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REVIEW PAPER

OVERVIEW OF SODIUM NITRITE AS A MULTIFUNCTIONAL MEAT-CURING INGREDIENT

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Sodium nitrite is a meat-curing ingredient responsible for developing and fixing the sensory product attributes, also having a strong antimicrobial activity. However, it is associated with nitrosamine formation. Therefore, there is consumer demand for low-nitrite or nitrite-free meat products. In order to develop these products it is essential to understand the complex functions of sodium nitrite in meat-curing. This review summarizes the positive effects of nitrite in meat-curing, and points out that sodium nitrite is involved in the formation of nitrosamines, on the other hand. Several data from recent reports on the potential alternatives to nitrite replacement are also summarized.

Keywords: sodium nitrite, color, flavor, novel alternatives, meat products

Introduction

Fresh meat and meat by-products are highly perishable as a result of their excellent concentrated nutrients, and they often tend to provide suitable media to microorganism growth (Zhou *et al*., 2010; Stoica *et al*., 2014; Sultana *et al*., 2014). Microorganisms and many other interrelated factors including endogenous enzymes, moisture, storage temperature, oxygen availability, light, NaCl (sodium chloride) presence have a major influence on lipid oxidation in meat and meat products (Zhou *et al*., 2010). Lipid oxidation raises many economic problems, being a major cause of meat quality deterioration (*i.e.* formation of undesirable odours and flavours, loss of the sensory, nutritive, and functional values of meat products) (Tuba and Aytunga, 2017). Food spoilage caused by microorganisms pose not only economic problems but also numerous human health problems, therefore their control is essential and decisive (Stoica *et al*., 2014; Stoica, 2018a). The incorporation of some preserving agents, either natural or synthetic that can act as either antimicrobials or antioxidants, or both, is a valuable solution for the meat industry (Sultana *et al*., 2014). A strong preserving agent is $NaNO_2$ (sodium

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nitrite), the sodium salt of HNO_2 (nitrous acid). $NaNO_2$ is considered the active curing ingredient responsible for the preservation of meat in combination with other ingredients like NaCl, and ascorbic acid or its derivatives. $NaNO_2$ is commonly used in industrially processed meat because it contributes to the microbiological safety of meat products, to a superior control of color formation (unique reddish-pink meat color), color uniformity, and product flavour (Honikel, 2008; Sindelar and Milkowski, 2011; Parthasarathy and Bryan, 2012; Xu *et al*., 2016; Cantwell and Elliott, 2017; De Maere *et al*., 2018; Lee *et al*., 2018; Liu *et al*., 2019). $NaNO_2$-curing is used in the manufacture of numerous processed meat products to extend their shelf-life by preventing microbiological spoilage and the growth of pathogens. Despite all the many beneficial attributes of $NaNO_2$ in regard to meat preservation, its use in the meat industry has not been without controversy (Sindelar and Milkowski, 2012). The toxicological implications of $NaNO_2$ has become more complex. $NaNO_2$ is generally perceived as an undesirable food additive because it is involved in the formation of highly carcinogenic compounds such as nitrosamines, during meat processing (Berger and Schmahl, 1986; Honikel, 2008; European Comission, 2011; Loh *et al*., 2011; Keszei *et al.,* 2012; Milkowski, 2011, 2012; Sultana *et al*., 2014; Sindelar and Stepien *et al*., 2016; Cantwell and Elliott, 2017; Iqbal, 2017; Majou and Christiean, 2018). Because of the ease of nitrosamine formation, sodium nitrite has raised concern over potential adverse effects on human health. In order to develop low-nitrite or nitrite-free meat products it is essential to understand the complex functions of sodium nitrite in meat-curing. This review summarizes the positive effects of nitrite in meat-curing. The study points out that sodium nitrite is involved in the formation of nitrosamines, on the other hand. Several data from recent reports on the potential alternatives to nitrite replacement are also summarized.

Functions of sodium nitrite in cured meat

The main functions of $NaNO_2$ in cured meat consist of the sensory characteristics development (color, flavor), as well as the protection against lipid and protein oxidation, and the microbiological safety of meat products.

Improvement and fixation of the color

Color is the most important indicator of meat product quality, and it is often considered an extremely important attribute for consumer acceptance (Carpenter *et al*., 2001; Krystallis and Chryssohoidis, 2005; Sindelar and Milkowski, 2012; Xu *et al*., 2016; De Maere *et al*., 2018). The development and fixation of a desirable red color is the most obvious effect of $NaNO_2$ addition (Sindelar and Milkowski, 2012). A very little amount of $NaNO_2$ is needed to induce a cured meat color. It was reported that a minimum level of 25-50 *ppm* (mg/kg) of $NaNO_2$ is sufficient to induce an acceptable cured color (Sindelar and Milkowski, 2011). However, higher levels of $NaNO_2$ would be necessary to achieve and maintain the acceptable reddish-pink meat color, especially during long product shelf life (Sindelar and Milkowski, 2011). The reddish-pink color of thermally-processed cured meat products is the result of highly complex chemical processes, culminating in the formation of nitrosomyglobin (Figure 1).

Figure 1. Visual effect* of different state of myoglobin (Mb). *Colored online

The color manifests itself in many different shades, depending on the nature of the ligand attached to iron and the oxidation state of the iron (De Maere *et al*., 2018). Raw meat is primarily found as one of three colors depending on the Mb (myoglobin) state and the bound ligand. Deoxy-Mb (ferrous myoglobin) is present when iron is in the ferrous state (Fe_2^+), nothing is bound to the ligand, and the meat is purple-red (Mancini and Hunt, 2005; De Maere *et al*., 2018). Oxy-Mb is the state in which the iron is in the ferrous (Fe_2^+) state, O_2 is bound to Mb, and the color is bright red (Mancini and Hunt, 2005; De Maere *et al*., 2018). The consumers associate this bright red color with meat freshness. Finally, ferric met-Mb is present when the iron is in the ferric (Fe_3^+) state, nothing is bound to the ligand, and the meat is brown (Mancini and Hunt, 2005; De Maere *et al*., 2018). Met-Mb is generated by the removal of a superoxide anion from the hematin, and its replacement by a water molecule. The Mb, oxy-Mb, and met-Mb are constantly interconverted in the fresh meat (being in equilibrium with one another), but the presence of met-Mb formation downgrades the quality of fresh meat. Thermal processing denatures met-Mb, and meat remains brown in color. Fortunately, the denatured met-Mb can be oxidized. In other words, met-Mb turns into oxy-Mb by means of a reductant agent that can reduce the met-Mb to either Mb or oxy-Mb (Pegg and Shahidi, 2000).

When $NaNO_2$ is added to meat in an aerobic environment, the first reaction that takes place is that it acts as a strong oxidizing agent on Mb to form met-Mb. Ferrous Mb becomes oxidized to ferric met-Mb. The first visual effect of $NaNO_2$ addition to meat is a color change, from red to brown, and the reduction of $NaNO_2$ to NO (nitrogen monoxide) (Skibsted, 2011). Reducing $NaNO_2$ to NO is a long and much more complex process (Pegg and Shahidi, 2000). $NaNO_2$ itself is not a nitrosylating agent, it is an agent that transfers NO in meat, with the intermediate formation of N_2O_3 (dinitrogen trioxide), the nitrous acid anhydride (Sebranek and Bacus, 2007) or NOCl (nitrosyl chloride). Both N_2O_3 and NOCl start from HNO_2. NOCl starts from HNO_2 in the presence of sodium chloride, and it is a more powerful nitrosating agent than N_2O_3 (Skibsted, 2011). All cured meats have undergone the addition of sodium chloride in varying amounts (Sebranek, 2009). Once $NaNO_2$ has been added to meat, it will easily dissolve due to its good solubility in the aqueous portion of the meat matrix, and the first meat curing step is HNO_2 formation, in the weak acidic (pH 5.5) conditions (Honikel, 2008; Pegg and Honikel, 2014) (Equation 1).

$$NO_2^- + H_2O \leftrightarrow HNO_2 + OH^- \quad (1)$$

Two molecules of HNO_2 can form water and N_2O_3 during dissociation (McKnight *et al*., 1997; Honikel, 2008) (Equation 2).

$$2HNO_2 \rightarrow H_2O + N_2O_3 \quad (2)$$

N_2O_3 is unstable and dissociates into NO and NO_2 (nitrite) as the temperature rises (Honikel, 2008; McKnight *et al*., 1997; Pegg and Shahidi, 1997) (Equation 3).

$$N_2O_3 \rightarrow NO + NO_2 \quad (3)$$

NO is a highly reactive free radical that ends the chain of free radical reactions, and once produced it can act as an oxidant (NO^+ - nitrosonium cation or nitrosyl), and a reducing agent (NO^- - nitroxyl anion), depending on the environment (Henry *et al*., 1997; Cammack *et al*., 1999; Sebranek and Bacus, 2007). In the biochemical systems, NO in solution has a half-life of a few seconds (Cammack *et al*., 1999). NO tends to react rapidly with a wide variety of compounds such as the oxidized protein and forms an intermediate unstable compound – nitrosylmetmyoglobin, which can be reduced to nitrosylmyoglobin or nitrosomyoglobin (bright red color) by a reducing agent such as ascorbic acid or its derivatives (Pegg and Shahidi, 2000; Honikel, 2008; Skibsted, 2011; Parthasarathy and Bryan, 2012).

NO_2 can readily react with water, forming again one molecule of HNO_2 (which reenters into the NO reactions) and one molecule of HNO_3 (nitric acid) which may dissociate to NO_3 (nitrate) (Honikel, 2008). This explains why NO_3 is present in meat products to which only $NaNO_2$ was added. When the meat product is thermally processed the nitrosylmyoglobin (extremely unstable compound) will denature and form nitrosylhemochrome (attractive reddish-pink compound) (Pegg and Shahidi, 2000; Sebranek and Bacus, 2007; Honikel, 2008; Sindelar and Milkowski, 2011; Parthasarathy and Bryan, 2012). Nitrosohemochrome is stable in the absence of oxygen or under vacuum or inert gas packaging.

The resting of the final meat product at cool temperature for a short curing time (12 - 24 hours) after $NaNO_2$ addition is critical to the characteristic appearance of reddish-pink color and a cured flavor. Another very important factor that governs the NO reactions during meat curing are ascorbic acid and its derivatives (cure-accelerators that facilitate the conversion of $NaNO_2$ to NO), which contribute to a faster development of cured meat characteristics. Ascorbic acid or its derivatives can be added to meat batters at ~500 *ppm* (Pegg and Honikel, 2014). These reductants may react with N_2O_3 binding the resulting NO, and form an intermediate agent for the nitrosylation of myoglobin (Skibsted, 2011). They may retard the oxidation of NO to NO_2 and the formation of NO_3, and also act to prevent potential N-nitrosamine formation, being one of the strongest arguments in favor of adding ascorbic acid to cured meat.

Excess levels of $NaNO_2$ (above 600 *ppm* of $NaNO_2$/kg of meat) lead to a discoloration known as nitrite burn, and meat exhibits a green color due to the formation of nitrihemin, a green-brown pigment. The nitrite burn is also connected to the pH value in meat products and low pH values reduce the level of nitrite causing nitrite burn. High levels of $NaNO_2$ result in high levels of HNO_2 during the conversion of NO_2 into NO. HNO_2, a temporary acid, denatures myoglobin, which supports the formation of a green/yellow color caused by nitrite burn in the first place (Feiner, 2016).

Development of flavour and antioxidant effect

$NaNO_2$ is essential to the flavour development of meat products (Sindelar and Milkowski, 2012). Its role in flavour development is still unknown. The unique flavour of cured meat products is primarily related to the chemical processes of $NaNO_2$ and its associated reactions (Sindelar and Milkowski, 2011, 2012). The sensory panels revealed that a low level of $NaNO_2$ (~50 *ppm*) was sufficient to develop the flavour differences between cured and uncured meat (Sindelar and Milkowski, 2011; Alahakoon *et al.*, 2015). Sensory research suggests that a combined effect from the suppression of lipid oxidation by $NaNO_2$ and the development of $NaNO_2$ - related flavour, through yet unknown reactions, is responsible for the development of cured meat flavour (Sindelar and Milkowski, 2011).

The oxidation of lipids is the main cause for the decline of nutritional and sensory food quality and shelf-life, manifested through loss of essential nutrients, changes in texture and color, and formation of undesirable off-flavor and odors. Lipid oxidation can contribute to the induction of protein oxidation that is believed to proceed through a free radical chain reaction similar to that of lipid oxidation, but the mechanism of protein oxidation has not yet been fully established. A good strategy to control lipid and protein oxidation in food is to add antioxidant compounds to supplement endogenous antioxidants. However, it seems that the classical lipid antioxidant strategies do not inevitably apply to muscle proteins, and the compounds capable of preventing lipid oxidation are not always able to prevent protein oxidation (Pedersen, 2018).

$NaNO_2$ is highly effective as an antioxidant (Sindelar and Milkowski, 2011; Skibsted, 2011; De Maerea *et al.*, 2018). NO reacts rapidly with most radicals including the lipid derived alkyl, alkoxyl, and peroxyl radicals and other radicals involved in initiating lipid oxidation, like hydroxyl radical and the superoxide radical anion. The interaction of NO with the hydroxyl radical leads to the formation of $ONOO^-$ (peroxynitrite). Although, $ONOO^-$ may deactivate by isomerisation to nitrate at meat pH, it is a strong oxidant that has the ability to initiate both the lipid and protein oxidation (Berardo *et al*., 2016; Pedersen, 2018). This stability of cured meat is attributed to the NO potential to bind and stabilize heme iron of meat pigments during the curing process and to react with other biomolecules in the meat matrix (Alahakoon *et al*., 2015). $NaNO_2$ acts as an antioxidant in a mechanism similar to the one responsible for the coloring development (Honikel, 2008; Sindelar and Milkowski, 2011, 2012). NO neutralizes the free radicals, quickly shuts down oxygen and other reactive oxygen species, and blocks the cycle of the lipid autooxidation (Honikel, 2008; Sindelar and Milkowski, 2011; Alahakoon *et al*., 2015). The oxidative stability of cured meat can be improved by adding a reducing agent to the product formulation, such as ascorbic acid, that can act synergistically with $NaNO_2$ in order to inhibit the oxidation of the meat product (Yun *et al*., 1987). The pro-oxidative activities of the ascorbic acid involve generation of oxidation initiators / pro-oxidant by reducing Fe_3^+ to Fe_2^+. An important initiator of lipid and protein oxidation in meat is the Mb while nitrosylmyoglobin from cured meat was found to act as a lipid antioxidant. The antioxidant activity of nitrosylmyoglobin was related to formation of a stable Mb-NO complex, which was formed upon heating of meat, and blocked any catalytic activity of the heme-iron and also prevented its release; lastly, that nitrosylmyoglobin formed in the meat curing acted as an antioxidant (Pedersen, 2018). The ability of $NaNO_2$ to effectively delay the development of oxidative rancidity, which is the main cause of meat product quality deterioration, is one of its most noteworthy properties (Sindelar and Milkowski, 2012).

Antimicrobial activity

$NaNO_2$ is a strong preserving agent, and destroys some groups of microorganisms against which sodium chloride has no effect (Amariei *et al*., 2013). The inhibitory mechanisms of $NaNO_2$ are not well known (Sindelar and Milkowski, 2012; Majou and Christiean, 2018; Pedersen, 2018). $NaNO_2$ has both bacteriostatic and bactericidal effects that can be explained by the so-called Perigo effect (combination of $NaNO_2$ and amine groups of microorganism proteins). It targets bacteria at multiple sites by inhibiting metabolic enzymes, limiting oxygen uptake, and breaking the proton gradient (Sebranek, 2009). The antimicrobial effect is associated with the generation of NO, HNO_2 or $ONOO^-$ (Sebranek, 2009; Sindelar and Milkowski, 2012; Majou and Christiean, 2018). The $NaNO_2$ reactions related to the development of cured meat color are also important for the antimicrobial properties attributed to $NaNO_2$ (Sindelar and Milkowski, 2012). NO binds to iron, and limits its availability for metabolic enzyme functionality and bacterial metabolism and growth (Tompkin, 2005). $NaNO_2$ has stronger antimicrobial properties against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella.* However, it is used in meat processing primarily due to its uniquely powerful antimicrobial property against growth of the heat resistant *Clostridium botulinum*, *i.e.* vegetative cells, spores and toxin production (Christiansen *et al*., 1974; Sindelar and Milkowski, 2012; Alahakoon *et al*., 2015; Hung *et al*., 2016; Christieans *et al*., 2018; De Maerea *et al*., 2018). The antimicrobial activity of $NaNO_2$ is influenced by some factors including the pH and a_w (water activity) of the meat product, the ingoing amount of $NaNO_2$, treatment temperature, sodium chloride and ascorbic acid or its derivatives (that promote the formation of $ONOO^-$), and the thermal process applied to the product (Tompkin, 2005; Majou and Christiean 2018). The antimicrobial effect in cured meats is manifested for an ingoing amount of 150 *ppm* $NaNO_2$. Stoica *et al*. (2018b) found that the ingoing amount of 55 *ppm* $NaNO_2$ had effect on the microbiological, and sensory attributes of parizer (a product similar to the frankfurter). Low nitrite-cured parizer was microbiologically stable under refrigeration conditions after 21 days, similar to conventionally nitrite-cured parizer. The addition of $NaNO_2$ in a lower concentration (55 *ppm*) showed that it was enough to produce a suitable pink color in low-nitrite parizer (Stoica *et al*., 2018b). Although $NaNO_2$ has very important functions in cured meat products, it is an additive which has very strictly regulated usage rules (EFSA, 2003). The EU legislation generally sets limits on the ingoing amount of $NaNO_2$ in all meat products at 150 *ppm* and in only sterilized meat products 100 *ppm* (European Commission, 2011). For traditional products cured by immersion - in a curing solution containing nitrites and/or nitrates, salt and other components, traditional dry cured products, the EU legislation generally sets limits on the ingoing amount of 175-180 *ppm* $NaNO_2$ (European Commission, 2011).

Much of the $NaNO_2$ added during product manufacturing is either depleted through a series of NO reactions or during product manufacture and storage (Sindelar and Milkowski, 2012; Pedersen, 2018). The residual $NaNO_2$ serves as a reservoir for the regeneration of cured meat pigment lost from oxidation and light-induced iron-NO dissociation. To maintain a cured meat color throughout extended shelf-life, it is generally accepted to use a small amount (10-15 *ppm*) of residual $NaNO_2$ (Sindelar and Milkowski, 2012).

Nitrosamine formation

Despite all sodium nitrite positive effects, its addition to meat has become a source of controversy. Under certain conditions, residual sodium nitrite may react with free amino acids and amines and form nitrosamines in cured-meat products. The use of sodium nitrite as meat-curing agent raises public concern because it can be a precursor of nitrosamines. However, the nitrosamines are only obtained under special conditions like the presence of secondary amines (Equation 5), NO_2 availability to react, low pH values and product temperatures greater than 130℃ (Honikel, 2008; Sindelar and Milkowski, 2011, 2012).

$$\textit{Primary amine} \quad RNH_2 + NO^+ \rightarrow RNH - N = O + H^+ \rightarrow ROH + N_2 \tag{4}$$

$$\textit{Secondary amine} \quad R_2NH + NO^+ \rightarrow R_2N - N = O + H^+ \tag{5}$$

$$\textit{Tertiary amine} \quad R_3N + NO^+ \textit{ no nitrosamine formation} \tag{6}$$

The nitrosamines occur only in small amounts during the frying or grilling of cured meat products, and are suspected to cause cancer in humans exposed to even small amounts for prolonged periods (Berger and Schmahl, 1986). Several epidemiological studies demonstrated a potential relationship between nitrosamines and the risk of different cancer types (Keszei *et al*., 2013; Loh *et al*., 2011; Stepien *et al*., 2016; Iqbal, 2017). The nitrosamines are easily avoidable by proper frying and grilling.

Future trends

Due to the involvement of nitrite in the formation of nitrosamines, meat scientists are currently focusing on the development of novel strategies to replace conventional sodium nitrite. It is very difficult to replace sodium nitrite in meat products, and the strategies to replace it must provide the same safety as curing with sodium nitrite, as well as the same sensory characteristics. Several critical reports showed that it is both feasible and possibly beneficial to use alternatives to sodium nitrite (Table 1).

Table 1. Possible sodium nitrite alternatives in processed meat

Alternatives	References
Glycated nitrosohemoglobin (G-NOHb)	Zhang *et al*., 2013; Liu *et al*., 2019;
Carboxyhemoglobin (COHb)	Pereira *et al*., 2014; Xu *et al*., 2016
Plant-based alternatives	Aleson-Carbonell *et al*., 2004; Sebranek and Bacus, 2007; Sindelar *et al*., 2007; Eyiler and Oztan, 2011; Sebranek *et al*., 2012; Mihalcea *et al*., 2018
Microbial sources	Ananou *et al*., 2010; Yu *et al*., 2015
High Hidrostatic Pressure (HPP) treatment	Liu *et al*., 2012; Myers *et al*., 2013

Zhang *et al* (2013) and Liu *et al* (2019) suggested that G-NOHb prepared from porcine blood cells had potential to replace some of the functions of sodium nitrite in meat products. Other researchers noted that addition of both COHb and $NaNO_2$ synergistically increased the nitroso pigment contents and synergistically decreased residual nitrite contents (Xu *et al*., 2016). Pereira *et al*. (2014) and Xu *et al*. (2016) showed that COHb has potential for use in meat product formulations. The addition of blood treated with carbon monoxide allows the production of better-colored meat products having lower residual nitrite levels. Many researchers have dealt with sodium nitrite replacement using plant-based sources (*e.g.* lemon albedo, tomato powder or extract, celery and starter culture, beet). Among them Mihalcea *et al*. (2018) showed a promising example of total sodium nitrite replacement, without compromising the sensory characteristics and microbial safety of parizer (a frankfurter-like product) by the addition of a sea buckthorn groat. The results obtained by Yu *et al* (2015) suggested that *Monascus* strains can be use as a sodium nitrite substitute in the meat processing. HHP (High Hidrostatic Pressure), a novel non-thermal technology, has an immense potential for ensuring the microbiological safety, simultaneously maintaining the sensory quality of meat products (Alahakoon *et al.,* 2015). Pietrzak et al (2007) revealed that the HHP treatment (600 MPa, 31℃, 6 min.) achieved the same antimicrobial ability as that of salt and sodium nitrite alone. The most effective strategy to sodium nitrite replacement 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 microorganisms along with better sensory qualities (Alahakoon *et al.,* 2015).

Conclusions

Sodium nitrite is a multifunctional additive in meat industry, being responsible for the unique properties of cured meat products like color and flavour. It acts as an antioxidant, preventing the development of warmed-over flavour, and it has a bacteriostatic impact, retarding the formation of the *Clostridium botulinum* toxin. Despite all its positive benefits in meat curing, several reports indicated that sodium nitrite is a source of controversy, owing to its potential carcinogenic effect in humans. Many researchers are currently focusing on the search for better strategies to reducing the residual nitrite in cured meat. The glycated nitrosohemoglobin, carboxyhemoglobin, some plant-based powders or extracts, microbial sources, and HHP processing may be effective as sodium nitrite alternatives. The issue is that the developing of these alternatives are many times more expensive compared with conventional sodium nitrite. This can be compensated if the consumers would like to pay for the healthier meat products.

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