, 2009). Aerobic colony counts may range from 1.5×10^3–2.1×10^8 cfu/g for fresh

Table 1. Sausage classification (adapted from Savic, 1985).

Classification	Characteristics	Examples
Raw sausages		
Fresh	Made from fresh comminuted, uncured, non-smoked meats. Must be refrigerated prior to heating by the consumer.	Breakfast sausage (USA), boerewors (South Africa), bratwurst (Germany), merguez (North Africa), siskonmakkara (Finland)
Fermented	Made from comminuted, cured or uncured, fermented and often smoked meats. Not heat-processed.	
Semidry (quickly fermented)	Stuffed in medium- and large-diameter artificial casings. "Tangy" flavour produced by fermentation. Length of smoking and fermentation depends on type but rarely exceeds a few days. Improved stability of stored refrigerated.	Variety of summer sausages, cervelats, mettwursts, Lebanon bologna (USA)
Dry (slowly fermented)		Different types of salamis, droë wors (South Africa, not fermented)
Heat-processed sausages		
Smoked precooked	Mostly comminuted, cured, non-fermented. Final cooking before consumption	Chinese pork sausages, kielbasa
Emulsion-type	Made from comminuted well-homogenized cured meats, fat, water and seasoning. Usually smoked, slightly cooked. Ready-to-eat product.	Frankfurters, wieners, bologna, mortadella
Cooked	Made from previously comminuted cooked fresh or cured raw materials. Final cooking after stuffing. With or without smoking. Ready-to-eat product.	Liver sausage, Braunschweiger

Table 2. Content of the major fresh sausage varieties produced world-wide.

Variety	Meat	Fat	Other ingredients	Casing	Reference
Breakfast sausage (United States of America)	Meat and meat by products of multiple species. May contain mechanically-separated product up to 20% of the meat portion.	Not more than 50% by weight	Salt, pepper, sage. Binders and extenders up to 3.5%. Paprika not permitted.		USDA-FSIS (1999, 2014), http://en.wikipedia.org/wiki/Breakfast_sausage, accessed on 25/11/2014
Fresh pork sausage (USA)	Pork, no pork by products	Not more than 50% by weight	Salt, pepper. Flavouring: either sage and sugar, or sage, chilli and ginger. Paprika, binders or extenders not permitted.	Narrow (26–28 mm) pig casing	USDA-FSIS (1999, 2014), Savic (1985)
Fresh beef sausage (USA)	Beef, no beef by products. May contain mechanically-separated beef up to 20% of the meat portion.	Not more than 30% by weight	Salt, pepper, red pepper, chilli, ginger, cardamom, fenugreek, sugar. Paprika, binders or extenders not permitted.	Sheep or goat. Narrow (16–18 mm), narrow-medium (18–22 mm), medium (20–22 mm) and wide (22–24 mm)	USDA-FSIS (1999, 2014), Savic (1985)
Whole hog sausage (USA)	Can use meat parts from entire hog, including muscle by-products like tongue and heart, in proportions consistent with the natural animal.	Not more than 50% by weight			USDA-FSIS (1999, 2014)
Italian sausage (Italy)	At least 85% pork meat. May contain mechanically-separated pork up to 20% of the meat portion.	Not more than 35% of finished product	Salt, pepper, fennel and/or anise. Optional: spices (including paprika), flavourings, red or green peppers, onions, garlic, parsley, sugar, dextrose and corn syrup.		USDA-FSIS (1999, 2014)
Boerewors (South Africa)	At least 90% meat of the bovine, ovine, porcine or caprine species. No by-products or mechanically separated meat are permitted	Not more than 30% by weight	Salt (1–5%), pepper. Vinegar, herbs, spices (coriander, nutmeg, allspice, etc.), harmless flavourants, cereal products or starch, permitted food additives	Natural animal casings	Rust, 1987; Romans *et al.*, 2001; http://en.wikipedia.org/wiki/Boerewors (accessed on 6/11/2014)
Merguez (North Africa)	Made from mutton or beef or a mixture of both		Salt, pepper. Sumac for tartness and paprika, cayenne pepper or harissa for red colour	Lamb casing	http://www.meatsandsausages.com/sausage-recipes/merguez (accessed on 06/11/2014)
Bratwurst (Germany)	Mainly pork and veal, but also pork and beef		Salt, pepper, marjoram, caraway, nutmeg, ginger, white of eggs	32–36 mm hog casings	http://www.meatsandsausages.com/sausage-recipes/bratwurst (accessed on 25/11/2014)

sausage to 1.4×10^3–3.1×10^7 cfu/g for frozen sausage (Farber, Malcolm, Weiss, & Johnston, 1988) and yeast counts varying from 5.0×10^3–4.7×10^8 cfu/g for fresh sausages (Dalton, Board, & Davenport, 1984; Dillon & Board, 1994). The genera involved in spoilage of fresh meats and sausages were discussed by Cocolin *et al.* (2004); Coma (2008); Crowley *et al.* (2005); Dalton *et al.* (1984); Dillon and Board (1994); Huffman (2002), and Olofsson, Ahrné, and Molin (2007).

A number of pathogens are associated with ground beef (Eisel, Linton, & Muriana, 1997; Farber *et al.*, 1988; Huffman, 2002; Hussain, Mahmood, Akhtar, & Khan, 2007; Little, Richardson, Owen, de Pinna, & Threlfall, 2008; Mrema, Mpuchane, & Gashe, 2006; Nortjé, Vorster, Greebe, & Steyn, 1999; Vorster, Greebe, & Nortjé, 1994). According to the United States Department of Agriculture (USDA-FSIS, 1999), sausage makers should ensure that their products are not contaminated by pathogens such as *Listeria*, *Escherichia coli* O157, *Salmonella*, *Trichinae* and *Staphylococcus* enterotoxin.

The intrinsic factors affecting bacterial growth, and therefore the spoilage potential of fresh sausage products, include pH, which should not be less than 5.5 (Cocolin *et al.*, 2004; Romans *et al.*, 2001); nutrient availability; water activity which is equal to, or higher than 0.97 (Cocolin *et al.*, 2004; Romans *et al.*, 2001; Thomas, Anjaneyulu, & Kondaiah, 2008); and oxidation/reduction potential.

The extrinsic factors include temperature where fresh sausages are usually stored at or below 4 °C before consumption (Cocolin *et al.*, 2004; Rust, 1987); particle surface area where grinding of the meat increases the spoilage characteristics; gaseous environment and packaging material (Cannon *et al.*, 1995; Romans *et al.*, 2001).

Free radicals

Apart from microbial spoilage, lipid oxidation or oxidative rancidity is the second most known spoilage factor of fresh meat and fresh meat products. The grinding of meat disrupts the integrity of muscle membranes and exposes the lipid membranes to metal ions, which facilitates interactions between prooxidants and unsaturated fatty acids (Kim *et al.*, 2013). Lipid oxidation therefore depends on light and oxygen access, the chemical composition of the meat, storage temperature and technological processes (e.g., grinding). This will have a negative effect on the quality of the meat leading to changes in sensory (colour, texture and flavour) and nutritional quality (Shah, Bosco, & Mir, 2014).

Fresh meat cuts and meat products owe their bright red colour to the presence of oxymyoglobin. During chilled storage, this red colour is lost due to exposure to high levels of oxygen. The red oxymyoglobin is then transformed to the brown-coloured metmyoglobin (Kim *et al.*, 2013).

Conventional chemical preservation of fresh sausages

Lipid oxidation and microbial growth during storage can be reduced by applying antioxidant and antimicrobial agents to the meat products, leading to a retardation of spoilage, extension of shelf-life, and maintenance of quality and safety (Kim *et al.*, 2013). Many preservatives used for this goal, are chemicals.

Antimicrobials

The most commonly used preservative in fresh sausages, is currently still sulphur dioxide (SO_2). It is usually added in the sulphite salt form as sodium metabisulphite (Varnam & Sutherland, 1995) and expressed as part per million (ppm) or mg/kg SO_2 (Gould & Russell, 2003). The antimicrobial effect of the sulphite salts is exerted through the undissociated SO_2 molecule. The degree of dissociation is dependant on the pH value and is reduced under acidic conditions. Even though the pH of meat (5.2–5.7) has a negative effect on the antimicrobial activity of the sulphite salts, it is still sufficiently powerful to act as an antimicrobial (Varnam & Sutherland, 1995). Another factor that may have an influence on the effectiveness of SO_2 is the presence of carbonyl compounds (keto- or aldehyde-groups) that bind with it. Thus for SO_2 to be effective, not only must the substrate be acidic, but fairly free of oxygen and sulphite binding compounds (Carr, Davies, & Sparks, 1976).

Bacteria are much more sensitive to SO_2 than yeasts and moulds. The bisulphites have lower activity than SO_2 against yeasts, and sulphites have none. Metabisulphite is more effective against Gram-negative bacteria, especially *Pseudomonas*. However, activity against fermentative Gram-negative bacteria, e.g. the Enterobacteriaceae (*Enterobacter, Citrobacter, Klebsiella*), is less marked possibly due to adaptation amongst members of this family to this preservative. *Brochothrix thermosphacta*, the dominating spoilage bacteria in British fresh sausages, is also relatively resistant to sulphite (Varnam & Sutherland, 1995).

The antimicrobial effect of sulphite on fresh pork sausage was illustrated by Dyett and Shelley (1966). The results suggested that sausages containing a sulphite concentration greater or equal to 450 mg/kg had a lower aerobic count. The study also showed that the presence of 400–500 mg/kg SO_2 in minced meat had a negative effect on the growth of Gram-negative bacteria even at temperatures as high as 22 °C and inhibited the growth of pathogenic organisms such as *Staph. aureus* and *Salmonella* Typhimurium. Therefore, most fresh sausages are by law preserved by these concentrations of SO_2.

Originally, research indicated that humans are reasonably tolerant to sulphur dioxide and, unless damaging doses are given, can recover unaffected. Recently, however, cases concerning the sensitivity of asthmatics to sulphur dioxide have been reported (Bañón *et al.*, 2007). Some of these were life threatening or fatal, due to seizures and anaphylactic shock. It may cause headaches, nausea, and diarrhoea in some humans (Gunnison *et al.*, 1987). Since some SO_2 is liberated as gas during cooking, this could give rise to respiratory problems, mostly in asthmatic people, thiamine absorption deficiency and disruption of carbohydrate metabolism, particularly in individuals who have an allergic reaction to SO_2. The toxic effect is, however, variable in humans and persons may tolerate different levels (Bañón *et al.*, 2007).

Antioxidants

To reduce lipid oxidation in meat products, several synthetic antioxidant preservatives, such as butylated hydroxyl toluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and propyl gallate (PG), are typically used to protect foods from lipid oxidation spoilage. Antioxidant use in food products is controlled by regulatory laws of a country or international standards. In general, the use of synthetic antioxidants is restricted due to possible carcinogenic effects (Kim *et al.*, 2013). The mode of action for antioxidants can be by: scavenging radicals; breaking chain reactions; decomposing peroxides; decreasing localized oxygen concentrations; and binding chain initiating catalysts (Shah *et al.*, 2014).

Antibrowning

Sulphur dioxide (SO_2) has been used in sausages not only as an antimicrobial but also to improve or maintain the colour of the sausages (Dyett & Shelley, 1966; Romans *et al.*, 2001).

Natural antimicrobial and antioxidant preservatives for fresh sausages

Chemical additives and preservatives are usually avoided due to possible allergic reactions in sensitive consumers of preserved food. The other major reason, however, is because consumers in general are nowadays more aware of the safety of any additives to food and are demanding natural products (Gyawali & Ibrahim, 2014). Researchers all over the world are therefore investigating a variety of safer and natural preservatives as alternatives to chemical and synthetic preservatives. In fresh meat and especially fresh sausage production, the alternative preservatives can be broadly divided into microbial-derived compounds, plant-derived compounds and animal-derived compounds.

Microbial-derived compounds

Many microorganisms, especially the lactic acid bacteria (LAB), have the ability to produce antimicrobials to improve their competitiveness. The compounds produced by these bacteria have historically long been used to preserve food. These compounds are small-molecular-mass organic molecules, which are divided into proteins (mainly bacteriocins) and non-proteins which include organic acids (lactic acid, propionic acid, butyric acid, acetic acid, etc.), hydrogen peroxide, diacetyl and other compounds (Sun, Li, Song, & Zhu, 2011). The application of microbial-derived compounds in the manufacture of fresh sausages is depicted in Table 3.

Bacteriocins

Bacteriocins are the most known antimicrobial peptides produced by lactic acid bacteria. In bacterial metabolism the bacteriocins are synthesized and secreted by the ribosome into the environment. Bacteriocins are known to be more effective against Gram-positive bacteria (e.g. *Listeria monocytogenes*) than Gram-negative ones due to the presence of the protective outer membrane on the cell membrane of Gram-negative bacteria (Sun *et al.*, 2011).

Bacteriocin production is a natural process during fermentation of foods. It may, however, be added to foods in the form of concentrated preservative preparations, shelf-life extenders or additives. According to Sun *et al.* (2011), the bacteriocins can help to reduce the addition of chemical preservatives in the food industry as well as the intensity of heat treatments. Many bacteriocins are active against endospore-forming bacteria and may, therefore, be applied as another hurdle in combination with other

Table 3. Application of microbial-derived natural preservatives in fresh sausage products.

Microbial preservative	Produced from	Activity against	Product tested	Reference
Bacteriocins				
Lacticin 3147	*Lactococcus lactis* subsp. *lactis*	*Listeria innocua, Clostridium perfringens, Salmonella* Kentucky	Fresh pork sausage	Scannell, Ross, Hill, and Arendt (2000)
Reuterin	*Lactobacillus reuteri*	*Listeria monocytogenes, Salmonella* spp.	Bratwurst-style fresh sausage in Turkey	Kuleaşan and Cakmakçi (2002)
Organic acids				
Citric acid (sodium citrate)		*Salmonella* Kentucky	Fresh pork sausage	Scannell, Hill, Buckley, and Arendt (1997)
Acetic acid and sodium lactate mixture		Total plate count (SR) Lipid oxidation ↑ Colour loss ↑	Fresh Italian pork sausage	Crist *et al.* (2014)
Sodium lactate and sodium acetate mixture		Total aerobic count	Merguez sausage	Ayachi *et al.* (2007)

'↑', increased; SR, significant reduction.

preservation hurdles (Gálvez, Abriouel, Lucas, José, & Burgos, 2011). The food composition, interaction of bacteriocins with food components, bacteriocin stability, pH and storage temperature may, however, influence the effectiveness of bacteriocins (Sun *et al*., 2011). It is recommended that the activity of any bacteriocin should be confirmed through applied studies in food model systems after *in vitro* studies have been performed.

Nisin is a heat-stable bacteriocin produced by *Lactococcus lactis* subsp. *lactis* and is the only antimicrobial that is approved for use in more than 50 countries world-wide (Gyawali & Ibrahim, 2014). Organisms that are inhibited by nisin include Gram-positive (*Staph. aureus, M. luteus*) and spore forming bacteria (*Bacillus cereus* and *Clostridium*) (Davies *et al*., 1999; Rajendran, Nagappan, & Ramamurthy, 2013). The cytoplasmic membrane of these bacteria is permeated by nisin, causing leakage of intracellular metabolites and disrupting the membrane potential (Lucera, Costa, Conte, & Del Nobile, 2012). Since nisin is ineffective against Gram-negative bacteria and fungi, its use and application as a broad-spectrum antimicrobial is restricted (Juneja, Dwivedi, & Yan, 2012). Nisin has not yet been evaluated in fresh sausages although studies have been performed on vacuum-packaged fresh beef which showed *L. monocytogenes* reduction (Zhang & Mustapha, 1999). Other studies on cured meat products have been discussed by Juneja *et al.* (2012).

Lacticin is another bacteriocin produced by *Lactococcus lactis* subsp. *lactis*. Since the organism producing this bacteriocin has GRAS status, the use of this bacteriocin food production is also regarded safe (Fallico McAuliffe, Ross, Fitzgerald, & Hill, 2011). A study by Scannell, Ross, Hill, and Arendt (2000) on the keeping quality of fresh pork sausage found that lacticin and nisin performed better against Gram-positive organisms than sodium metabisulphite. The combination of organic acids with either of these bacteriocins enhanced their antimicrobial activity against *Listeria innocua* and *Salmonella* Kentucky and was even more effective against *Clostridium perfringens*. This study also suggested that lacticin combined with sodium lactate or sodium citrate can be used as an alternative preservative of fresh pork sausage since it gave lower total aerobic plate counts throughout storage than sodium metabisulphite.

Pediocin is produced by *Pediococcus acidilactici* or *P. pentosaceus* and has been shown to be effective against *L. monocytogenes* and other Gram-positive pathogens on meat surfaces (Coma, 2008; Siragusa, Cutter, & Willett, 1999). Pediocin has GRAS status in certain food applications (Juneja *et al*., 2012). Research on some meat products indicated that pediocins (especially pediocin PA-1), could be more effective than nisin especially when used in combination with nisin, lysoszyme, organic acids, sodium dodecyl sulphate or ethylenediaminetetraacetic acid (EDTA). Pediocin has not yet been evaluated in fresh sausages. A study has been performed on fresh chicken to control *L. monocytogenes* (Juneja *et al*., 2012).

Reuterin (β-hydroxypropionaldehyde) is produced by *Lactobacillus reuteri* and has a broadspectrum antimicrobial activity towards food pathogens and spoilage organisms and has GRAS status. The bio-preservative effect of reuterin originates from its high resistance to heat, proteolytic and lipolytic enzymes, as well as good solubility in water. It is stable over a wide range of pH values (Gyawali & Ibrahim, 2014). Reuterin has a higher antimicrobial effect on Gram-negative than on Gram-positive pathogens. The application of reuterin in a fresh sausage model system is indicated in Table 3. Furthermore, a study by El-Ziney, van den Tempel, Debevere, and Jakobsen (1999) on ground pork found that reuterin at 100 AU/g reduced *E. coli* O157:H7 by 5 log cfu/g after 1 day storage at 4 °C while *L. monocytogenes* was reduced by 3 log cfu/g after 7 days of storage. It is speculated that reuterin causes oxidative stress response by modifying the thiol groups in proteins and small molecules (Langa, van den Tempel, Debevere, & Jakobsen, 2013).

The combination of bacteriocins with other compounds usually results in better antimicrobial activities. Nisin molecules alone, for example, usually interact with food components in raw meats, limiting its activity. It also has poor solubility at close to neutral pH. However, a combination with other hurdles such as organic acids, lysozyme, chelators, vacuum packaging or MAP, improved its effectivity against *B. thermosphacta, E. coli* O157:H7 and *L. monocytogenes* (Gálvez *et al*., 2011).

Other bacteriocins (e.g. sakacins, carnobacteriocins, bifidocins, lactococcins or pentocins) have not yet been evaluated in fresh sausages. On raw meats or poultry meats, however, they showed variable antimicrobial effects against pathogenic and spoilage bacteria (Gálvez *et al*., 2011).

Organic acids

The organic acids are natural antimicrobials produced during lactic acid fermentation and have generally recognized as safe (GRAS) status for meat products. Application of these acids on meat surfaces, mainly by spraying or dipping, is a well-known and widely-used practice. Many studies, as indicated by Mani-López, García, and López-Malo (2012), have tested the efficacy of organic acids. Organic acids, however, may have negative impacts on colour and flavour and it is recommended that sensory studies should always be applied when an organic acid is evaluated for possible use as natural antimicrobials in meat and meat products. Another limiting factor in using organic acids in fresh meat products is that some acids (e.g. citric acid) need low pH for optimum antimicrobial activity (Mani-López *et al*., 2012).

According to Mani-López *et al.* (2012) acetic acid, acetates, diacetates and dehydroacetic acid have effectivity as antimicrobials against yeasts and bacteria in dairy products and meat and meat products. Lactic acid and lactates are effective against bacteria in meat and meat products and fermented foods while sodium propionate is effective against moulds in meat products. An *in vitro* study by Álvarez-Ordóñez, Fernández, Bernardo, and López (2010) indicated that acetic acid is the best antimicrobial against *Salmonella* Typhimurium with a decreasing order of effectivity of other organic acids as follows: acetic > lactic > citric > hydrochloric.

The applications of organic acids in fresh sausage model systems are given in Table 3. According to Gálvez *et al.* (2011), organic acids and salts in conjunction with bacteriocins result in increased inactivation of bacteria and inhibition of growth as well as increased solubility and activity of bacteriocin molecules. Ayachi, Daoudi, and Benkerroum (2007) reported that the addition of a mixture of organic acids (sodium lactate 90% and sodium acetate 10%) at different concentrations (from 0 to 20 g/kg) on Merguez sausages significantly reduced microbial cell loads during storage at 8 °C. A study by Crist *et al.* (2014) indicated that vinegar and sodium lactate mixtures increased shelf life by reducing total plate counts, however, lipid oxidation and colour loss increased over time. This study recommended that antioxidants such as BHA/BHT should be used in combination with sodium lactate or vinegar/sodium lactate mixtures to prevent colour degradation and rancidity.

Plant-derived compounds

Extracts from fruits, vegetables, herbs and spices are rich sources of essential oils. The FDA (2014) has recently published a revised list of plants and generally recognizes the essential oils, oleoresins and extractives of these plants as safe. The essential oils (EOs) of leaves (e.g., oregano, rosemary, thyme, sage, basil, marjoram); flowers or buds (e.g., clove); bulbs (e.g., onion, garlic); seeds (e.g., parsley, caraway, nutmeg, fennel); rhizomes (e.g., asafoetida); fruits (e.g., pepper, cardamom) or other parts (e.g. bark) of plants are responsible for the antimicrobial (Tiwari, Valdramis, Bourke, & Cullen, 2011) and antioxidant (Shah *et al.*, 2014) activities by plants. These EOs have also been used extensively in foods as flavouring agents and/or for their medicinal properties since the earliest recorded history (Tiwari *et al.*, 2011).

The addition of EOs to foods may result in either destroying microbial cells or in inhibiting the production of secondary metabolites such as mycotoxins (Tiwari *et al.*, 2011). In general, the EOs are more inhibitory against Gram-positive than Gram-negative bacteria (Busatta *et al.*, 2008; Oussalah, Callet, Saucier, & Lacroix, 2007), however, some EOs from oregano, clove, cinnamon, citral and thyme are effective against both groups. The antimicrobial activity of EO's are mainly ascribed to the phenolic compounds, terpenes, aliphatic alcohols, aldehydes, ketones, acids and isoflavonoids. The principle constituents from these compounds responsible for the antimicrobial effect include carvacrol, thymol, citral, eugenol and their precursors (Tiwari *et al.*, 2011). The phenolic compounds are the most abundant and important phytochemicals. They exhibit antimicrobial, antioxidant, anti-allergic, anti-inflammatory and cardio-protective properties by acting as reducing agents, hydrogen donors, oxygen quenchers and metal chelators (Mariem *et al.*, 2014).

The efficacy of EOs is dependant on factors such as the chemical structure of their components, the concentration, interactions with the food matrix, matching the antimicrobial spectrum of activity with the target microorganism(s) and the method of application (Tiwari *et al.*, 2011). The combination of EOs with other natural antimicrobials or even other chemical preservatives also shows positive effects.

A large number of antimicrobial and antioxidant studies on a variety of plant-derived compounds were performed on fresh meat and fresh meat products, especially ground meat and patties. These studies are summarized in excellent reviews (Hygreeva, Pandey, & Radhakrishna, 2014; Jayasena & Jo, 2013; Shah *et al.*, 2014). The antimicrobials and antioxidants mentioned in these reviews could also be evaluated in fresh sausages. Other studies not listed by these reviews include *Capsicum annuum* extract in minced beef meat (Careaga *et al.*, 2003), Ghardaq (*Nitraria retusa*) extract in beef patties (Mariem *et al.*, 2014), olive leaf, blueberry and *Zizyphus jujuba* extracts in meatballs (Gök & Bor, 2012) and makampom (*Phyllanthus emblica* L.) extract in raw ground pork (Nanasombat *et al.*, 2012).

The applications of plant-derived compounds in fresh sausage studies are depicted in Table 4. A study on boerewors investigated the preservative effect of citrus seed extract (Citrox). Although the shelf life increased, lipid oxidation decreased and red colour loss was slower with the addition of Citrox, the sensory attributes were negatively affected (Van Schalkwyk, Hugo, Hugo, & Bothma, 2013). *Laurus nobilis* essential oil was evaluated in a fresh Tuscan sausage stored at 7 °C for 14 days (Da Silveira *et al.*, 2014). The shelf life increased and low levels of rancidity were reported. Although the sensory characteristics were affected, it was acceptable by consumers.

Plant-derived compounds may be used in conjunction with lowered levels of chemical preservatives. In a study on boerewors, rosemary in combination with chitosan and lowered levels of SO_2, increased the shelf life and red colour of the product (Mathenjwa *et al.*, 2012). A study on Green tea and grape seed extract combined with low levels of SO_2 in raw beef patties increased shelf life, decreased lipid oxidation and decreased colour loss (Bañón *et al.*, 2007). The natural preservatives may also be used in conjunction with different packaging materials. In a study on reduced pork back-fat sausages using thymol combined with modified atmosphere packaging (MAP), the shelf life was extended (Table 4; Mastromatteo, Incoronato, Conte, & Del Nobile, 2011).

Animal-derived compounds

Natural preservative compounds derived from animals which have shown effectiveness in fresh meat and meat

Table 4. Application of plant-derived natural preservatives in fresh sausages.

Preservative	Activity against	Product tested	Quality attributes	Reference
Garlic (fresh [F], powder [P] or oil [O])	Aerobic plate count (SR for F and P; NS for O)	Fresh chicken sausage	Lipid oxidation ↓, shelf life ↑	Sallam *et al.* (2004)
Laurus nobilis (bay leaf) essential oil	Psychrotrophic count (SR) Total aerobic count (SR) LAB count (SR) Coliform count (SR)	Fresh Tuscan sausage	Shelf life ↑, low oxidation levels, sensory attributes affected but still acceptable	Da Silveira *et al.* (2014)
Rosemary and ascorbic acid mixture		Fresh pork sausage	Red colour loss ↓ Off-odour production ↓	Martínez, Cilla, Beltrán, and Roncalés (2007)
Rosemary, chitosan and SO_2 mixture	Total bacteria count (SR) Yeast and mould count (SR) Coliform count (NS) Enterobacteriaceae count (NS)	Boerewors	Shelf life ↑ Red colour ↑	Mathenjwa *et al.* (2012)
Origanum marjorana	*E. coli* (~2 LR)	Fresh sausage	Shelf life ↑, sensory properties ↓ with increased concentration	Busatta *et al.* (2008)
Citrus seed extract (Citrox)	Total plate count (SR) Coliform count (NS) Yeast and mould count (SR)	Boerewors	Shelf life ↑, lipid oxidation ↓, red colour loss ↓, sensory properties ↓	Van Schalkwyk *et al.* (2013)
Thymol combined with MAP	*Pseudomonas* spp. (SR)	Reduced pork back-fat sausages	Shelf life ↑	Mastromatteo *et al.* (2011)

LAB, lactic acid bacteria; '↑' and '↓', increase and decrease; LR, log reduction; SR, significant reduction; NS, no significant reduction.

products will be discussed in this section. Chitosan, lactoferrin and lysozyme are the major animal-derived antimicrobials which will be discussed in this section. Since studies on lactoferrin and lysozyme have not yet been performed on fresh sausages, only the applications of chitosan in fresh sausages are summarized in Table 5.

Chitosan

Chitosan is a deacetylated form of chitin and the second most abundant biopolymer in the world after cellulose. It consists of N-acetylglucosamine residues joined by β(1–4) glycosidic links (Friedman & Juneja, 2011). It is derived from the shell of crabs and shrimps and the cell

Table 5. Application of chitosan as an animal-derived natural preservative in fresh sausage products.

Preservative	Activity against	Product tested	Quality attributes	Reference
Chitosan	Total bacterial and yeast and mould counts (SR) Coliform and Enterobacteriaceae counts (NS)	Boerewors	Shelf life ↑	Mathenjwa *et al.* (2012)
Chitosan, rosemary and SO_2 mixture	Total bacterial and yeast and mould counts (SR) Coliform and Enterobacteriaceae counts (NS)	Boerewors	Shelf life ↑ Red colour ↑	Mathenjwa *et al.* (2012)
Chitosan in 0.9% saline at pH 6.2	*Saccharomyces ludwigii* (Inh) *Lactobacillus viridescens* and *Listeria innocua* (Ina) Native microflora (1–3 LR)	Skinless pork sausages	Shelf life ↑	Sagoo, Board, and Roller (2002)
Chitosan and rosemary extract combination		Fresh pork sausages	Shelf life ↑ Lipid oxidation ↓	Georgantelis *et al.* (2007)
Chitosan (0.5% and 1% combined with nitrites (150 ppm)	Lactic acid bacteria (SR) *Brochothrix thermosphacta* (SR) Enterobacteriaceae and *Pseudomonas* (SR) Yeasts and moulds (SR)	Fresh pork sausages	Shelf life ↑ Lipid oxidation ↓	Soultos, Tzikas, Abrahim, Georgantelis, and Ambrosiadis (2008)

'↑' and '↓', increase and decrease; LR, log reduction; SR, significant reduction; NS, no significant reduction; Inh, inhibition; Ina, inactivation.

wall of fungi (Gyawali & Ibrahim, 2014; Roller *et al.*, 2002). Although it is not generally regarded as a safe (GRAS) compound, it is speculated that it will receive GRAS status in the future (Zivanovic, Davis, & Golden, 2015).

Although chitosan exhibits antimicrobial activities against a range of foodborne microorganisms (Georgantelis, Ambrosiadis, Katikou, Blekas, & Georgakis, 2007), it is more effective against Gram-negative than Gram-positive bacteria (Friedman & Juneja, 2011). The antimicrobial and antioxidant activity of chitosan are due to its ability to cause permeability of the cell membranes, water-binding capacity and inhibition of various enzymes (Coma, 2008; Georgantelis *et al.*, 2007; Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades, & Roller, 2001; Kanatt, Chander, & Sharma, 2008).

Chitosan's use as a food preservative has been limited due to its insolubility at neutral and higher pH (Du, Zhao, Dai, & Yang, 2009). However, a study by Chantarasataporn *et al.* (2014) indicated that water-based chitosan, in the forms of oligochitosan and nanowhisker chitosan, significantly inhibited microbial activity and extended the shelf life in raw minced pork meat (Table 5). Chitosan in combination with lowered levels of SO_2 and rosemary extract in a boerewors model system indicated an increase in shelf life and red colour (Table 5; Mathenjwa *et al.*, 2012).

Lactoferrin

Lactoferrin (Lf) is an iron-binding glycoprotein isolated from milk which has a wide range of antimicrobial activity against bacteria (e.g., *L. monocytogenes, E. coli, Klebsiella* and *Carnobacterium*) and viruses (Gyawali & Ibrahim, 2014). It has been approved in the USA for application in meat products (Juneja *et al.*, 2012; USDA-FSIS, 2010). The mode of action is speculated to be either limiting microbial access to nutrients via iron chelation and/or destabilizing the Gram-negative outer membrane (Gyawali & Ibrahim, 2014).

A study on Turkish meatballs indicated that Lf and a mixture of Lf and nisin increased the shelf life of the product by significantly reducing the counts of the t*otal aerobic bacteria, coliforms, E. coli, total psychrophilic bacteria, Pseudomonas spp. and yeasts and moulds* (Colak, Hampikyan, Bingol, & Aksu, 2008). No studies have been performed on fresh sausages.

Lysozyme

Lysozyme is a bacteriolytic enzyme. It is isolated from mammalian milk and avian eggs and has GRAS status for direct addition to foods (FDA, 1998). According to Juneja *et al.* (2012) the white lysozyme from eggs has bacteriolytic activity due to the hydrolysis of the β-1,4 linkage between the N-acetylmuramic acid and N-acetyl-glucosamine in the peptidoglycan of the Gram-positive microbial cell wall. Lysozyme has better effectivity against Gram-negative bacteria when used in combination with detergents and chelators (e.g. EDTA), nisin and lactoferrin (Branen & Davidson, 2004). Lysozyme is widely reported for its application as a preservative for meat, meat products, fish, fish products, milk and dairy products, and fruits and vegetables (Gyawali & Ibrahim, 2014). However, literature is not available on its use in fresh sausages.

In a study on minced meat using a combination of chitosan and lysozyme, the shelf life of the product was increased by inhibiting the growth of *B. cereus*, *E. coli* and *Pseudomonas fluorescens* while a significant reduction was noticed in the *Staph. aureus* counts. This combination also decreased lipid oxidation (Rao, Chander, & Sharma, 2008).

The combination of lysozyme with nisin and EDTA was evaluated in ostrich patties. *L. monocytogenes* was significantly reduced, the total viable count and lactic acid bacteria count decreased by 1 log and 2 log, respectively, while Enterobacteriaceae and *Pseudomonas* were not affected. Shelf life therefore increased, the colour of the product was not affected while off-odours developed (Mastromatteo, Lucera, Sinigaglia, & Corbo, 2010). A lysozyme and nisin mixture also showed inhibition of *B. thermosphacta* and a significant reduction of *Carnobacterium* spp. in cores of lean and fat pork tissue (Nattress, Yost, & Baker, 2001).

Possible other natural preservatives

Research on a few other natural preservatives has only been evaluated *in vitro*, and has not yet been tested in food preservation and/or applied in food systems. These preservatives may find applications in fresh sausage preservation in future. An example includes algae and mushrooms which have been reported to have antimicrobial, antiviral, antioxidant, antifouling, anti-inflammatory, cytotoxic, antimitotic activities (references cited in Gyawali & Ibrahim, 2014). Saponins are naturally occurring glycosides in some plants which showed promising results as a broad-spectrum antimicrobial (Juneja *et al.*, 2012). Flavonoids are bioactive pigment compounds in some plant parts and showed broad-spectrum antimicrobial activities as well as an antifungal preservative (Juneja *et al.*, 2012).

Conclusions

Microbial-, plant- and animal-derived natural compounds were investigated for their antimicrobial, antioxidant and antibrowning properties in fresh sausages. Lacticin and reuterin as bacteriocins and citric acid, sodium lactate and sodium acetate as organic acids produced by microorganisms, have been evaluated in fresh sausages and showed antimicrobial activity against Gram-positive and Gram-negative pathogens.

The plant-derived compounds have been investigated extensively since a vast range of these compounds show excellent antimicrobial, antioxidant and anti-browning activities in meat products and fresh sausages. Chitosan is the only animal-derived antimicrobial that has yet been evaluated in fresh sausages and showed increased shelf life of these products.

It was evident that combinations of the natural antimicrobials with either other natural antimicrobials, or lowered levels of synthetic/chemical antimicrobials, or with other hurdles, such as specific packaging material, will enhance the performance of all three types of antimicrobials discussed in this review.

A vast range of research has been performed on meat and meat products. Many studies are however, *in vitro* studies and need to be evaluated in food systems since complex food matrices such as fresh sausages, may have an effect on the activity of the natural preservative. Also, sensory studies should always form part of these studies to ascertain consumer's perception.

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