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Trends in Food Science & Technology 45 (2015) 37–49

TRENDS IN FOOD SCIENCE & TECHNOLOGY

Alternatives to nitrite in processed meat: Up to date

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Nitrite has been used in different meat products mainly to maintain their microbial quality, flavor, and color and to prevent lipid oxidation. Since consumer demand for organic or natural meat products has increased due to the concerns of health risk of synthetic additives, the meat industry is currently focusing on the development of nitrite alternatives. This paper reviews the potential alternatives to replace nitrite salts that are used completely or partially in the manufacturing of meat products.

Introduction

The consumption of animal products including meat and meat products has increased globally with an increase in the household income (Nam, Jo, & Lee, 2010). In parallel, the demand for safe and high quality meat and meat products has also increased with the novel concepts of all-natural and clean-label (Jayasena & Jo, 2013).

Spoilage by microbes, autolytic enzymes and lipid oxidation can cause deterioration of meat and meat products, which has considerable economic and environmental impact (Jayasena & Jo, 2013). Several thermal and non-thermal meat preservation techniques, including refrigeration, freezing, drying, and smoking, are presently being used in the meat processing industry (Sindelar & Milkowski, 2011).

Meat curing which includes the addition of salt, nitrite, and sometimes nitrate to fresh meat cuts, enables preservative effect by removing moisture and reducing the water activity of the meat (Parthasarathy & Bryan, 2012). In addition to the preservative action, particularly against *Clostridium botulinum*, the curing process imparts several other distinctive properties that are common to all cured meat products which is attributable to the sodium nitrite present in the curing mixture (Sindelar & Milkowski, 2011). These other properties include contribution to the formation of an unique color, texture, and flavor to cured meat products and protection of meat lipids from oxidation (Sindelar & Milkowski, 2011). In the modern meat processing industry, meat curing is a well-developed segment that uses advanced techniques. In addition, nitrite plays a vital role in normal human body functions (Sindelar & Milkowski, 2012).

However, over the years, great concerns have been expressed regarding the exposure of consumers to certain harmful products that may be formed in meat and meat products during and after curing. The foremost concern is that certain reaction products after curing may be carcinogenic to humans (Cassens, 1997a; Sen & Baddoo, 1997). This concern has led researchers to seek ways to reduce the risk of nitrosamine formation and alleviate potential human health concerns. One such way is the substitution of nitrite with alternative ingredients having comparable characteristics without causing any health hazards (Sindelar & Milkowski, 2011). Over the past several decades, studies have conducted to counter this difficult challenge; however, until date, these attempts remained unsuccessful in identifying an effective single replacement material possessing all the properties of nitrite (Sindelar & Milkowski, 2011). One possible approach to resolve this problem is the use of hurdle technology in meat curing; in this approach, low levels of sodium nitrite are combined with other

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http://dx.doi.org/10.1016/j.tifs.2015.05.008

compounds and/or other processing technologies. It is imperative that products treated with these secondary compounds or technologies be safe for human consumption.

This review provides an overview of the published data on the potential alternatives to completely or partially replace nitrite salt in meat and meat products.

Nitrite in processed meat

The precise discovery of meat curing may never be known, but it is generally accepted to be associated with preservation methods using salt as early as 3000 B.C. (Sindelar & Milkowski, 2011). With the invention of refrigeration and food packaging technologies, the original role of curing technology of meat and meat products gradually changed from that of preservative to that of development to diverse convenient products (Pegg, 2004).

The main functions of nitrite in cured meat include the formation of the characteristic reddish-pink color and flavor associated with cured meats in addition to serving as an effective antioxidant and antimicrobial agent alone or in combination with other ingredients (Pegg, 2004; Sindelar & Milkowski, 2011). Nitrate can be reduced to nitrite to perform the same function (Sindelar & Milkowski, 2011). Therefore, naturally present or artificially supplemented nitrate in brine solutions should be converted to nitrite by the meat microflora or by the addition of bacteria possessing nitrate reductase activity (Sebranek & Bacus, 2007).

Cured color

Nitrite – the true curing ingredient – is considered a multifunctional food additive that forms nitric oxide during the curing process. Formation of nitric oxide from the intermediates is facilitated by reductants such as ascorbate. It has been recognized that nitrous acid (HNO_2) is formed from nitrite under acidic conditions such as that in postmortem muscles (Pegg & Shahidi, 2000). According to Honikel (2004), dinitrogen trioxide (N_2O_3) is formed from nitrous acid and will subsequently form nitric oxide or will react with other substrates in meat.

Nitric oxide will react with iron of both myoglobin (Fe^{+2}) and metmyoglobin (Fe^{+3}) to form cured color (Pegg & Shahidi, 2000). Comminuted meat quickly turns into brown color with the addition of nitrite due to metmyoglobin formation since nitrite acts as a strong heme pigment oxidant and is, in turn, reduced to nitric oxide. Nitric oxide reacts with metmyoglobin and subsequent reduction reactions convert the oxidized heme to reduced nitric oxide myoglobin for typical cured color subjected to cooking (Pegg & Shahidi, 2000).

Nitric oxide reaction with myoglobin forms the nitrosylmyoglobin complex, which outline the basis for unique cured meat color (Parthasarathy & Bryan, 2012). Nitrosylmyoglobin is bright red in color (Parthasarathy & Bryan, 2012) and is an extremely unstable compound. During thermal processing, it is converted to a stable, attractive reddish-pink compound – nitrosohemochrome – because of the denaturation of the protein moiety of the myoglobin pigment (Parthasarathy & Bryan, 2012).

Although a minute amount of nitrite (2–14 ppm) is sufficient to develop a cured color in the meat, a higher amount is necessary to avoid non-uniform curing and to preserve the developed reddish-pink color throughout the meat's shelf-life (Sebranek & Bacus, 2007; Sindelar & Milkowski, 2011). As the residual nitrite levels in cured meat products gradually decline due to oxidation- and light-induced fading over the storage period (Cassens, 1997b), a residual nitrite level of 10–15 ppm is generally recommended as a reservoir primarily for the regeneration of cured meat color (Sindelar & Milkowski, 2011).

Cured flavor

The characteristic flavor of cured meat products can also be attributed to the chemical reactions of nitrite and its associated reactions as described above. Sensory evaluation revealed that a low level of nitrite (50 ppm) was sufficient to develop the unique flavor differences between cured and uncured meat. However, the principle mechanism and the compounds responsible for this unique flavor remain unknown (Sindelar & Milkowski, 2011). Shahidi (1998) proposed that this characteristic feature could be due to the nitrite-related suppression of oxidation products, which manipulates the development of rancid-flavor compounds. Sindelar and Milkowski (2011) suggested that cured meat flavor could be the result of a combination of nitrite-related flavors and aroma. Hydrocarbons, ketones, alcohols, phenols, esters, furans, pyrazines, aldehydes, and other nitrogen containing compounds, and increased carboxylic acids, sulfur, and nitrite/nitrate containing compounds have been found in cured meat compared to uncured meat (Ramarathnam, Rubin, & Diosady, 1993). Alcohols and phenols undergo nitrosation reactions and could impact volatile compounds as well. Increases in sulfur compounds could be attributed to S-nitrosothiol formation and reduction to disulfide bonds during meat curing (Ramarathnam *et al.*, 1993).

Antioxidant effect

Another remarkable property of nitrite is its ability to retard the development of rancidity during storage and the subsequent warmed-over flavors developed upon heating of meat and meat products (Parthasarathy & Bryan, 2012; Pegg & Shahidi, 2000). The antioxidant activity of nitrite is attributed to the potential of nitric oxide to bind to and stabilize heme iron of meat pigments during the curing process. Oxygen and other reactive oxygen species rapidly react with, and are sequestered by nitric oxide (Ford & Lorkovic, 2002). Nitric oxide, as a free radical, can also terminate lipid autooxidation (Pegg & Shahidi, 2000). In addition, it binds free irons and stabilizes heme iron (Bergamaschi & Pizza, 2011) which can reduce lipid oxidation by limiting prooxidant activity of iron. This lowers the amount of free iron released during cooking

and chelates free radicals including lipid-derived alkyl, alkoxyl, and peroxyl radicals that accelerate lipid oxidation in meat products (Parthasarathy & Bryan, 2012). Antioxidant effect of nitrite has also been reported at levels as low as 40 ppm (Al-Shuibi & Al-Abdullah, 2002).

Antimicrobial effect

Nitrite alone or in combination with other salts can inhibit the growth of several aerobic and anaerobic microorganisms. Nitrite targets bacteria at multiple sites by inhibiting metabolic enzymes, limiting oxygen uptake, and breaking the proton gradient. In addition, nitric oxide bound to iron, thus limits iron availability which is necessary for enzyme functionality and bacterial metabolism and growth (Tompkin, 2005). Iron–sulfur complexes and heme ion centers of enzymes are often the targets of nitrite due to the high reactivity of iron and nitrite (Cui, Joannou, Hughes, & Cammack, 1992).

Moreover, nitrite is well-known to suppress the outgrowth of *C. botulinum* spores in cured meat products (Sindelar & Milkowski, 2011) and to completely control botulism. Nitrite has been reported to contribute to controlling the growth of several other pathogens such as *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus* and *Clostridium perfringens* (Parthasarathy & Bryan, 2012; Pradhan *et al*., 2009).

Fate of nitrite in meat systems and health risk issues

Nitrite can react with or binds to several constituents within the meat systems as it is an extremely reactive compound. Its reactivity is increased by the heat applied during the thermal processing of cured meat products (Cassens, 1997b). In particular, the nitrite ion at pH < 7 is extremely reactive; it can interact with various constituents of the meat such as amines, amino acids, sulphurhydryl, phenolic compounds, myoglobin, and reductants such as ascorbic acid (Toth, 1983).

The added nitrite in meat products could be completely recovered as nitrate, nitrosylmyoglobin, gaseous nitrogen compounds, and residual nitrite (Varnam & Sutherland, 1995). Due to the combination of nitrite with meat pigments and other compounds, the detectable amount declines rapidly during storage. Therefore, the residual amount of nitrite is considerably lower compared to the amount of the ingoing/added nitrite level (Cassens, 1996). In addition, it has been reported that only 10–20% of the added nitrite could be analytically detected in cured meat immediately after processing (Cassens, 1997a). Sebranek *et al*. (1973) reported the following fate of nitrite in cured meat as the percentage of nitrite added: losses with protein 20–30%, with myoglobin 5–15%, as nitrate 1–10%, as nitrite 5–20%, as a gas 1–5%, with sulphydryl groups 5–15%, and with lipid 1–5%. These results indicate that the largest portion of the reacted or bound nitrite is associated with the protein component of the meat.

Nitrite can act as a nitrosating agent in the formation of nitroso compounds (Cassens, 1995). N-nitroso compounds belong to six fundamental categories: volatile N-nitrosamine, non-volatile N-nitrosamine, N-nitrosamide, N-nitrosated heterocyclic carboxylic products, N-nitrosated glycosylarnines, and Amadori compounds (Hiramoto, Kido, & Kikugawa, 1993). Several epidemiological studies have demonstrated a potential relationship between nitrate, nitrite, and N-nitroso compounds and the risk of cancer (Alexander & Cushing, 2011). Certain nitroso compounds belong to a family of potent human carcinogens known as N-nitrosodimethylamine; these nitrosamines are easily formed by interaction of a secondary amino compound with nitrite under favorable conditions such as near acidic pH and a product temperature of >130 °C (Cassens, 1995). Although general health risks associated with nitrite are known, Alexander and Cushing (2011) have reported that there is no supportive evidence to prove the relationship between processed meat consumption and cancer risk. The exposure to only high overdoses of nitrite and nitrate from different sources has been associated with increased incidence of health risks (Gangolli *et al*., 1994; Sanchez-Echaniz, Benito-Fernandez, & Mintegui-Raso, 2001).

Meanwhile, studies conducted in the recent past have suggested that nitrite is an important molecule for human health; it plays a prominent role in human physiology especially, in the endogenous production of nitrite in the human body. The major endogenous source of nitrate and nitrite in mammals is the L-arginine–nitric oxide pathway that is active in numerous cell types throughout the body (Moncada & Higgs, 1993). Consequently, nitrite and nitrate are produced in the blood and tissues by rapid oxidation of nitric oxide. The reaction of nitric oxide with oxyhemoglobin in the blood mainly results nitrate and methemoglobin (Moncada & Higgs, 1993).

Recent studies report that nitric oxide is involved in controlling blood flow in cardiac muscle and potentially other tissues. Moreover, nitrite has a major role in mitochondrial respiration and activation of the alpha form of the estrogen receptor, and applies an antiapoptotic effect to avoid cell death (Bryan & Ignarro, 2010). In addition, the normal production of nitric oxide and nitrite may prevent various types of cardiovascular diseases, including hypertension, atherosclerosis, and stroke (Sindelar & Milkowski, 2012). Oral microflora including *Veillonella* spp., *S. aureus*, *Staphylococcus epidermidis*, *Nocardia* spp., and *Corynebacterium* spp. play a major role in nitrite-producing in human saliva. This nitrite producing activity of aforementioned microorganisms is occurred with the contribution of combination of reducing substances in salivary secretions, reducing bacterial metabolites, mammalian nitrate reductase in the papilla linguae, and nitrate reductase enzymes of the microorganisms colonizing the tongue (Li *et al*., 1997). Since most of the ingested nitrite is formed in saliva, swallowing saliva along with the food may result in the formation of

nitroso compounds (Sindelar & Milkowski, 2011). Therefore, processed meats are not the primary source of nitrate or nitrite. In addition, vegetables, beer, and cereals can also vital sources of nitrate, nitrite and N-nitroso compounds (Sindelar & Milkowski, 2011).

Nitrite is difficult to replace as a preservative because it can serve multiple functions simultaneously. Although some studies have revealed that nitrite is not positively related to health hazards in humans, a reduction or elimination of nitrite usage is a key issue for the meat industry. Therefore, several studies are ongoing to examine the antimicrobial and sensorial effects of various alternative compounds and/or technologies that can be used as nitrite substitutes. Therefore, organic or uncured meat production currently plays a major role in the meat industry in order to avoid direct usage of direct nitrite/nitrate. In this case, nitrite is introduced indirectly through other ingredients into uncured meat products to achieve the quality, shelf-life, and safety improvements expected by modern-day consumers.

Potential nitrite alternatives in processed meat

The challenge for the meat industry is to search for strategies to reduce supplemented and residual nitrite in cured meat in order to minimize the nitrite intake. There is a considerable interest toward the development of alternatives from natural sources and other preservation techniques that are considered to be comparatively healthier. This interest is further accelerated by the pressure generated from consumer demand for salt- and nitrite-reduced meat products.

The residual nitrite concentration in most of uncured products is generally lower than that in conventionally cured products (Sebranek & Bacus, 2007; Sindelar, Cordray, Sebranek, Love, & Ahn, 2007). Although it is difficult to replace nitrite by a single antimicrobial agent owing to its broad-spectrum activity (Pegg & Shahidi, 2000), a combination of nitrite and different antimicrobial agents may be effective. However, any improvement measures with regards to consumption safety should be undertaken without altering the unique characteristics of the natural and organic processed meat products.

Plant-based alternatives

Considerable amount of nitrates are present in some vegetables, which can be used as sources of nitrite. Vegetables such as celery, spinach, radish, and lettuce have been reported to contain more than 2500 mg nitrate/kg (Santamaria, 2006). Celery juice and celery powder are frequently used as natural nitrate sources because they do not impart any off-flavors owing to their high compatibility with processed meat products (Sebranek & Bacus, 2007). Celery powder contains approximately 3% nitrate (Sindelar *et al*., 2007) and is used by several meat processors along with a bacterial starter culture that reduces nitrate to nitrite during the manufacturing process while retaining the properties of typical cured meat products (Sebranek & Bacus, 2007). Celery powder was originally available in its nitrate form; however, subsequently, pre-converted celery powder was developed by some processors, in which nitrate was converted to nitrite since the incubation step increased the processing time (Sebranek & Bacus, 2007). It is considered that commonly used pre-converted celery powder contains 10,000−15,000 ppm sodium nitrite (Sindelar, Terns, Meyn, & Boles, 2010). However, the addition of celery powder to processed meat is generally limited to 0.2−0.4% of the formulation weight because at levels higher than this, off flavors may develop (Sindelar *et al*., 2007). Since celery powder contains a significant amount of naturally occurring nitrate it will not be the best alternative source of nitrite for meat without using in combination with nitrate reducing bacterial cultures to produce a standard cured meat product. However, celery powder has only a very little amount of pigments and a mild taste that does not detract the flavor of meat.

Recently, spray-dried Swiss chard powder was used as a natural source of nitrate. This product is similar to celery powder and contains 3.0−3.5% nitrate and as for celery powder, this product should also be used at a concentration of 0.15−0.3% (Sebranek, Jackson-Davis, Myers, & Lavieri, 2012). High concentrations may negatively affect the sensory attributes. The main advantage of Swiss chard powder is that it does not contain allergens (Sebranek *et al*., 2012). In addition, Gabaza, Claeys, Smet, and Raes (2013) showed that fresh and dried spinach can be used as a source of nitrate and that *Staphylococcus carnosus* can convert nitrate to nitrite. Sebranek and Bacus (2007) reported that the nitrate level of spinach juice is approximately 3227 ppm. The residual nitrite content of fermented spinach-treated pork samples (50 g/L) was lower than that of nitrite-treated samples (Gabaza *et al*., 2013).

Phenolic compounds, organic acids, and flavonoids are the key antimicrobial and antioxidant compounds in most plant extracts. These compounds can damage the cell membrane, which may lead to the leakage of cellular components, thereby inactivating or destroying microorganisms (Oussalah, Caillet, Saucier, & Lacroix, 2006). The antioxidant property of these compounds can be attributed to their characteristic to function as donors in the free radical chain reaction of lipid oxidation (O'Grady, Maher, Troy, Moloney, & Kerry, 2006). The antioxidant and antimicrobial properties of cranberry may be attributable to its organic acid content (citric acid, quinic acid, and malic acid), and to the presence of anthocyanins, flavonol glycosides, and proanthocyanidins (Chen, Zuo, & Deng, 2001; Lee, Reed, & Richards, 2006). In addition, the residues of tomato processing industries, including seeds and peels, contain highly biologically active compounds such as carotenoids (e.g., lycopene, β-carotene, phytoene, phytofluene, and lutein; Choksi & Joshi, 2007). Moreover, carotenoids in tomato are among the most important groups of natural pigments used as food colorants (Francis,

Barringer, & Whitemoyer, 2000). Both cranberry and tomato extracts showed pH reductions that increase the amount of nitrite involved in the curing reactions when added together with nitrite and thus reduce the residual nitrite concentration. Hence, the decreased pH observed in these studies due to cranberry and tomato extracts may have accelerated the nitric oxide production that led to the depletion of residual nitrite (Pegg & Shahidi, 2000; Xi, Sullivan, Jackson, Zhou, & Sebranek, 2011).

Cranberry powder is a source of natural antimicrobial agents, particularly effective against *L. monocytogenes* growth in natural and organic processed meats (Qiu & Wu, 2007). Although lemon and lime powders, and grape seed extract are less effective against *L. monocytogenes*, they have the potential to control this organism in cured cooked meat when combined with cranberry powder (Xi *et al.*, 2011). These authors further reported that nitrite (150 ppm initial nitrite) along with cranberry powder at 1, 2, and 3% concentration reduced the growth of *L. monocytogenes* by 2–4 log CFU/g as compared with only nitrite. However, the disadvantage regarding cranberry products is that it is slightly acidic in nature as they contain organic acids, which may eventually limit the amount of cranberry product to avoid quality defects (Xi *et al.*, 2011). Therefore, selection of the optimum concentration of this product for supplementation to processed meat is a crucial factor.

Deda, Bloukas, and Fista (2007) examined the quality parameters of frankfurters produced with different levels of sodium nitrite and tomato paste. Frankfurters with low levels of sodium nitrite (50 and 100 ppm) and 12% tomato paste showed the highest redness, whereas frankfurters with 12% tomato paste alone showed the lowest levels of residual nitrite. Therefore, the amount of nitrite added to frankfurters can be reduced from 150 to 100 ppm when combined with 12% tomato paste without any negative effect on the quality of the final product. In addition, Eyiler and Oztan (2011) stated that tomato powder retarded the oxidation reaction and improved the consumer acceptability in case of frankfurters. Furthermore, these authors observed that increase in the amount of tomato powder resulted in increased redness values in the final product, which is in agreement with the results of Deda *et al.* (2007). In addition, tomato powder has been shown to reduce the residual nitrite level in frankfurters as well as to act as a natural colorant (Eyiler & Oztan, 2011). Hayes, Canonico, and Allen (2013) stated that tomato pomace powder, when incorporated at a concentration of 1.5%, did not have any detrimental effects on the physicochemical properties of pork luncheon roll. The pork luncheon roll formulated with 50 ppm nitrite and 1.5% tomato pomace powder had similar or enhanced sensory attributes and no negative effects on the texture, sensory qualities or the microbial stability as compared to those formulated with 100 ppm nitrite alone. Several studies that tested tomato-based ingredients in meat products reported lower contents of nitrite and thiobarbituric acid reactive substances (TBARS) and improvements in sensory qualities, including color and odor (Calvo, Garcia, & Selgas, 2008; Candogan, 2002; Sanchez-Escalante, Torrescano, Djenane, Beltran, & Roncales, 2003; Yilmaz, Simsek, & Isiki, 2002). Therefore, it is obviously appropriate to use tomato in combination with nitrite in meat products to avoid quality defects and to obtain maximum benefits.

The antioxidative components in garlic are S-alkenyl cysteine sulfoxide and other sulfur components such as diallyl disulphide and diallyl trisulphide (Sasse, Colindres, & Brewer, 2009). Antibotulinal properties of the majority of spice extracts can be attributed to their constituents such as eugenol, isoeugenol, D-borneol, citronellol, menthol, cinnamic acid aldehyde, and rosemarin acid (Ueda, Yamashita, & Kuwabara, 1982). Moreover, the application of spices such as rosemary, thyme, sage, and garlic can reduce the content of heterocyclic aromatic amines, thereby reducing the formations of carcinogens in cooked cured meat (Murkovic, Steinberger, & Pfannhauser, 1998). The active constituents in sage and rosemary are rosmanol, rosemadial, carnosol, carnosic acid, and epirosmanol (Murkovic *et al.*, 1998).

Cui, Gabriel, and Nakano (2010) used combinations of sodium nitrite and spice extracts from sage, clove, and nutmeg and found them to successfully inhibit the growth of *C. botulinum* (sage) or inactivate (clove and nutmeg) the organism. The combined antibotulinal efficacy of nutmeg, sage, and clove extracts observed in this meat model system can be useful in the development of minimally processed meat products, particularly those with low levels of sodium nitrite (approximately 10 ppm; Cui *et al.*, 2010). Ismaiel and Pierson (1990) noted diverse antibotulinal activities of sodium nitrite (50–100 ppm) and oregano essential oil (400 ppm) in ground pork. Furthermore, Nevas, Koronen, Lindstrom, Turkki, and Korkeala (2004) examined the antibacterial properties of essential oils derived from several spices against 12 bacterial strains including *Escherichia coli*, *L. monocytogenes*, *Salmonella* Typhimurium, *C. botulinum*, *C. perfringens* etc. The authors found that oregano, savory, and thyme essential oils showed the broadest range of antibacterial activity by inhibiting the growth of all tested organisms. However, *C. botulinum* and *C. perfringens* were the most sensitive among all organisms. Since spice extracts could not provide all the functions that nitrite alone could do, it can be suggested to use spice extracts in combination with an appropriate amount of nitrite.

Balentine, Crandall, O'Bryan, Duong, and Pohlman (2006) demonstrated that processed meat treated with rosemary at a concentration of 3000 ppm could maintain the red color for longer period and showed lower TBARS value. Doolaege *et al.* (2012) investigated the effects of different doses of rosemary extracts (0, 250, 500, and 750 ppm) combined with low sodium nitrite levels (40, 80, and 120 ppm). The addition of rosemary extract positively retarded lipid oxidation in liver pate. Furthermore, it was found that the concentration of sodium nitrite added to liver pate could

be reduced from 120 to 80 ppm when rosemary extract was added at all three concentrations, without any negative effect on lipid oxidation, antioxidant level, and color stability.

Incorporation of citrus co-products in meat products is another recent trend adopted for reducing the residual nitrite concentration. The extracts of citrus co-products are rich in dietary fibers, antioxidant fibers, and bioactive compounds such as organic acid and polyphenols which can be used as functional ingredients in meat products (Perez-Alvarez, 2008).

Fernandez-Gines, Fernandez-Lopez, Sayas-Barbera, Sendra, and Perez-Alvarez (2003) investigated the effects of different concentrations of orange dietary fiber (0.5, 1, 1.5, and 2%) on the residual nitrite levels in a bologna-type cooked sausage. The maximum reduction (69.57%) in the residual nitrite level was obtained with 2% orange dietary fiber in combination with 0.02% oregano essential oil (Garcia-Martinez, 2009). The incorporation of orange dietary fiber into dry-cured meat products resulted in reduced residual nitrite levels as compared with that in the control and a higher redness value at levels >5 g/kg (Fernandez-Lopez *et al*., 2007).

Several researchers studied the use of lemon albedo (raw or cooked, dehydrated raw or dehydrated cooked) in cooked and dry cured meat products for reducing the levels of residual nitrite. Reduction in residual nitrite levels due to the bioactive constituents of raw and cooked lemon albedo (Fernandez-Gines, Fernandez-Lopez, Sayas-Barbera, Sendra, & Perez-Alvarez, 2004). Aleson-Carbonell, Fernandez-Lopez, Sendra, Sayas-Barbera, and Perez-Alvarez (2004) investigated the influence of various concentrations (0, 25, 50, 75, and 100 g/kg) of raw and cooked lemon albedo on the residual nitrite levels in dry-cured sausages and found that raw albedo was more effective in reducing the residual nitrite content and delaying lipid oxidation at all tested concentrations. Samples treated with 50 g/kg dehydrated raw albedo and 75 g/kg dehydrated cooked albedo showed sensory properties similar to those of the control. However, it is important to select the best concentration since higher concentrations may exert a negative effect on sensory attributes. Table 1 presents other potential plant-based alternatives for nitrite that can be used effectively in meat and meat products.

Organic acids

The use of ions of organic acids such as lactate, acetate, sorbate, and benzoate has been part of the food industry for several years and has many different applications in wide range of foods. Sodium and potassium lactate are widely incorporated in both fresh and cured meats (Brewer, Rostogi, Argoudelis, & Sprouls, 1995; Kim *et al*., 2006).

Presently, lactate is the most popular organic acid used as an antimicrobial agent in meat system since it is also known to enhance meat flavor owing to the salty taste (Doores, 2005). Lactate has also been shown to improve color stability of fresh meat and to function as an antioxidant

Table 1. Studies on potential usage of plant based alternatives for nitrites in meat products.

Potential alternatives	Concentrations used	Type of meat product/media	Effects	References
Green tea extract	1–2%	Ground beef meat	Reduced lipid oxidation and stabilized the meat color	Mustafa (2013)
Tea catechin with modified atmospheric packaging	200 mg/kg	Beef patties	Improved lipid stability and color stability	Tang *et al*. (2006)
Rosemary and oregano extract	0.02% of each	Raw pork batters	Higher antioxidant activity and prevention of color deterioration	Hernandez-Hernandez, Ponce-Alquicira, Jaramillo-Flores, and Legarreta (2009)
Grape seed extracts	0.00015–0.125%	Aqueous media	Impact on growth of *L. monocytogenes*	Bisha,Weinsetel, Brehm-Stecher, & Mendonca (2010)
Oregano and cranberry, with sodium lactate	Oregano and cranberry (50:50) at 750 ppm with 2% sodium lactate	Cooked ground beef	Impact growth of *L. monocytogenes*,	Apostolidis, Kwon, and Shetty (2008)
Aqueous extract of *Coptis* rhizome with sodium nitrite	Sodium nitrite 6–8 ppm with 0.05% *Coptis* extract	Broth media	Synergistic antibotulinal activity, reducing sodium nitrite from 6–8 ppm–2 ppm with 0.05% *Coptis* extract	Cui *et al*. (2010)
Anka rice with nitrite	Anka rice 0.5% 25 ppm nitrite	Low-nitrite Chinese sausages	No difference with 100 ppm nitrite added, color stability	Liu, Wu, and Tan (2010)
Annatto (*Bixa orellana* L.) powder	60% in sausage and 0.08, 0.31, and 0.16% (v/v) in broth	Sausage	Higher redness value, no growth of *C. perfringenes*, control of *C. botulinum* growth	Zarringhalami, Sahari, and Hamidi-Esfehani (2009)
Grape seed extract, pine bark extract and oleoresin rosemary	1% each ingredient	Cooked beef	Impact growth of *L. monocytogenes*	Ahn, Grun, and Mustapha (2007)

(Brewer *et al*., 1995; Shelef, 1994). The addition of lactate may improve color stability by replenishing the reduced form of nicotinamide adenine dinucleotide (NADH) when lactate is converted to pyruvate by lactate dehydrogenase, thereby increasing the metmyoglobin reducing activity. In addition, deoxymyoglobin can convert nitrite to nitric oxide; the generation of excess deoxymyoglobin in turn results in the production of more nitric oxide and reduction in the residual nitrite level (Kim *et al*., 2006).

Ions of organic acids such as lactate, sorbate, and citrate can exert antimicrobial effect specifically by changing water activity, migrating through the cell membrane, lowering the intracellular pH, and affecting cellular metabolism via inhibition of adenosine triphosphate (ATP) generation (Maas, Glass, & Doyle, 1989). Sodium, potassium, and calcium lactates are similar in their effectiveness in inhibitory activity against aerobic and anaerobic microorganisms in meat (Shelef, 1994). Maas *et al*. (1989) investigated comminuted raw turkey containing 0, 2.0, 2.5, 3.0 or 3.5% sodium lactate against a 10-strain mixture of *C*. *botulinum* spores from proteolytic type A and B and showed that sodium lactate exhibited an antibotulinal effect. Meng and Genigeorgis (1994) also examined the effect of sodium lactate on toxigenesis caused by proteolytic and nonproteolytic *C*. *botulinum* spores inoculated in processed 'sous-vide'-type beef and chicken breast. Their results showed that sodium lactate delayed toxin production in beef and chicken at >2.4 and > 1.8%, respectively. Furthermore, Choi and Chin (2003) stated that sodium lactate influenced the growth of *L*. *monocytogenes* delaying their lag phase by 2 wk, in addition affecting the growth of *C*. *botulinum*. Schlyter, Glass, Loeffelholz, Degnan, and Luchansky (1993) demonstrated antilisterial effects of sodium diacetate (0.1, 0.3 and 0.5%) alone or in combination with sodium nitrite (30 ppm), sodium lactate (2.5%) or pediocin (5000 AU/mL) in slurries prepared from ready-to-eat turkey breast meat. The increased antilisterial activity was due to the synergistic effect of the combined treatments.

In addition, inhibition of the germination and outgrowth of *C*. *perfringens* by buffered sodium citrate alone and in combination with sodium diacetate during abusive chilling of roast beef and injected pork has been evaluated by Thippareddi, Juneja, Phebus, Marsden, and Kastner (2003). The incorporation of sodium citrate into roast beef formulation resulted in reductions in the population of *C*. *perfringens* by 0.98, 1.87, and 2.47 log CFU/g at 0.5, 1.0, and 2.0% concentrations, respectively. Moreover, Juneja and Thippareddi (2004) conducted the same experiment for ground turkey, but with two additional organic acids (sodium lactate and sodium acetate). According to their study, sodium lactate and sodium acetate at 1% concentration could control *C*. *perfringens* germination and outgrowth (<1.0 log CFU/g). Houtsma, Heuvelink, Dufrenne, and Notermans (1994) showed that lactate mediated inhibition of proteolytic *C*. *botulinum* toxin formation was more effective at lower temperatures, and that the effect was not due to lowering the water activity of the broth system used. Jones and Betts (2009) demonstrated that addition of 2% potassium lactate had an inhibitory action on non-proteolytic *C*. *botulinum* in broth studies where 2% salt was present, the pH was 5.5 and the broths were stored at 30 °C.

Furthermore, the use of benzoates as antibacterial compounds has received only a little attention compared to other weak acid preservatives such as sorbates and lactates. Islam, Chen, Doyle, and Chinnan (2002) have shown that a solution containing benzoate at a concentration of 25% (w/v) inhibited the growth of *L*. *monocytogenes* on the surface of frankfurters. In addition, Jones and Betts (2009) demonstrated that 2000 ppm sodium benzoate had an inhibitory action on non-proteolytic *C*. *botulinum* in broth studies where no salt was present.

Moreover, studies conducted on bacon using potassium sorbate with low levels of nitrite have shown some success, producing products of acceptable color and taste (Gray & Pearson, 1984). However, complete replacement of nitrite with sodium sorbate is not possible since samples which contain sorbate solely being significantly unacceptable due to increasing rancidity (Al-Shuibi & Al-Abdullah, 2002). A previous study has shown allergic reactions to sorbate used as a nitrite alternative in meat, which caused many to disregard its use (Gray & Pearson, 1984). Sorbates are not currently permitted as additives in any organic products and their permitted uses in non-organic meat products are very restricted. They are permitted in pate and in jelly coatings of cooked, cured or dried meat products in combination with p-hydroxybenzoates (Al-Shuibi & Al-Abdullah, 2002).

Lactates tend to be added to foods for their taste, buffering ability and humectant properties (Davidson, Sofos, & Branen, 2005). Lactic acid is currently permitted as an additive in the production of organic foodstuffs of either plant or animal origin, with no specific upper limit of usage set.

Bacteriocins and other microbial sources

Antimicrobial proteins or peptides produced by bacteria are known as bacteriocins. These are synthesized in bacterial ribosomes and can inhibit other bacteria (Klaenhammer, 1993). Bacteriocins constitute a diverse group of antibacterial proteins that can kill closely related bacteria by various mechanisms such as by inhibiting cell wall synthesis, permeabilizing the target cell membrane, or by inhibiting enzyme activity (Klaenhammer, 1993). Bacteriocinsare considered as safe and effective natural food preservatives.

Nisin – a low molecular weight bacteriocin produced by certain strains of *Lactococcus lactis* subspp. *Lactis* – has been used as a food preservative for more than 30 years. These bacteriocins have a potential for application in hurdle technology as effective natural preservatives (Galvaz, Abriouel, Lopez, & Omar, 2007). Fowler and Gasson (1991) demonstrated that spores of *Clostridium* species

that had survived a heat treatment of 3 min at 121.1 °C were ten times more sensitive to nisin. Nisin is widely used as a preservative in heat processed foods as it can increase the sensitivity of spores to heat in diverse species (Fowler & Gasson, 1991).

Scott and Taylor (1981) reported that 50 ppm of nisin can inhibit the outgrowth of spores of *C. botulinum* type A in an agar medium; however, dosage of up to 125 ppm of nisin failed to prevent the outgrowth of these spores in cooked meat medium. Caserio, Stecchini, Pastore, and Gennari (1979) reported that 150 ppm of nitrite did not completely suppress the outgrowth of spores of *C. perfringens* in frankfurters. However, a combination of 200 ppm of nisin and 75 ppm of nitrite could inhibit the outgrowth of spores. Moreover, Rayman, Airs, and Hurst (1981) found that 75–100 ppm of nisin in combination with 40 ppm of nitrite could completely inhibit the outgrowth of spores of *Clostridium sporogenes* in meat slurries at 37 °C for 56 days. However, several studies evaluated the addition of nisin to meat products in order to reduce the nitrite levels (Calderon, Collins-Thompson, & Usborne, 1985; Houben & Krol, 1985) and found that only high level of nisin could achieve better control of *C. botulinum*.

Enterocins at 4800 AU/g reduced the number of *Listeria innocua* by 7.98 log cycles in cooked ham and by 9 log cycles in pate. In deboned chicken breasts, 4800 AU/cm^2 of enterocins reduced the *L. innocua* counts by 5.26 log cycles as compared to that in the control. Kouakou *et al.* (2009) investigated laboratory fermentation mixtures in which *L. monocytogenes* was co-cultured at 4 °C with bacteriocin-producing bacterium in lean pork meat without the addition of nitrite, and a strong antilisterial effect was noted after 1 week of culturing. Furthermore, enterocin AS-48 was tested alone or in combination with chemical preservatives against *L. monocytogenes* in a cooked ham model system. AS-48 (20, 40, and 60 μg/g) alone was active against *L. monocytogenes,* whereas the antilisterial effect was improved when AS-48 (40 μg/g) was combined with nitrite/nitrate, pentasodium tripolyphosphate, sodium benzoate or potassium sorbate. The most effective combination was determined to be AS-48-nitrite/nitrate (0.007%) because it could reduce listeria counts below detection level throughout the storage period (Ananou *et al.*, 2010).

In addition, the effectiveness of enterocin CCM 4231 in controlling *L. monocytogenes* contamination in dry-fermented salami has been examined. The addition of enterocin resulted in the reduction of *L. monocytogenes* by 1.67 log cycles immediately after bacteriocin treatment (Laukova, Czikkova, Laczkova, & Turek, 1999).

The cumulative findings suggest that nisin may be acceptable alternative to nitrite, or, when used as an adjunct, it can permit the reduction of nitrite levels in cured meat without compromising the safety aspects. However, further experiments are required prior to the use of nisin as a partial replacement for nitrite.

Apart from the natural colorants mentioned in previous sections, microbial conversion of metmyoglobin to nitrosylmyoglobin has generated considerable interest recently. As depicted by Table 2, several bacteria types have been tested, some of which demonstrated the ability of converting metmyoglobin to nitrosylmyoglobin, thereby providing a way to produce nitrite alternatives in meat products. Bacterial nitric oxide synthase has been detected in a *Nocardia* species (Chen & Rosazza, 1995) and *Lactobacillus fermentum* IFO 3956 (Morita, Sakata, & Nagata, 1998). Nitric oxide is derived from L-arginine by the bacterial nitric oxide synthase. However, it remains unclear how *Staphylococcus xylosus* can produce nitric oxide without the addition of nitrite and nitrate. On the other hand, we cannot assume that *S. xylosus* contains bacterial nitric oxide synthase (Morita *et al.*, 1998). In fact, the utilization of nitrate/nitrite as an alternative electron acceptor in the respiratory chain by staphylococci seems like a possible pathway for nitric oxide generation. The reduction of nitrate into nitrite by *S. carnosus* has been suggested to be due to a membrane-bound nitrate reductase involved in respiratory energy conservation (Pantel, Lindgren, Neubauer, & Gotz, 1998). The expression of nitrate reductase by staphylococci is induced by anaerobic growth conditions, and the expression level is the highest in the presence of nitrate during exponential growth phase (Pantel *et al.*, 1998).

HHP treatment

During HHP treatment, packaged food is placed inside a pressure vessel and subjected to water pressure from 100 to 900 MPa, which is isostatically transmitted inside the

Table 2. Major bacteria species associated with conversion of metmyoglobin to nitrosylmyoglobin in different studies.

Microorganisms	Growth mediums	References
Chromobacterium violaceum	Microbiological media	Arihara *et al.* (1993)
Kurthia sp.		
Lactobacillus fermentum	Smoked fermented sausages	Moller, Jensen, Skibsted, and Knochel (2006)
Lactobacillus fermentum	Chinese style sausage	Zhang, Kong, and Xiong (2007)
Pediococcus acidilactici	Broth medium	Gundogdu, Karahan,& Cakmakc (2006)
Lactobacillus plantarum		
Staphylococcus xylosus	Broth medium	Morita *et al.* (1998)
	Raw meat batters	Li, Kong, Chen, Zheng, and Liu (2013)
Staphylococcus carnosus, Staphylococcus simulans, Staphylococcus saprophyticus	Sausages	Gotterup *et al.* (2008)

pressure vessel. This treatment affects the cellular metabolism and membrane integrity of food microflora (Rendueles *et al.*, 2011). HHP does not inhibit or destroy any unique cellular site or cell function; however, cell death occurs due to multiple internal cell damage including ion exchange modifications and changes in the fatty acid composition, ribosome morphology, cell morphology, protein denaturation, and inhibition of enzyme activity (Simpson & Gilmour, 1997).

Meat products must be subjected to sufficient hurdles in order to inhibit the growth of pathogenic or spoilage microorganisms. Specifically, the combination of high pressure with another hurdle technique can result in a synergy between the different hurdles and thereby improve the inhibitory effect. HHP has been used for the inactivation of pathogens in food items, including meat products, without affecting the flavor of foods to a large extent (Cheftel & Culioli, 1997). Moreover, Cheftel and Culioli (1997) suggested the existence of a synergy between salt and HHP resulting from the pressure sensitization of bacterial cells, thereby limiting their regrowth.

HHP is a novel non-thermal preservation technique with immense potential for ensuring microbiological safety, while simultaneously maintaining the sensory quality of the food. In fact, the use of HHP could allow limiting of the amounts of salt and nitrite added to the meat products for prolonging their shelf-life. The feasibility of HHP application in low-salt meat products has been investigated (Omana, Plastow, & Betti, 2011; Pietrzak, Fonberg-Broczek, Mucka, & Windyga, 2007). Duranton, Guillou, Simonin, Cheret, and Lamballerie (2012) studied the response of spoilage bacteria to high pressure treatment in combination with different concentrations of salt and nitrite. This study demonstrated the combined effect of high pressure (up to 500 MPa a 20 °C for 6 min) with sodium chloride (0–3%) or sodium nitrite (0–100 ppm) on the outgrowth of endogenous flora of pork meat, including aerobic mesophiles, lactic acid bacteria (LAB), and Enterobacteriaceae members; a combination of high pressure with 1.5% and 3% of salt was found to reduce the microbial counts to <2 log CFU/g at the end of storage period.

Pietrzak *et al.* (2007) revealed that a reduction in the quantity of added salt and sodium nitrite did not significantly affect the microbiological quality of cooked pork ham over 8 weeks of storage 4–6 °C because HHP treatment (600 MPa, 31 °C, 6 min) achieved the same antimicrobial ability as that of salt and sodium nitrite alone. These results indicate that HHP treatment can significantly improve the shelf-life of vacuum-packed ham, including the hams with reduced level of curing ingredients in brine. Myers *et al.* (2013) determined the effect of nitrite and HHP on the growth of *L. monocytogenes* on ready-to-eat sliced ham. The use of HHP at 600 MPa for 3 min resulted in an immediate reduction of 3.9–4.3 log CFU/g in the *L. monocytogenes* populations. Hayman, Baxter, O'Riordan, and Stewart (2004) investigated the feasibility of using HHP to extend the shelf-life and improve the safety of refrigerated ready-to-eat meats containing salt and sodium nitrite. They found that HHP treatment at 600 MPa and 20 °C for 180 s was sufficient to control the levels of aerobic bacteria, anaerobic bacteria or lactobacilli to below the detectable limits or at lower levels over 95 days of storage at 4 °C.

Presently several scientists are studying HHP treatment as an alternative approach to inactivate *C. botulinum* spores. The effects of HHP under diverse temperature-time combinations on the inactivation of spores of *C. botulinum* type A in phosphate buffer has also been reported by Reddy, Solomon, Tetzloff, and Rhodehamel (2003) and Reddy, Tetzloff, Solomon, and Larkin (2006).

The combined effect of HHP (200 MPa for 10 min and 400 MPa for 10 min) and enterocin LM-2 (256 and 2560 AU/g) on the refrigerated shelf-life of sliced cooked ham has been evaluated (Liu *et al.*, 2012). The combination of HHP (400 MPa) and enterocin (256 or 2560 AU/g) was found to inhibit the growth of *L. monocytogenes* and *Salmonella* Enteritidis in sliced cooked ham up to undetectable levels and to extend the shelf-life of refrigerated sliced ham to 70 or 90 days, respectively. Table 3 reveals further

Table 3. Microbial inactivation by high hydrostatic pressure (HHP) alone and combined preservation treatments in meat products.

Treatment	Product	Effect	Reference
HHP at 600 MPa	Dry cured ham	Absence of *L. monocytigenes* after 120 days	Hugas, Garriga, and Monfort (2002)
HHP at 400 MPa	Cooked ham	1.9 log CFU/g reduction of *L. monocytogenes* after 42 days	Aymerich, Jofre, Garriga, and Hugas (2005)
HHP at 450 MPa	Iberian ham	3.6 log CFU/g reduction of *L. monocytogenes* after 60 days	Morales, Calzada, and Nunez (2006)
HHP at 400 MPa with pediocin, sakacin and enterocin	Meat homogenates	*L. monocytogenes* was $< 10^2$ CFU/g for 61 days	Garriga, Aymerich, Costa, Monfort, and Hugas (2002)
HHP at 400 MPa with potassium lactate	Sliced cooked ham	Inhibition of *L. monocytogenes* and *Salmonella* during 84 days	Aymerich *et al.*(2005)
HHP at 600 MPa	Cooked ham, dry cured ham and marinated beef loin	*Listeria monocytogenes, Salmonella enterica, Staphylococcus aureus* below the detection limit during 120 days storage	Jofre, Aymerich, Grebol, and Garriga (2009)

findings related to microbial inactivation in meat products by HHP alone or in combination with other hurdles.

Conclusion

Nitrite is considered as a multifunctional food additive in meat curing. Several studies have indicated that nitrite intake should be limited owing to its potential carcinogenic effect in humans. In contrast, some studies have elucidated the beneficial effect of nitrite on human health; however, the consumer demand for natural or nitrite-free meat products remains high. Hence, the challenge for the meat industry is to search for effective strategies to reducing the residual nitrite in cured meat and to search for better alternatives for nitrite for the preparation of uncured/naturally cured meat products. Emerging technologies such as HHP and several plant-based extracts, microbial sources, and organic acids can be effectively used in processed meats as nitrite alternatives. However, no single substitute for nitrite is available that can simultaneously provide all the functions of nitrite. Therefore, the most effective approach is to use hurdle technologies for meat curing, in which low levels of sodium nitrite is used in combination with other compounds and/or with other processing technologies possessing inhibitory activities against the most prevalent pathogenic microbes along with better sensory qualities. However, further investigations are necessary to confirm the safety of these compounds and/or technologies on human health prior to implementation in food industry.

Acknowledgments

This work was carried out with the support of Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ011617), Rural Development Administration, Republic of Korea and Research Institue of Eco-friendly Livestock Science, Institute of Green Bio Science and Technology, Seoul National University.

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