OVERVIEW OF SODIUM NITRITE AS A MULTIFUNCTIONAL MEAT-CURING INGREDIENT

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Received on 27 February 2019
Revised on 15 April 2019

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 NaNO₂ (sodium nitrite) which is used in meat-curing processes as a preservative and as a curing agent. This review paper aims to provide an overview of sodium nitrite as a multifunctional meat-curing ingredient.
nitrite), the sodium salt of HNO₂ (nitrous acid). NaNO₂ 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. NaNO₂ 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). NaNO₂-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 NaNO₂ in regard to meat preservation, its use in the meat industry has not been without controversy (Sindelar and Milkowski, 2012). The toxicological implications of NaNO₂ has become more complex. NaNO₂ 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 NaNO₂ 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 NaNO₂ addition (Sindelar and Milkowski, 2012). A very little amount of NaNO₂ is needed to induce a cured meat color. It was reported that a minimum level of 25-50 ppm (mg/kg) of NaNO₂ is sufficient to induce an acceptable cured color (Sindelar and Milkowski, 2011). However, higher levels of NaNO₂ 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 nitrosomyoglobin (Figure 1).

![Figure 1. Visual effect* of different state of myoglobin (Mb). *Colored online](image)

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 ($\text{Fe}^{2+}$), 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 ($\text{Fe}^{2+}$) state, $\text{O}_2$ 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 ($\text{Fe}^{3+}$) 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). NaNO₂ itself is not a nitrosylating agent, it is an agent that transfers NO in meat, with the intermediate formation of N₂O₃ (dinitrogen trioxide), the nitrous acid anhydride (Sebranek and Bacus, 2007) or NOCl (nitrosyl chloride). Both N₂O₃ and NOCl start from HNO₂. NOCl starts from HNO₂ in the presence of sodium chloride, and it is a more powerful nitrosating agent than N₂O₃ (Skibsted, 2011). All cured meats have undergone the addition of sodium chloride in varying amounts (Sebranek, 2009). Once NaNO₂ 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 HNO₂ formation, in the weak acidic (pH 5.5) conditions (Honikel, 2008; Pegg and Honikel, 2014) (Equation 1).

\[ \text{NO}_2^- + \text{H}_2\text{O} \leftrightarrow \text{HNO}_2 + \text{OH}^- \]  

(1)

Two molecules of HNO₂ can form water and N₂O₃ during dissociation (McKnight et al., 1997; Honikel, 2008) (Equation 2).

\[ 2\text{HNO}_2 \rightarrow \text{H}_2\text{O} + \text{N}_2 \text{O}_3 \]  

(2)

N₂O₃ is unstable and dissociates into NO and NO₂ (nitrite) as the temperature rises (Honikel, 2008; McKnight et al., 1997; Pegg and Shahidi, 1997) (Equation 3).

\[ \text{N}_2 \text{O}_3 \rightarrow \text{NO} + \text{NO}_2 \]  

(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).

NO₂ can readily react with water, forming again one molecule of HNO₂ (which reenters into the NO reactions) and one molecule of HNO₃ (nitric acid) which may dissociate to NO₃⁻ (nitrate) (Honikel, 2008). This explains why NO₃⁻ is present in meat products to which only NaNO₂ 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 NaNO₂ 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 NaNO₂ 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 N₂O₅ binding the resulting NO, and form an intermediate agent for the nitrosylation of myoglobin (Skibsted, 2011). They may retard the oxidation of NO to NO₂ and the formation of NO₃, 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 NaNO₂ (above 600 ppm of NaNO₂/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 NaNO₂ result in high levels of HNO₂ during the conversion of NO into NO₂. HNO₂, 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**

NaNO₂ 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 NaNO₂ and its associated reactions (Sindelar and Milkowski, 2011, 2012). The sensory panels revealed that a low level of NaNO₂ (∼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 NaNO₂ and the development of NaNO₂-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).

NaNO₂ 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, alkoxy radicals, and peroxy 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). NaNO₂ 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 NaNO₂ 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 Fe³⁺ to Fe²⁺. 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 NaNO₂ 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**

NaNO₂ 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 NaNO₂ are not well known (Sindelar and Milkowski, 2012; Majou and Christiean, 2018; Pedersen, 2018). NaNO₂ has both bacteriostatic and bactericidal effects that can be explained by the so-called Perigo effect (combination of NaNO₂ 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, HNO₂ or ONOO⁻ (Sebranek, 2009; Sindelar and Milkowski, 2012; Majou and Christiean, 2018). The NaNO₂ reactions related to the development of cured meat color are also important for the antimicrobial properties attributed to NaNO₂ (Sindelar and Milkowski, 2012). NO binds to iron, and limits its availability for metabolic enzyme functionality and bacterial metabolism and growth (Tompkin, 2005). NaNO₂ 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 NaNO₂ is influenced by some factors including the pH and aₜw (water activity) of the meat product, the ingoing amount of NaNO₂, 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 NaNO₂. Stoica et al. (2018b) found that the ingoing amount of 55 ppm NaNO₂ 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 NaNO₂ 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 NaNO₂ 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 NaNO₂ 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 NaNO₂ (European Commission, 2011).

Much of the NaNO₂ 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 NaNO₂ 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 NaNO₂ (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), NO₂ availability to react, low pH values and product temperatures greater than 130°C (Honikel, 2008; Sindelar and Milkowski, 2011, 2012).

\[
\text{Primary amine} \quad RNH_2 + NO^+ \rightarrow RNH - N = O + H^+ \rightarrow ROH + N_2 \quad (4)
\]

\[
\text{Secondary amine} \quad R_2NH + NO^+ \rightarrow R_2N - N = O + H^+ \quad (5)
\]

\[
\text{Tertiary amine} \quad R_3N + NO^+ \quad \text{no nitrosamine formation} \quad (6)
\]

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...
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**

<table>
<thead>
<tr>
<th>Alternatives</th>
<th>References</th>
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<tbody>
<tr>
<td>Glycated nitrosohemoglobin (G-NOHb)</td>
<td>Zhang et al., 2013; Liu et al., 2019;</td>
</tr>
<tr>
<td>Carboxyhemoglobin (COHb)</td>
<td>Pereira et al., 2014; Xu et al., 2016</td>
</tr>
<tr>
<td>Plant-based alternatives</td>
<td>Aleson-Carbonell et al., 2004; Sebranek and Bacus, 2007;</td>
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<td></td>
<td>Sindelar et al., 2007; Eyiler and Oztan, 2011;</td>
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<td></td>
<td>Sebranek et al., 2012; Mihalcea et al., 2018</td>
</tr>
<tr>
<td>Microbial sources</td>
<td>Ananou et al., 2010; Yu et al., 2015</td>
</tr>
<tr>
<td>High Hydrostatic Pressure (HPP)</td>
<td>Liu et al., 2012; Myers et al., 2013</td>
</tr>
</tbody>
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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 NaNO₂ 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 Hydrostatic 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°C, 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.

References


