Meat: Reduction of Nitrate and Nitrite Salts in Meat Products –
What Are the Consequences and Possible Solutions?

Régine Talon and Sabine Leroy
Université Clermont Auvergne, INRAE, MEDIS, Clermont-Ferrand, France

The use of nitrite and nitrate salts as additives in the manufacture of cured meat products is regulated by European directives. The nitrite ion can be toxic, and nitrate becomes so upon reduction to nitrite. However, nitrites have multiple functions, as they contribute to sensorial qualities (colour, flavour) and to the inhibition of some microorganisms. The European Commission is still pressing for further reductions in the levels of these additives. Although currently there are no compounds that replace nitrites in all their functions, some maintain the sensorial or hygienic qualities of cured meat products and, together with a reduced level of nitrites, could be an alternative. The use of nitrate/nitrite salts is still controversial, however, as some studies have highlighted their health benefits.

Introduction
Numerous foodstuffs contain nitrates and, to a lesser extent, nitrites. The average dietary intake of nitrates in France is 141 mg/day/person, with 75% provided by vegetables and fruits, 14% by water and 6% by animal products. The average dietary intake of nitrites is 2 mg/day/person, 40% of which comes from vegetables and fruits, 39% from processed animal products, 15% from other foodstuffs and 5% from water (EFSA, 2008a).

Sodium and potassium salts of nitrates (E251, E252) and nitrites (E249, E250) are commonly used as additives in the manufacture of meat products, such as salt-cured meats. Nitrites that are added or that derive from the reduction of nitrates are the active curing agent. They have a very broad spectrum of action and contribute to sensorial properties (colour, flavour) and the microbiological safety of cured products, notably regarding spore-forming bacteria.

The use of these additives is regulated by European directives 95/2/EC, 2006/52/EC and 1129 (11 November 2011), which authorize the addition of 100 to 150 mg of nitrites (E249, E250) per kg for heat-treated meat products, and 150 mg of nitrites with 150 mg of nitrates (E251, E252) per kg for non-heat-treated meat products. These directives were drawn up on the basis of the toxicity of nitrites, while the toxicity of nitrates is estimated from its reduction to nitrites. As a comparison, the mean nitrate content of lettuce is 4000 mg/kg. The acceptable daily intake (ADI) is 3.7 mg/kg bodyweight/day for nitrates and 0.07 mg/kg bw/day for nitrites (EFSA 2010, 2017).

In 2017, EFSA experts estimated that the exposure to nitrates solely from their use as a food additive was less than 5% of the overall exposure to nitrates in food and did not exceed the ADI, and thus, that exposure to nitrites as food additives was within safe levels. The toxicity of nitrites is due to their potential to form carcinogenic substances called nitrosamines (R₂N₂O), both in foodstuffs and in the body (Hammes, 2012). The residual nitrite and/or nitrate concentration in sausages and cooked ham was below 20 mg/kg, so the probability of forming nitrosamines in meat products was low (Honikel, 2008). Nitrosamines could, however, be generated when meat products were heat-treated at temperatures above 130 °C, such as during the grilling of bacon (Honikel, 2008).

Given the potential risk associated with nitrites, the European Commission is considering reducing levels of nitrites or nitrates or both in some foodstuffs, including meat products. The main risks associated with reducing or eliminating nitrites in the manufacture of cured meats are the growth of unwanted microorganisms, a reduction of about one-third in shelf-life, a greyish colour instead of a stable pink or red colour, and a loss of characteristic taste of these products. Numerous questions on the future of these products, therefore, remain unanswered. Is, for instance, this reduction compatible with the concomitant decrease in salt that food professionals must provide for consumer health? How can these reductions be achieved while ensuring the safety and sensory qualities of these foods?

To our knowledge, there are presently no compounds that replace nitrates in all their functions, but some more or less efficient alternatives are used or are being tested (Table 61.1).

Do Nitrate-Rich Plant Extracts Offer a Viable Alternative?
To avoid adding nitrates yet retain the sensorial and microbiological properties of meat products, nitrate-rich plant extracts have been tested. Sebranek and Bacus (2007) proposed that spices, spinach or celery juice were viable alternatives; they reported that the nitrate concentration in a powder from celery juice concentrate was high (2.75%) and that its addition to a meat...
TABLE 61.1
Role of Nitrate/Nitrite in Cured Meats and Examples of Alternatives

<table>
<thead>
<tr>
<th>NO₃ (nitrate)/NO₂ (nitrite)</th>
<th>Alternatives</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Colour</strong></td>
<td>Nitrate-rich plant extracts: celery, spinach ... = addition of nitrate</td>
</tr>
<tr>
<td>NO₃ → NO₂ via nitrate reductase of staphylococci</td>
<td>Natural colourants: tomato extract, paprika ... + decreased level of NO₂</td>
</tr>
<tr>
<td>NO₂ → NO + myoglobin = nitrosomyoglobin red colour stable</td>
<td>from 150 to 100 mg/kg</td>
</tr>
<tr>
<td><strong>Flavour</strong></td>
<td>Addition of bacteria: staphylococci with a nitric oxide synthase activity</td>
</tr>
<tr>
<td>Interaction with meat components = flavourful derivatives of nitroso-compounds</td>
<td>No alternatives</td>
</tr>
<tr>
<td>Inhibition of lipid oxidation = avoid rancidity</td>
<td>Antioxidant compounds: vitamin E, ascorbic acid, citrate, polyphenols</td>
</tr>
<tr>
<td><strong>Microbiological qualities</strong></td>
<td>Extracts of aromatic plants: rosemary, oregano, sage ... + decreased level of NO₂</td>
</tr>
<tr>
<td>No effect on growth for lactic acid bacteria</td>
<td>from 150 to 80 or 60 mg/kg</td>
</tr>
<tr>
<td>Altered survival of staphylococci and physiology (nitrosative stress)</td>
<td>Plant extracts: essential oil of savory, nutmeg, sage or clove + decrease level of NO₂ (10 mg/kg)</td>
</tr>
<tr>
<td>Inhibition of spoilage bacteria <em>Enterobacteria and Brochothrix thermophaacta</em></td>
<td>Acidifying lactic acid starter (pH &lt; 5.0)</td>
</tr>
<tr>
<td>Inhibition of pathogenic bacteria <em>Clostridium botulinum, Listeria monocytogenes, Salmonella</em></td>
<td></td>
</tr>
</tbody>
</table>

**Source:** Adapted from: Honikel, 2008; Ras et al., 2017; Sindelar and Milkowski, 2011; Christieans et al., 2017; Vermassen et al., 2014; Weiss et al., 2010.

batter in the manufacture of sausages resulted in a nitrite concentration of 100 mg/kg. Products made in this way are categorized as “clean label” food. Nitrites added in this “natural” way must be reduced to nitrites, which is possible via the action of starters with nitrate reductase activity, such as the species *Staphylococcus carnosus* and *S. xylosus*. The addition of “natural” nitrites to cooked meat products also called for the addition of starters that reduced nitrites to nitrites for the development of colour and thus led to manufacturing changes; in particular, the temperature should be kept at 42 °C for 90 min before the usual manufacturing procedure of these products (Sebranek and Bacus, 2007).

The addition of plant extracts with a *Staphylococcus* starter culture to cure brines yielded cooked ham with a colour and low level of lipid oxidation similar to that treated with nitrites, while ensuring lower residual levels (Krause et al., 2011).

The Standing Committee on the Food Chain and Animal Health considers that when a starter and nitrate-rich plant extracts are added, the nitrites obtained via the enzymatic activity of this starter should be considered as an additive and must be subject to authorization. Natural cherry extracts, which contain high levels of ascorbic acid, can be added instead of the acid itself to facilitate the conversion of nitrites into nitric oxide (NO) (Pegg and Honikel, 2015).

The use of nitrate-rich plant extracts rather than nitrites as an additive may therefore seem like an alternative, but it is in fact only a “false” solution, because the risk associated with the nitrite residue is the same.

---

**Role of Nitrites/Nitrites in Colour**

**Colour Formation**

Colour is formed through the reduction of nitrites to nitrites via the nitrate reductase activity of the two staphylococci used as starters, and also by numerous chemical reactions of nitrites with meat pigments (Talon et al., 1999; Honikel, 2008). These enzymatic and chemical reactions depend on pH, the pigment concentration in the raw material, the redox potential and temperature. Nitrate reductase activity differed greatly between the two species commonly used to initiate colour formation: *S. carnosus* and *S. xylosus* (Gøtterup et al., 2008; Sanchez et al., 2015; Leroy et al., 2017). Most *S. carnosus* strains reduced nitrites effectively, whereas fewer than 50% of *S. xylosus* strains did so. Various chemical reactions convert nitrites into derivatives such as NO, which binds to the iron of the haem group of myoglobin to form nitrosomyoglobin, a stable, red pigment that accounts for the typical colour of salt-cured meats (Honikel, 2008).

Various studies have shown that nitrite concentrations between 25 and 50 mg/kg are, in general, sufficient to produce a satisfactory colour in cured meats (Sindelar and Milkowski, 2011). However, higher concentrations are needed to develop and maintain acceptable cured meat colour of products with a long shelf-life.

**Alternatives**

**Addition of Natural Colourants**

The addition to meat batters of tomato extract (2.5–3.0%) or paprika as natural colourants at reduced nitrite levels (100 mg/kg instead of 150 mg/kg in the control) yielded a colour close to that of the control, confirming that the nitrite level could be reduced without affecting colour (Bazán-Lugo et al., 2012). Deda et al. (2007) showed that colour could be preserved in frankfurters by the addition of 12% tomato paste when the nitrite level was 100 mg/kg.

**Addition of Bacteria**

The lactic acid bacteria *Lactobacillus plantarum, L. fermentum* and *Pediococcus acidilactici*, the staphylococci *S. xylosus* and...
Meat: Reduction of Nitrates and Nitrites

S. carnosus when used as starters, and four other Staphylococcus species produced nitrosylmyoglobin in a laboratory culture supplemented with metmyoglobin (Morita et al., 1998; Gündoğdu et al., 2006; Göttlerup et al., 2007; Ras et al., 2017). The formation of nitrosylmyoglobin by S. xylosus was also noted in meat batter (Li et al., 2013). The bacteria could produce NO from arginine via nitric oxide synthase (NOS) (Crane et al., 2010). The gene encoding NOS has not been found in the genomes of lactic acid bacteria, but it has been identified in various species of coagulase-negative staphylococci, including two used as starters, S. carnosus and S. xylosus (Jansens et al., 2014; Ras et al., 2017). NO production via NOS has been demonstrated in a strain of S. xylosus (Ras et al., 2018).

Role of Nitrates/Nitrites in Flavour

Flavour Development, Antioxidant Role

Various sensory studies have shown that consumer panels are able to distinguish between food products made with different quantities of nitrates (Sindelar and Milkowski, 2011). In the absence of nitrates, products have a taste described as “meaty”, whereas they have a taste typical of cured meat when made in the presence of nitrates. Concentrations of about 50 mg/kg seemed sufficient for differences to be perceived by a panel (Gray et al., 1981). Pegg and Shahidi (2000) reported that 40 to 70 mg/kg nitrites was enough to ensure the development of the aroma characteristic of cured meats.

Despite this reported sensorial perception, the molecules responsible for this characteristic flavour and the mechanisms involved are largely unknown. Flavourful compounds are formed by the interaction of nitrite and/or NO with different ingredients of meat, resulting in nitroso-compounds. The nitrite can react with haem and non-haem proteins, low-molecular-weight peptides or amino acids, and lipids (Honikel, 2008). Nitrite and NO also have an antioxidant role (Sindelar and Milkowski, 2011); NO links to iron in myoglobin and thus prevents its oxidation and transformation into a potent oxidant able to peroxidize lipids. NO also chelates free radicals, and nitroso-compounds have antioxidant properties (Sebranek and Bacus, 2007; Sindelar and Milkowski, 2011).

There were more organoleptic compounds in ham cooked with nitrites than without: 49 versus 37 out of a total of 53, and lipid oxidation products were found at lower levels in nitrite-containing ham (Guillard, 1998). Likewise, the concentration of volatile compounds associated with lipid oxidation was lower in sausages made with a nitrate/nitrite mixture than in control sausages without nitrate/nitrite (Hospital et al., 2012). This antioxidant effect was noted at nitrate and nitrite concentrations above 75 mg/kg in fermented dry sausages. It should also be noted that the antioxidant power of a nitrate/nitrite mixture was well above that of nitrate alone (Christeians et al., 2017). In another study, 20 mg/kg nitrite was sufficient to inhibit lipid oxidation in fish, chicken, pork and beef (Sindelar and Milkowski, 2011).

Alternatives to Nitrates and Nitrites

There is an abundant literature on antioxidants tested in meat products (Weiss et al., 2010). Some, like vitamin E, lycopene, ascorbic acid, and extracts of rosemary, oregano and sage, trap or stabilize free radicals that cause oxidation reactions, while others, such as citrates and polyphenols, chelate metals that initiate oxidation. For example, rosemary extract added to liver pâté enabled a reduction in nitrites from 150 mg/kg to 80 mg/kg (Doolaege et al., 2012). A combination of rosemary (E392) and 60 mg/kg nitrite preserved the colour and flavour of cooked ham.

Role of Nitrates/Nitrites in Microbiological Qualities

Nitrates serve as a reservoir of nitrites, which are the active molecules involved in inhibition of various bacterial populations. The mechanisms responsible for the inhibition are poorly understood and depend on the bacterial species. Furthermore, inhibition depends on factors like pH, salt concentration and the presence of reducing agents like ascorbate and iron (Sindelar and Milkowski, 2011). The nitrite ion is a stronger inhibitor at acid pH (as in meat), doubtless because of the various chemical reactions that yield NO and nitrous and nitric acids (Honikel, 2008). Inhibition can also be influenced by iron, which binds to and inactivates nitrite. Thus, the presence of liver in certain cured meats can inhibit nitrites because it contains high levels of iron (EFSA, 2003).

We shall now review the effects of nitrate/nitrite on different bacterial populations, i.e., starters, spoilage bacteria and pathogenic bacteria.

Growth, Survival and Activity of Starters

Bacterial starters composed of a mixture of lactic acid bacteria and staphylococci are added during the manufacture of dry sausages. The microbial ecology of sausages depends on numerous endogenous factors, such as the composition of the raw material, its initial quality (pH, water activity and microbial load), salt, saccharides, spices, additives (nitrates and nitrites), added starters, casings, exogenous factors (temperature and humidity) and microbial interactions (Hammes, 2012).

In general, the growth and survival in sausages of lactic acid bacteria added as starters are not affected by the different concentrations of nitrate and/or nitrite used. Thus, populations of Lactobacillus sakei alone or in the presence of Pediococcus pentosaceus increased more than 2.5 log-fold and then remained stable in dry sausages made with different concentrations of nitrate (150–300 mg/kg) or nitrite (150 mg/kg) or both (80/80 mg/kg, 120/120 mg/kg) (Marco et al., 2006; Christeians et al., 2017). L. plantarum grew and survived in the absence or presence of nitrate/nitrite mixtures (150/150; 112.5/112.5; 75/75 mg/kg) in dry sausages (Hospital et al., 2012; Hospital et al., 2014).

The survival of two staphylococci used as starters (S. xylosus, S. carnosus) was modified by the presence of nitrate and/or nitrite. It was greater in a sausage made without nitrate/nitrite or with 75/75 mg/kg nitrate/nitrite than in the presence of greater quantities (Hospital et al., 2012; Hospital et al., 2014).

Very few studies have examined the impact of nitrate/nitrite on the physiology of starters. The absence or very low levels of...
these additives favoured the formation of volatile compounds via degradation of saccharides or amino acids (Hospital et al., 2012), a finding linked to results showing that these additives affected the survival of staphylococci (Hospital et al., 2014). Inoculation of S. xylosus into a meat matrix in the presence of nitrate/nitrite resulted in the modulation of expression of 24% of its genes, reflecting a large change in its physiology in this condition (Vermassen et al., 2014). Notably, nitroso-compounds generated by nitrate/nitrite exerted nitrosative stress, and the bacterial strain responded by overexpression of genes involved in iron homeostasis and those encoding antioxidant enzymes (Vermassen et al., 2014).

**Growth and Survival of Spoilage Bacteria**

*Enterobacteriaceae* in dry sausages containing 150/150 or 112.5/112.5 mg/kg nitrate/nitrite decreased in number during fermentation and were completely eliminated during drying in the presence of the highest concentrations (Hospital et al., 2012). In control (without nitrate/nitrite) or in sausages made with 75/75 mg/kg nitrate/nitrite, *Enterobacteriaceae* multiplied during fermentation and then decreased faster in samples with nitrate/nitrite than without (Hospital et al., 2012). Similarly, the growth of the spoilage bacterium *Brochothrix thermosphacta* in minced pork stored at 10 °C under vacuum for 12 days was slowed by the presence of nitrate/nitrite compared with a control (Hospital et al., 2014).

**Inhibition of Pathogenic Bacteria**

Numerous studies have shown that nitrite is valuable in controlling spore-forming bacteria like *Clostridium botulinum*, and also *Listeria monocytogenes* and *Salmonella*.

**Clostridium botulinum**

*Botulinum* poisoning results from the consumption of food containing preformed *botulinum* toxin, produced by *Clostridium botulinum* during the growth phase of vegetative cells following the germination of spores. Toxin poisoning often occurs after consumption of poorly sterilized preserves or of “homemade” ham and cured meat. Nitrites are used to control the growth of *C. botulinum*, thereby avoiding toxin production in cured meat products. The growth of *C. botulinum* is inhibited because nitroso-compounds interact with the iron-sulfur clusters of respiratory chain enzymes of this strictly anaerobic bacterium, reduce the level of intracellular ATP, and reduce the efficiency of active transport systems by blocking enzyme pathways (Milkowski et al., 2010).

There are two groups of *C. botulinum*: group I-proteolytic *C. botulinum* (toxins A, B, F), the spores of which are heat-resistant and can germinate and grow at a water activity >0.94 (10% NaCl) and pH > 4.6, and group II-neprolytic *C. botulinum* (toxins B, E, F), the spores of which are heat-sensitive and can germinate and grow at a water activity >0.97 (5% NaCl) and pH > 5.0, but with growth possible from 3 °C, which is not the case with the proteolytic strains (EFSA, 2003). Keto-Timonen et al. (2012) have shown that group II *C. botulinum* inoculated into heat-treated meat products (Wiener or Bologna sausages and cooked ham) prepared with nitrites (0, 75 or 120 mg/kg) survived. Type B *botulinum* toxin was produced during storage at 8 °C in the products without nitrite, whereas no toxin was present in the products prepared with nitrite during 5 weeks of storage (Keto-Timonen et al., 2012). When ground pork was inoculated with group I *C. botulinum* and subjected to short (80 °C, 7 min) and long (80 °C, 1 h) heat treatments in the presence of 100, 200 or 300 mg/kg nitrite, the probability of toxin production was reduced from 96% to 35% and from 86% to 23% for the short and long treatments, respectively (EFSA, 2003). In the presence of ascorbate, the probability of toxin production was decreased from 26% to 1% and from 8% to 0% for the short and long treatment, respectively (EFSA, 2003).

Various studies have suggested that 50–100 mg/kg nitrite is necessary to inhibit the growth of *C. botulinum* and hence the production of toxin in heat-treated meat products (EFSA, 2003; Cui et al., 2010; Keto-Timonen et al., 2012). In non-heat-treated meat products, however, the addition of 150 mg/kg nitrite is considered necessary to inhibit *C. botulinum*.

**Salmonella**

In France, the prevalence of *Salmonella* on the surface of pig carcasses was about 17.6% in 2006/2007 (EFSA, 2008b). This degree of contamination is an issue when the cuts from contaminated carcasses are used for the manufacture of dry cured meats consumed as such. Even if products like dry sausages are, during processing, subject to technological stages (fermentation, drying) that in theory have an unfavourable effect on bacterial growth and survival, the presence of *Salmonella* at the end of drying is today a reality. Since 2007, the prevalence of *Salmonella* in French finished products has increased, as seen from batch withdrawals and two cases of food poisoning following the consumption of dry sausage in June 2010 and December 2011. This emergence is often ascribed to upstream conditions (method of feeding animals) and to changes introduced in recent years by industrial manufacturers, both technologically and in terms of formulations (reduction of fat, salt and nitrite levels). France is not the only country affected, and cases of salmonellosis associated with pork consumption have been reported elsewhere in Europe.

In studies on sausages inoculated with *Salmonella enterica* serovar *Typhimurium* and made with different concentrations of nitrate or a nitrate/nitrite mixture, when these compounds were absent or when only nitrate was added (150, 200 or 205 mg/kg) a 2–2.5 log growth was observed during the fermentation phase, followed by a decrease during maturation and storage, albeit insufficient to eliminate these bacteria (Hospital et al., 2014; Christieans et al., 2017). In the presence of a nitrate/nitrite mixture, the growth of *Salmonella* was low (1 log) or nonexistent, and a population decrease was observed during the maturation phase; an absence of *Salmonella* was only noted at the end of storage (90 days). In these two studies, *Salmonella* was not eliminated by the time the sausages could potentially be marketed (Hospital et al., 2014; Christieans et al., 2017).

**Listeria**

*Listeria* in sausages grew during fermentation and then decreased slightly during maturation in the absence of nitrate/
Meat: Reduction of Nitrate and Nitrite Salts

nitrate or in the presence of nitrate only (Hospital et al., 2012; Christieans et al., 2017). In the presence of a nitrate/nitrite mixture, the Listeria population remained stable or decreased during fermentation, and a 2.5 log decrease was noted at the end of maturation in the presence of 150/150 mg/kg nitrate/nitrite, whereas 120/120 to 75/75 mg/kg nitrate/nitrite only caused a 1–1.5 log reduction. At the end of storage (90 days), Listeria was no longer detected only in the sausages made with at least 120/120 mg/kg nitrate/nitrite. These results prompted the recommendation by the code of practice in France (Code des usages, 2020) that 120 mg/kg nitrate/nitrite be used instead of 150 mg/kg (Christieans et al., 2017).

Alternatives

Plant Extracts

Numerous studies have envisaged controlling health risks by partially replacing nitrates with plant extracts that have antimicrobial activity. As an example, cranberry powder (3%) together with 0.4% celery powder when used instead of nitrates prevented the growth of Listeria monocytogenes in frankfurters (Xi et al., 2012). Clostridium perfringens was inhibited in mortadella sausages prepared with essential oil of savory and 100 mg/kg nitrite (Coutinho de Oliveira et al., 2011). Likewise, a synergistic effect was noted in the control of C. botulinum in a meat matrix by combination of 10 mg/kg nitrite with nutmeg, sage or clove (Cui et al., 2010).

Lactic Acid Bacteria

Salmonella persisted during the manufacturing of dry sausage in the presence of nitrates at weakly acid pH (>5) and water activity of 0.90 or 0.85 at the end of drying (Hospital et al., 2012; Christieans et al., 2017). In dry fermented sausages made with lactic acid bacteria that yielded a pH of 5.1–5.2 at the end of fermentation and of 5.4–5.5 at the end of drying, or a pH of 4.8–4.9 at the end of fermentation and of 4.8–5.0 at the end of drying, approximately 3.5 log growth of Salmonella occurred during the fermentation phase at moderate pH, whereas only 2 log growth was recorded at the more acidic pH (Christieans et al., 2017).

Thus, it appears that new hurdle strategies can be envisaged by acting on two levels: (i) processing, notably by optimizing the fermentation stage, and (ii) using substitutes that compensate for reductions in salt and nitrates while preserving the traditional characteristics of the products.

Conclusion

The use of nitrates/nitrates in the manufacture of cured meats remains controversial. Should it be banned, given the toxicity of nitrates? Or should nitrates/nitrates be authorized in more limited amounts, given that they enhance the safety and sensorial qualities of cured meats? What about the consumption of leafy plants, which contain large amounts of nitrate disproportionate to those added to meat products? These questions remain unanswered, especially as several studies (Milkowski et al., 2010; Hammes, 2012; Parhasarathy and Bryan, 2012) tend to highlight the human health benefits of the consumption of moderate amounts of nitrate/nitrite, which are responsible for the formation of NO, deficiency in which can lead to several diseases. Lundberg et al. (2011) even go so far as to suggest that “we may have to reconsider our current thinking and realize that inorganic nitrate may not necessarily be a threat to human health. Instead, in some years we might even consider it as an essential nutrient.”

REFERENCES


EFSA. 2017. Re-evaluation of sodium nitrate (E251) and potassium nitrate (E252) as food additives. Scientific opinion. EFS Journal, 15 (6), 4787.


