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Volatile *N*-nitrosamines in meat products: Potential precursors, influence of processing and mitigation strategies

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## ABSTRACT

Meat products can be contaminated with carcinogenic *N*-nitrosamines, which is ascribed to the reaction between a nitrosating agent, originating from nitrite or smoke, and a secondary amine, derived from protein and lipid degradation. Although in model systems it is demonstrated that many amine containing compounds can be converted to *N*-nitrosamines, the yield is dependent of reaction conditions (e.g. low pH and high temperature). In this paper the influence of the composition of the meat products (e.g. pH,  $a_w$ , spices) and processing (e.g. ageing, ripening, fermentation, smoking, heat treatment and storage) on the presence and availability of the amine precursors and the *N*-nitrosamine formation mechanism is discussed. In addition, this paper explores the current *N*-nitrosamine mitigation strategies in order to obtain healthier and more natural meat products.

Keywords

*N*-nitroso compounds, amine, cured meats, nitrite, proteolysis, lipolysis.

## Introduction

*N*-Nitrosamines, a subgroup of the *N*-nitroso compounds, have been detected in all kinds of foodstuff, including meat products. Most volatile *N*-nitrosamines are strong mutagens and their intake can lead to organ specific tumours (Lijinsky, 1999). Based on epidemiological studies and evidence of animal experiments, the International Agency for Research on Cancer (IARC) recognised *N*-nitrosodimethylamine (NDMA) and *N*-nitrosodiethylamine (NDEA) as probably carcinogenic for humans. Other *N*-nitrosamines commonly found in meat products, e.g., *N*-nitrosodibutylamine (NDBA), *N*-nitrosopiperidine (NPIP) and *N*-nitrosopyrrolidine (NPYR), *N*-nitrosomorpholine (NMOR) are classified as possibly carcinogenic to humans (IARC, 1998) (Fig. 1).

In general, *N*-nitrosamines are formed by the reaction of a nitrosating agent and a secondary amine. In foodstuff, and especially in meat products, the main sources of nitrosyl donors are sodium nitrite (by curing) and gaseous nitrogen oxides (by smoking) (Hotchkiss & Parker, 1990). Secondary amines may be introduced into the food product by different routes. The occurrence of NDEA, NDBA and NMOR is often related to the migration of amine precursors from packaging materials (Sen & Baddoo, 1986; Sen, et al., 1993; Kataoka, et al., 1997; Domanska & Kowalski, 2003), while NPIP and NPYR may originate from the use of pyrrolidine and piperidine containing spices like black pepper and paprika (Nakamura et al., 1981). In particular in meat products, the presence of biogenic amines and other protein degradation products are considered to be an important source of amine precursors. Among the protein degradation products, the secondary amines such as dimethylamine are supposed to be directly

nitrosated (Mottram et al., 1975). However biogenic amines such as spermidine, spermine, cadaverine and putrescine, which contain only primary amine groups, are suggested to undergo deamination and cyclization reactions to form the corresponding nitrosatable secondary amine (Lijinsky & Epstein, 1970; Warthesen et al., 1975). Besides the presence of precursors, the reaction mechanism of the *N*-nitrosamine formation is influenced by environmental conditions (time, T, pH,...) (Fridman et al., 1971). As a consequence meat processing steps (fermentation, drying and heat treatments,...) may provide optimal conditions for the formation of *N*-nitrosamines during the production of meat products (Domanska-Blicharz et al., 2004; Yurchenko & Mölder, 2007; Drabik-Markiewicz et al., 2010, 2011; Jurak, et al., 2013; De Mey, et al., 2014d; Herrmann, et al., 2015a).

The general assumption of the inevitable conversion of protein degradation products to *N*-nitrosamines strengthen the unhealthy image of cured meat products (Jakszyn & Gonzalez, 2006). However, recent studies of commercial meat products demonstrated low *N*-nitrosamine contents when produced under good manufacturing practices (GMP) (Yurchenko & Mölder, 2007; De Mey, et al., 2014a; Herrmann et al., 2015a). Therefore, this paper will give a critical overview of experimental evidence of the actual contribution of biogenic amines and other lipid and protein degradation products in the formation of *N*-nitrosamines in meat and meat products. Hereby, important meat process conditions which influence the formation of *N*-nitrosamines are discussed. In this way, a better understanding of the real contribution of amine precursors and the influence of the meat processing will lead to a realistic assessment of the risk of *N*-nitrosamine formation and effective mitigation strategies for the meat products of tomorrow.

## Potential meat-born precursors in model systems

In general, *N*-nitrosamines are formed from the reaction of a nitrosating agent and a secondary amine. In meat, the degradation of proteins and lipids may deliver many possible precursors of *N*-nitrosamines. For instance, it was demonstrated in buffer solutions that NDMA can be formed through dimethylamine from glycine, choline, lecithin, sarcosine, creatine, creatinine, betaine (fig. 2), while NDEA can be formed from the amino acid alanine (Ender & Čeh, 1971; Pensabene et al., 1975). As listed in Table 1, proline, putrescine and spermidine could be converted to NPYR, while the amino acids hydroxyproline and ornithine and the biogenic amine spermine did not result in the formation of NPYR (Huxel et al., 1974; Warthesen et al., 1975; Hildrum, 1975a). Furthermore, the addition of piperidine or cadaverine in buffer solutions gave rise to the formation of NPIP (Warthesen et al., 1975; Nakamura et al., 1981).

Although it is clear that many innocuous compounds, which may be present in meat, can be considered as *N*-nitrosamine precursors, it must be emphasized that the nitrosation can be influenced by the composition and production process of the meat product. Therefore, forced by the persistent problem of NPYR contamination of fried bacon in the 1970s, more complicated model systems than simple buffers were used to simulate the heat treatment of meat products. In Table 1 a summary is given of the NPYR formation from potential precursors using different kind of model systems. Bills et al. (1973) demonstrated the synthesis of NPYR when nitrite and pyrrolidine, spermidine, proline, or putrescine were added in an oil-water mixture. Hereby, an intensive heat treatment (up to 170°C) was necessary to obtain yields between 0.4 and 2.0%. For putrescine, the yield was considerably lower (0.04%). In a low-moisture system (5% H<sub>2</sub>O)

heated to 160°C, yields of 22% and 1% were obtained when putrescine or ornithine were added, respectively (Warthesen et al., 1975). Nevertheless, due to differences in reaction conditions between the oil-water mixture and low-moisture system, especially with regard to the incubation time, no conclusions can be made concerning the influence of the moisture content of both model systems on the reaction yield of the *N*-nitrosamine formation.

In dry samples, pyrrolidine ring containing compounds (e.g., L-proline, glycyl-L-proline, L-prolylglycine and pyrrolidine) were converted to NPYR when nitrite was added and heated at 170°C (Huxel et al., 1974). The intensive heating was essential for the deamination and cyclization of aliphatic amines such as putrescine, since diazonium ions were formed prior to cyclisation to pyrrolidine (fig. 3) (Lijinsky & Epstein, 1970, Gray & Collins, 1977a). In addition, Hildrum (1975a) suggested the possible formation of dozens of different *N*-nitroso compounds from spermidine and spermine, since both molecules possess secondary amine groups which are all conceivable for nitrosation. In that study,  $\gamma$ -butenyl(3-propenyl)nitrosamine (BPN) was identified as the principal *N*-nitrosamine from spermidine, and to much lesser extent NPYR was detected (Fig. 4). In the case of amino acids like proline, the heat treatment induced decarboxylation to form the corresponding amine (Fig. 5). Moreover, *N*-nitrosamino acids like the non-carcinogenic *N*-nitrosoproline (NPRO) can be decarboxylated to NPYR at elevated temperatures (up to 100°C) (Bills et al., 1973; Janzowski, 1978).

In the context of *N*-nitrosamine contamination of meat products, the formation of NPIP is less intensively studied than its structural homologue NPYR. Besides piperidine, introduced through the use of spices such as pepper (Nakamura et al., 1981), the biogenic amine cadaverine is

suggested to be an important precursor of NPIP in meat products (Lijinsky & Epstein, 1970). Warthesen et al. (1975) demonstrated that more than 20% NPIP could be produced in a high temperature-low-moisture model system when nitrite and cadaverine were added. Moreover, as illustrated in Fig. 6, the parental amino acid, lysine, could also act as precursor of NPIP. On the one hand, lysine can be converted to cadaverine by decarboxylase active microorganism (Suzzi & Gardini, 2003). Subsequently, this biogenic amine is deaminated and cyclized to piperidine prior to nitrosation. On the other hand, in the presence of sugars, lysine can undergo carbonyl-assisted decarboxylative deamination during pyrolysis. In this way, the maillard product pent-4-en-1-amine can be generated, which on its turn may cyclize to piperidine (Nikolov & Yaylayan, 2010). As a consequence, the heating of a mixture containing glucose, lysine and nitrite may result in the production of NPIP (Heyns & Roper, 1973). However, it must be emphasized that in the study of Warthesen et al. (1975) mainly *N*-nitrosopiperic acid (NPCA) was formed from lysine instead of NPIP in this high temperature-low-moisture model system (Fig. 6).

The experiments conducted in the different model system, namely dry samples, oil-water and low-moisture systems, strongly confirm the involvement of amino acids and biogenic amines in the *N*-nitrosamine contamination of proteinaceous foods. However, food matrices are very complex, containing several interfering compounds which can influence the availability of the precursors. In Table 2, a summary is given of recent studies, wherein the role of added precursors in the formation of *N*-nitrosamines during the actual processing of meat products was investigated. For many precursors, similar results were obtained in these food systems but not always. For instance, in cooked ham, the precursors putrescine and spermidine will both lead to the formation of NDMA instead of the formation of NPYR (Drabik-Markiewicz et al., 2011). To



induce the formation of *N*-nitrosamines in meat products, extreme high concentrations of the precursors, higher than can be naturally expected, must be present in the product. In addition, as will be discussed in the next paragraph, intensive meat processing conditions are often necessary for the conversion of the precursor to the corresponding *N*-nitrosamines.

#### Formation of *N*-nitrosamines during meat processing

As described in previous paragraph, in the presence of nitrite, several amine containing compounds, e.g. proteins, free amino acids and (biogenic) amines can be converted to their corresponding *N*-nitrosamines. However, in the complex matrix of meat products, the occurrence and availability of these precursors and the subsequent formation of *N*-nitrosamines is influenced both by composition and processing (Fig. 7). Therefore, an understanding of the actual contribution of realistic processing factors and biochemical reactions are indispensable to prevent or at least control the formation of *N*-nitrosamine formation in meat products.

#### Meat quality and ageing

Although *N*-nitrosamines in meat products are mainly considered to be process contaminants (Behnlian et al., 2014), raw meat materials can already contain *N*-nitrosamines. In pork and beef meat, and to a lesser extent in horse, goat, veal, and game meat NDMA (5-15 µg/kg) and NDEA (5-20 µg/kg) can be detected. The presence of these carcinogenic compounds is higher in fresh meat cuts from non-ecologically bred animals. It is believed that precursors for the endogenous formation of *N*-nitrosamines may originate mainly from ingesting feed and water, contaminated with nitrate and amines, or possibly also from breathing polluted air (containing

nitrogen oxides) (Rywotycki, 2003a, 2003b, 2007). Fortunately, the contamination of raw unprocessed meat with volatile *N*-nitrosamines is still rare (Jakszyn & Gonzalez, 2006).

In contrast, the freshness of the raw meat material plays an important role since during the storage chemical alterations can occur which may lead to the formation of *N*-nitrosamines during the consecutive meat processing. For instance, as demonstrated during prolonged storage of high oxygen packaged pork, the lipid and protein oxidation of the meat delivers degradation products which may function as the necessary amine precursors. Mainly the degradation of sarcoplasmic proteins and the formation of carbonyls due to protein and lipid oxidation, respectively, resulted in an increased NDEA formation when the aged pork meat was subsequently treated with nitrite (Yang et al., 2013; Sun et al., 2014). Also the oxidation of myofibrillar proteins during freeze-thaw cycles induces to the formation of NDEA (Yang et al., 2012). In addition, in fatty meat products, the susceptibility of the adipose tissue to rancidity is decisive for the formation of *N*-nitrosamines. In fresh meat products, unsaturated fatty acids may act as nitrite scavengers and thus inhibiting the formation of *N*-nitrosamines (Combet et al., 2010). Nevertheless, as the ageing progresses, the oxidation of the adipose tissue will results in the formation of malondialdehyde, a secondary product of the lipid oxidation, which can catalyse the *N*-nitrosamine formation (Kurechi et al., 1980). Also, protein degradation may cause the release of free amino acids. For instance, during the cold storage (2°C) of green bellies, a dramatic increase of the free proline content was observed, which may act as a precursor during the further production of bacon (Gray & Collins, 1977b).

Meat product formulation

Nitrite salts are almost indispensable additives for the production of meat products. As has already been summarized by Sebranek and Bacus (2007), the functional properties of these salts are very diverse: (1) they provide the meat products an attractive red colour by the formation of nitrosomyoglobin (in raw meat products) and nitrosomyochromogene (in heated meat products), (2) they act as a preservative against spoilage bacteria (i.e., *Enterobacteriaceae*) and pathogens (i.e., *Clostridium Botulinum*) and (3) they have anti-oxidative properties and thus inhibit the development of rancidity and off-odors. Nevertheless, the use of nitrite is controversial since it can cause direct food intoxications (lethal oral dose: 33 mg/kg body weight) and the formation of carcinogenic *N*-nitrosamines (Schuddeboom, 1993). Therefore, the addition of these additives in meat products is legally restricted to 150 mg/kg (expressed as NaNO<sub>2</sub>/kg) (Directive, 2006). Although the limitation of the amount of added nitrite, resulted in a drastic reduction of the *N*-nitrosamine content in meat products (McCutcheon, 1984), the risk for the formation of *N*-nitrosamines still exists since the nitrosating agent can also be formed from other precursors. In air, especially in combustion smoke, NO<sub>x</sub> compounds may act as nitrosating agents (Hotchkiss & Parker, 1990; (Habermeyer & Eisenbrand, 2009) and additional amounts of nitrite/nitrate can be introduced from spices, herbs and other vegetables sources (Atawodi et al., 1991; Özcan & Akbulut, 2008), As a matter of fact, the use of plant materials for the natural reddening of alleged nitrite-free meat products must be avoided since the suggested plant-based powders, i.e. celery powder may contain high concentrations of nitrate which can be reduced to nitrite and thus may form a risk for the formation of *N*-nitrosamines (Sindelar, 2006).

Moreover, many spices may contain alkaloids which may function as *N*-nitrosamine precursors. In the course of *N*-nitrosamine contamination of meat products, the piperidine and pyrrolidine

derivatives act as the major sources for the formation of *N*-nitrosamines NPIP and NPYR, respectively. For instance, black pepper contains pyrroperine and pyrrolidine as precursors of NPYR as well as piperine and piperidine which lead to the formation of NPIP (Nakamura et al., 1981). Piperidine can directly be nitrosated to form NPIP, while the main pungent compound of pepper, piperine (3 -- 8%) (Schulz et al., 2005), is firstly cleaved into piperic acid and piperidine. Subsequently, both resulting fractions can be nitrosated to give several unknown C-nitroso compounds and the *N*-nitrosamine NPIP (Osawa et al., 1982). The use of spices that contain a considerable amount of these precursors, may form a risk for the formation of *N*-nitrosamines. Since it was demonstrated that mixtures of spices and nitrite may contain *N*-nitrosamines, especially NPIP and NPYR (Gough & Goodhead, 1975), the use of such kind of premixes is strongly discouraged (USDA, 2012). Nevertheless, in a recent study, high amounts of piperine and piperidine in the spices, which were used in the premixes with nitrite, did not necessarily result in the formation of NPIP. This suggests that the extent of *N*-nitrosamines formation may be related to the storage conditions of the premixes and the processing of the spices, which influences the total composition of the spice products (De Mey et al., 2014b). For instance, many spices contain anti-oxidants, such as polyphenols and other compounds with nitrite scavenging properties (Baliga et al., 2003; Tsai et al., 2007). Nevertheless, the inhibitory or catalysing effect of natural polyphenols on the *N*-nitrosamines is highly dependent on the structure, environmental conditions (i.e., pH) and the alkalinity of the amine precursors (Kikugawa & Kato, 1991).

Besides the fact that certain food ingredients may introduce nitrosating agents and/or amine precursors, some commonly used functional additives in meat processing may also influence the *N*-nitrosamine formation. Polyphosphates, which are added to meat products to increase the

water binding capacity, enhanced the formation of *N*-nitrosodiethylamine in pasteurised pork ham (Rywotycki, 2002). The increased *N*-nitrosamine formation in meat products which contain phosphates may be ascribed to the nucleophilic catalysis of anions on the nitrosation reaction (Fan & Tannenbaum, 1973). However, other studies both in model systems (Theiler et al., 1981) as well as in meat models (Herrmann et al., 2015b), could not confirm the role of phosphate as enhancer of the *N*-nitrosamine formation.

In the study of Devik (1967) the maillard reaction, a common reaction of reducing sugars and amino acids in food systems, was considered as a possible source of *N*-nitrosamines. However, in later studies these findings could not be confirmed and were ascribed the false-positive results due to the presence of pyrazines (Havre & Ender, 1971). In contrast, the addition of reducing sugars such as dextrose, lactose and maltose in a fried bacon model resulted in a decrease of the carcinogenic *N*-nitrosamine content (Theiler et al., 1984), probably due to the formation of the nondiazable melanoidins which may act as nitrite scavengers (Kato et al., 1987).

Although sodium chloride, in contact with nitrite or nitrogen oxide, may form nitrosyl chloride as nitrosating agent, this mechanism occurs mainly at pH-values below 2.5. At pH-values above 2.5, the major nitrosating species is nitrous anhydride, which can be formed from nitrous acid originating from nitrite. Increasing the ionic strength of the medium through the addition of sodium chloride stabilises the charged nitrite ions and thus limits the formation of the nitrous anhydride (Hildrum, 1975a). As a consequence the addition of sodium chloride to slightly acid foods such as meat products showed an inhibitory effect on the *N*-nitrosamine formation (Theiler et al., 1981; Rywotycki, 2002, 2007). Nevertheless, the inhibitory effect of sodium chloride is

minimal whereby the nowadays trend to reduce the sodium chloride content of meat products does not implicate and additional risk for the formation of *N*-nitrosamines (Herrmann, 2014)

## Processing

### Fermentation and ripening

Extreme protein degradation especially takes place during the production of dry fermented meat products. The fermentation and consecutive ripening process, are characterized by the growth of microorganisms and an extended proteolysis and lipolysis, respectively. Hereby the free amino acids can be decarboxylated to the corresponding biogenic amines. Since the intake of biogenic amines can cause food poisoning (Silla Santos, 1996), the reduction of biogenic amines in fermented meat products is intensively studied. The reduction of biogenic amines content reduces the risk of immediate intoxication (Suzzi & Gardini, 2003). Moreover, it is believed that the biogenic amines are the principal source of nitrosatable amine precursors in meat products. Therefore, foods which are heavily contaminated with biogenic amines may give rise to the formation of *N*-nitrosamine and thus the intake of these foods may induce cancer. However, more recent studies could not demonstrate a direct relationship between the *N*-nitrosamine contamination and the biogenic amine contents in commercial dry fermented sausages (De Mey et al., 2014a). This observation was also confirmed by means of a dry fermented sausage model. The addition of an extremely high concentration of cadaverine to the dry fermented sausage model did not result in the formation of NPIP during the production (De Mey et al., 2014c). In contrast, the same study demonstrated that the addition of an extremely high concentration of the alkaloid piperidine to the fermented meat model resulted in the formation of small amounts of

NPIP. These observations support the hypothesis of Lijinsky and Epstein (1970) that an additional process, most likely a heat treatment, is necessary to induce the cyclization and deamination of the primary amines to form cyclic secondary amines. So, the presence of high concentrations of biogenic amines does not necessarily lead to the formation of *N*-nitrosamines. In addition, since the formation yield of NPIP was still minute in the study of De Mey et al. (2014c), it indicates that the environmental conditions were far from optimal for the nitrosation reaction. As known, the *N*-nitrosamine formation is promoted in an acidic environment (Mirvish, 1975), however it was demonstrated in a liquid model system that a pH decrease, similar as observed during fermentation, is not sufficient enough to promote the *N*-nitrosamine formation (De Mey et al., 2014d). In addition, in the same study, the reduction of the water activity, as can be observed during drying, inhibited the *N*-nitrosamine formation. Therefore, the combination of fermentation and drying during the production of meat products may not lead to favourable conditions for the formation of *N*-nitrosamines. As a consequence, besides obtaining a shelf stable product, the fermentation and drying processes will provide flavour and odour compounds to the product without health concerns about additional formation of *N*-nitrosamines (Ordóñez et al., 1999). Nevertheless, prolonged ripening processes may lead to off-odours and discoloration by extensive lipid and protein oxidation.

#### Heat treatment

As discussed earlier, the *N*-nitrosamine formation can take place at low temperatures, as prevails during curing and storage of meat products, however at low temperatures it is in particular observed during the endogenous formation of *N*-nitrosamines in the strong acidic environment of

the stomach. Hereby the low temperature inhibits the acid-catalysed denitrosation. In contrast, as it is the case for many chemical reactions, increasing the temperature is an important to deliver the necessary activation energy for nitrosation. In slightly acid to neutral foodstuff, the intensity of the heat treatment is a critical factor in the formation of *N*-nitrosamines (Fridman, Mukhametshin, & Novikov, 1971). Also in meat products it could be observed that the heat treatment caused an increase in the concentrations of various *N*-nitrosamines (Rywotycki, 2007; Yurchenko & Mölder, 2007; Drabik-Markiewicz et al., 2011; Li et al., 2012).

During a case study of fried bacon in the 1970s, it became clear that especially the formation of *N*-nitrosopyrrolidine showed a high dependency of the heat treatment (Pensabene et al., 1974). As can be seen in figure 5, two possible pathways were identified; (1) at temperatures between 100 - 150°C, NPYR is mainly formed via the intermediate *N*-nitrosoproline, which is a non-carcinogenic *N*-nitrosamino acid (Lee et al., 1983), and (2) at temperatures higher than 175°C, proline, abundantly present in collagen, is firstly decarboxylated to pyrrolidine prior to nitrosation (Nakamura et al., 1976). The conversion of pyrrolidine and proline to NPYR was confirmed during the heating of cured meat (Drabik-Markiewicz et al., 2009, 2010). Hereby, it was demonstrated that an oven temperature above 200°C was necessary to form a considerable amount of NPYR in a complex matrix such as meat products. Moreover, in accordance to the experiments in model systems (Bills et al., 1973), the addition of hydroxyproline, another important constituent of collagen (Belitz et al., 2004), did not give rise to an increased formation of NPYR (Drabik-Markiewicz et al., 2009). Although hydroxyproline can be nitrosated to form the non-volatile and non-carcinogenic *N*-nitrosohydroxyproline, the further decarboxylation to *N*-nitrosohydroxypyrrolidine was not detected during the frying of bacon (Gray et al., 1977c).



The conversion of biogenic amines to *N*-nitrosamines is clearly promoted by elevated meat processing temperatures. Although, as discussed earlier, in studies using simplified model systems it was demonstrated that putrescine and spermidine are the precursors of NPYR (Bills et al., 1973; Hildrum et al., 1975b; Warthesen et al., 1975), these results could not be reproduced in the meat matrix (Drabik-Markiewicz et al., 2011). Nevertheless, the presence of both biogenic amines may result in the formation of NDMA when meat products are subjected to intensive heat treatments (Drabik-Markiewicz et al., 2011). The addition of cadaverine, in contrast to its presence in non-thermally processed meat products such as dry fermented sausages (De Mey et al., 2014c), may give rise to an increased content of NPIP when lean meat products are extremely intensively heated ( $>200^{\circ}\text{C}$ ) (Drabik-Markiewicz et al., 2011). In addition, the presence of adipose tissue may form an additional risk during frying of meat products. The addition of sodium nitrite results in the formation of pseudo-nitroso unsaturated lipid derivatives, which may decompose again at elevated temperature and therefore act as a source of nitrosating agents (Liu et al., 1988).

Although it is clear that the *N*-nitrosamine formation shows a high dependency of temperature, the final concentration of *N*-nitrosamines is also influenced by the method of cooking. Boiling may lower the amount of *N*-nitrosamines since these contaminants can dissolve in the cooking water. In addition, keeping the total time of the cooking as short as possible, as can be done in a microwave oven, will result also in lower *N*-nitrosamine levels (Li et al., 2012). Also during baking and pan frying, the *N*-nitrosamines are not necessarily increasing, except NPIP. (Herrmann et al., 2015a). Since the boiling points of the most occurring volatile *N*-nitrosamines are situated between  $150^{\circ}\text{C}$  and  $220^{\circ}\text{C}$  (Williams et al., 1964), the heat induced formation may

often be compensated by evaporation of these volatile compounds. In the study of Drabik-Markiewicz et al. (2011) the regression model indicated maximum levels of NDMA and NPYR after a heat treatment around 200°C, while at higher temperature a slight reduction is expected. Due to its relatively high boiling point of 218°C, the loss of NPIP by evaporation is not observed which results in a net increase of the NPIP levels during realistic baking processes (Herrmann et al., 2015a).

It is clear that an intensive heat treatment has a large contribution in the formation of *N*-nitrosamines in meat products. Additionally, heat treatments such as grilling and roasting, are of high risk since the meat products come in direct contact with flames and smoke. Kocak et al. (2012) observed an increased risk of *N*-nitrosamine contamination when the meat was grilled above a inappropriately prepared -- still flaming and smoking - charcoal fire. On the one hand, the smoke may contain increased concentrations of NO<sub>x</sub> compounds which may act as precursors of nitrosating agents (Paul et al., 2008). On the other hand, combustion smoke may already contain *N*-nitrosamines. In contrast to cigarette smoke, which contains mainly tobacco specific *N*-nitrosamines like *N*-nitrosonornicotine, *N*-nitrosoanatabine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and *N*-nitrosoanabasine (Zhou et al., 2007), combustion smoke produced by burning wood mainly contains NDMA, and to a lesser extent NPIP and NMOR (Kataoka et al., 1997). As a result, grilled and smoked meat products are commonly more contaminated with *N*-nitrosamines (Yurchenko & Mölder, 2007), even when these products were prepared without the addition of nitrite salts, like doner kebab and grilled lamb (Özel et al., 2010; Kocak et al., 2012).

## Storage conditions

In addition, storage conditions may play a role in the increase of *N*-nitrosamines in perishable processed meat products (Domańska & Kowalski, 2002). For instance, the increase of NDMA content during the shelf life of edible offals was related to the microbial growth. Due to microbial activity, the generation of amine precursors is continued and the reduction of nitrate to nitrite may occur (Domańska & Róžańska, 2003; Domanska-Blicharz et al., 2004). Furthermore, among the microbial population, the existence of *N*-nitrosamine generating microorganisms was reported by Ayanaba and Alexander (1973). Nevertheless, even in canned products, which are considered to be safe due to sterilization, a gradual increase of *N*-nitrosamines has been observed. Jurak et al. (2013) observed especially an increase in NDEA during the storage of canned liver paste at room temperature.

## Current mitigation strategies

### Irradiation

It is clear that the addition of sodium nitrite to meat products is the major cause of the *N*-nitrosamine formation in meat products. In addition to the coloring and anti-oxidative properties, sodium nitrite functions also as antimicrobial against the growth of *Enterobacteriaceae* and the pathogen *Clostridium botulinum* (Honikel, 2008). In this regard, the microbial stability of meat products can alternatively be achieved by gamma irradiation treatments instead of the addition of nitrite as antimicrobial agent. Thereby, the amount of added nitrite, and thus indirectly the risk of *N*-nitrosamine formation, can be reduced in irradiation-sterilized (30 kGy) meat products

(Fiddler et al., 1981). However, due to its colour forming properties, the total omission of nitrite in meat products is not advisable.

In this regard, the application of irradiation has an additional benefit, as the breakdown of residual nitrite in meat products can be achieved by irradiation. In case of dry-cured ham, an irradiation treatment (5 kGy) after the sun-drying period of almost two months resulted in a decrease (26-70%) of the residual amount of nitrite during the further ripening period (ca. 11 months) of this product (Wei et al., 2009). Although the reduction of sodium nitrite in cooked sausages could also be observed after mild irradiation (5 kGy) (Ahn et al., 2004b), this dose was not always effective (Jo et al., 2003). A clear reduction of the residual nitrite level could be achieved when cooked sausages were irradiated at 10 kGy or more (Ahn et al., 2004a). In a model system it was demonstrated that the breakdown of sodium nitrite is dose-dependent whereby a reduction of 50% was observed after an irradiation of 10 kGy, while 40 kGy was needed for the total destruction. Moreover, the breakdown products from the irradiated nitrite were not able to act as nitrosating agents and thus the formation of *N*-nitrosamines can thus be inhibited (Ahn et al., 2003). In addition, the reduction of nitrite by irradiation is more effective in vacuum packages than in aerobic packages due to its sustained lower redox potential at vacuum conditions (Ahn et al., 2002a). However, an effective irradiation dose for the reduction of nitrite reduced also the nitrosomyoglobin content of cooked sausages and therefore the red colour of these products was negatively influenced (Ahn et al., 2004a, 2004b). Lowering the irradiation dose could prevent color deficiencies, however no effective reduction of the residual nitrite level could be achieved (Jo et al., 2003).

Furthermore, irradiation can be applied to reduce the amount of biogenic amines, which are mentioned earlier as possible precursors of *N*-nitrosamines. The degradation of putrescine, spermidine and spermine in aqueous solutions was achieved at an irradiation dose of 5 kGy, while other biogenic amines such as cadaverine, tryptamine, histamine and tyramine were broken down at an irradiation dose of 15 kGy or higher. However, higher irradiation doses are necessary for the reduction of biogenic amines in non-aqueous foods due to the low moisture content and the presence of other compounds (Kim et al., 2004). In the case of ripened meat products, varying effects on the biogenic amine content were observed. In pepperoni, a total reduction of putrescine was seen at an irradiation dose of 10 kGy or higher, while cadaverine and phenylethylamine were not degraded by irradiation up to 20 kGy (Kim et al., 2005). Meanwhile, in smoked Egyptian sausages, cadaverine, together with histamine, tyramine and putrescine, was significantly reduced after irradiation (6 kGy) (Rabie & Toliba, 2013). Besides a reduction of the biogenic amine levels, even an increase has been observed. In the case of dry cured ham, the irradiation promoted the formation of spermidine, phenylethylamine, cadaverine and tryptamine (Wei et al., 2009). The divergent results of the irradiation treatment may be explained by the fact that irradiation, besides the direct breakdown of biogenic amines, also results in a reduction of microorganisms in food. Dependent of the sensitivity of certain microbial species to irradiation, the population will be altered. In general Gram-positive bacteria will be more resistant than Gram-negative bacteria (Aymerich et al., 2008). As a result, the possibility exists that specific decarboxylase active strains may predominate and give rise to an increased amount of some biogenic amines while the total biogenic amine content tends to decrease.

Ultimately, irradiation can be applied for the direct reduction of *N*-nitrosamines. In an aqueous solution, an irradiation dose of 5 kGy is already enough to break down NDMA and NPYR. Nevertheless, the influence of the matrix composition may not be underestimated since the availability of water is indispensable for the hydrolysis of *N*-nitrosamines. In non-aqueous solutions such as ethanol the irradiation dose needed for the breakdown of *N*-nitrosamines is significantly higher (20 kGy) (Ahn et al., 2002b). As a consequence, irradiation doses needed for the reduction of *N*-nitrosamines may depend on the composition of the meat products. For instance, an irradiation dose of 5 kGy has been reported to be sufficient (Jo et al., 2003), while in other studies higher irradiation doses (> 10 kGy) were needed for the breakdown of volatile *N*-nitrosamines in cooked sausages (Ahn et al., 2002a). In dry fermented sausages, an irradiation dose higher than 10 kGy was needed for the breakdown of NDMA and NPYR (Byun et al., 2004).

In summary, gamma irradiation is a promising treatment for the reduction of volatile *N*-nitrosamines, both directly as well as indirectly by the breakdown of the precursors, i.e., biogenic amines and residual nitrite in meat products. Nevertheless, for meat products, the application of irradiation has some drawbacks, i.e., generation of off-flavours, colour deficiencies and possible toxic substances (Behnlian et al., 2014). For instance, 2-alkylcyclobutanones are uniquely generated from fatty acids and triglycerides during irradiation of fat containing foods. However, to date, the consumption of irradiated foods contaminated with 2-alkylcyclobutanones is considered to be harmless for human health (Song et al., 2014). As a consequence, the irradiation of foods is still legally authorized although the consumer's acceptance is dependent on the education by the local health authorities (Diehl, 2002).

*N*-nitrosamine blockers

Concerning the prevention of *N*-nitrosamine formation in meat products the addition of *N*-nitrosamine blockers is since many years an important strategy. These nitrite scavengers compete for the nitrosating species by reducing nitrous acid ( $\text{HNO}_2$ ), which resulted from the protonation of the nitrite, to yield nitrogen oxide (NO) (Skibsted, 2011). Numerous antioxidants may inhibit the formation of *N*-nitrosamines, and are listed as E-numbers (Table 3). For example, ascorbic acid, its isomer erythorbic acid and salts thereof, which can be used to enhance the color by accelerating the formation of nitrosomyoglobin (Skibsted, 2011), are able to reduce the formation of *N*-nitrosamines (Archer et al., 1975). The addition of ascorbate and erythorbate was especially recommended for the reduction of the *N*-nitrosamine content of fried bacon (Havery et al., 1978), but their protective action is also observed in several other types of cured meat products, e.g., frankfurters (Fiddler et al., 1973), pasteurized canned pork meat (Rywotycki, 2002), cooked sausages (Pourazrang et al., 2015), and dry fermented sausages (De Mey et al., 2014c). Although the use of these antioxidants leads to a significant reduction of the *N*-nitrosamine content, the effectiveness is limited due to the lack of solubility in the fat fraction of meat products (Sebranek, 1979). In contrast, fatty acid esters of ascorbic acid, e.g. L-ascorbyl palmitate, and lipid-soluble compounds of vitamin E, e.g. alpha tocopherol, can also reduce the *N*-nitrosamine formation in lipophilic environment, as can be found in fried bacon (Fiddler et al., 1978; Sen et al., 1976). Moreover the addition of these antioxidants prevents the formation of lipid oxidation, which on its turn is responsible for the initiation of protein oxidation. As a consequence, the formation of nitrosatable degradation products such as dimethylamine and diethylamine can be inhibited by preventing initial oxidation reactions (Yang et al., 2013).

However, in a recent study (Hermann et al., 2015b), L-ascorbyl palmitate did not provide an additional protection to *N*-nitrosamine formation in cooked sausages when compared to the use of erythorbic acid alone.

Despite the effectiveness of these legally allowed antioxidants, the consumers' aversion towards traditional (chemical) additives, forces manufacturers to search for other, preferably natural and sustainable antioxidants (Zink, 1997). The inhibitory effect on the *N*-nitrosamine formation of several kinds of compounds in the diet, e.g. vitamin C and E, phenolic and sulphur compounds, as can be found in fruits and vegetables is well described (Bartsch et al., 1988; Tanaka, 1998; Scotter & Castle, 2004). However, many studies were focused on the investigation of the inhibitory effect on the gastric nitrosation of dietary amines. In this approach, juices from strawberry, garlic, kale and orange or black tea inhibited the formation of NDMA during the consumption of an amine rich meal (Krul et al., 2004; Choi et al., 2007). In addition, during the gastric digestion of meat products, the endogenous formation of NDMA was inhibited when vitamin E was added to the sausages. Moreover the inhibitory effect of vitamin E was even greater when this antioxidant was added to the feed of the slaughter animals instead of adding it directly to the meat product (Meineri et al., 2013). Despite the collected evidence that the introduction of phenolic and sulphur compounds to the diet is able to prevent mostly the gastric nitrosation, only a few peer-reviewed papers investigated the effect of potential natural compounds on the exogenous *N*-nitrosamine formation in meat products. Most studies were restricted to evaluate these kind of compounds, or the vegetal sources thereof, with regard to their antioxidative or the nitrite scavenging properties and merely suggest that this will reduce the risk of *N*-nitrosamine formation. For example, citrus co-products are promising sustainable



ingredients which can be used for the reduction of the residual nitrite level in meat products and therefore are suggested as potential *N*-nitrosamine blocking ingredients (Viuda-Martos et al., 2009; Fernández-López et al., 2006). Nevertheless, other studies investigated the mechanism behind the potential *N*-nitrosamine blocking properties while providing evidence of actual reduction of the *N*-nitrosamine levels. For instance, tea catechins were effective in preventing the oxidation of meat lipids and proteins and thus inhibited the formation of *N*-nitrosamines when nitrite was added to meat models (Yang et al., 2013). In real dry cured meat products, such as dry cured sausage and bacon, the introduction of tea and grape seed polyphenols could decrease the amount of *N*-nitrosamines, although to a lesser degree than ascorbic acid but more effective than alpha tocopherol (Li et al., 2013a; Wang et al., 2015).

#### Nitrite reduction and natural nitrite-free meat products

In previous sections, the majority of the mitigation strategies are based on the avoidance of the formation of the nitrosating species from nitrite. Instead of attempts to breakdown or scavenge the available nitrite precursor, it might be simpler to reduce the ingoing amount of nitrite since the formation of *N*-nitrosamines is proportional to the nitrite concentration (Mirvish, 1975). To counter the *N*-nitrosamine problem of fried bacon in the 70s, the USDA took the initiative to regulate the maximal ingoing amount of nitrite (Cassens, 1997). Following this measure, the current European legislation allows the addition of 150 mg/kg sodium nitrite in most meat products. According to the European Food Safety Authority, the addition of 150 mg/kg sodium nitrite is recommended for the microbial stability, although lower amounts may be sufficient depending the product properties such  $a_w$  and pH (Directive, 2006). The Danish regulation

imposes a substantially lower level of maximal 60 mg/kg ingoing sodium nitrite. This level should be still sufficient for the reduction of lipid oxidation (Sato & Hegarty, 1971) and colour forming properties (Lin & Sebranek, 1979; Demasi et al., 1989), although for colour stability an amount of ca. 80-100 mg/kg is recommended (Dineen et al., 2000; Juncher et al., 2003). In comparison to commercial Belgian products, which were produced according Directive 2006 (max. 150 mg/kg NaNO<sub>2</sub>), the *N*-nitrosamine content of the Danish meat products (max. 60 mg/kg NaNO<sub>2</sub>) was not significantly lower (Herrmann et al., 2015a). Nevertheless, the positive correlation between the added amount of nitrite and the concentrations of several *N*-nitrosamine was confirmed in a cooked sausage model (Herrmann et al., 2015b).

Another approach is to completely eliminate the use of nitrite. However, due to the multiple technological advantages of nitrite, the production of a qualitative nitrite-free meat products is challenging. The benefits and drawbacks of alternatives for nitrite curing of meat products are reviewed in several papers (Shahidi & Pegg, 1992; Sebranek & Bacus, 2007; Weiss et al., 2010). In a nutshell, most alternatives are based on the replacement of sodium nitrite by vegetal sources of nitrate. However, the inherent variability of nitrate in plants, in combination with the necessity of microbial nitrate reduction, implies the risk of high residual nitrite levels. As a consequence, this strategy will not provide a solid solution to prevent *N*-nitrosamine formation. With regard to the colour formation in nitrite-free products, a number of strategies have been investigated. The formation of the coloring pigments nitrosylmyoglobin and nitrosomyochromogene in non-heated and heated meat products, respectively, can be achieved by the use of a nitrosylmyoglobine generating starter cultures (Kawahara et al., 2006; Zhang et al., 2007; Li et al., 2013b) or the addition of preformed cooked cured meat pigment (Shahidi & Pegg, 1992). Moreover, the

formation of a naturally occurring pigment identified as zinc protoporphyrin was observed during a prolonged ripening of nitrite-free cured ham such as Parma (Wakamatsu et al., 2004; Parolari et al., 2009). Understanding the formation pathway, and the influence of product and process conditions on the kinetics, may provide opportunities to accelerate the formation of this pigment. This may allow the development of an innovative technology for the natural reddening of other nitrite-free meat products (Becker et al., 2012; De Maere et al., 2014). Nevertheless, to cover all technological advantages of nitrite, no single ingredient solution can be given. As an example, recently, a nitrite-free frankfurter type sausage was proposed by using cochineal and paprika as colorant, sodium hypophosphite as antimicrobial agent and butylated hydroxyanisole (BHA) as antioxidant (Eskandari et al., 2013). Although these kind of proposals for the elimination of nitrite suggest to deliver more healthy and safer meat products, the monitoring of *N*-nitrosamine formation has rarely been included in the evaluation of these meat products.

## Conclusion

In general, it is known that *N*-nitrosamines are formed by the reaction of secondary amines with a nitrosating agent. In meat products, the latter precursor mainly originates from nitrite or NO<sub>x</sub> compounds in smoke. With regard to the source of secondary amines, various model systems, which differ especially in water and fat content, were used to study the nitrosation potential of several amine containing compounds. Many compounds, which can occur in meat and meat products, were demonstrated to be able to act as precursors for the formation of carcinogenic *N*-nitrosamines. In the case of amino acids (i.e., proline, ornithine and lysine) and biogenic amines

(i.e., putrescine and cadaverine) an intensive heat treatment (above 160°C) was often necessary to obtain substantial amounts of carcinogenic *N*-nitrosamines (e.g., NPYR and NPIP) *in vitro*.

In meat products, proteins and lipids can be degraded to amine precursors during the ageing, fermentation, ripening and storage. In the case of dry fermented sausages, especially free amino acids and biogenic amines are formed. However, due to the very mild acidic conditions and low water activity, the abundant presence of these compounds is not a substantial risk for the formation of *N*-nitrosamines. The necessary deamination and cyclization is thermally catalyzed and takes mainly place during baking and grilling. During mild heating processes (under 160°C), such as pasteurisation and sterilisation, the formation of *N*-nitrosamines is limited. Moreover, as long as GMP conditions are respected and fresh meat materials, which contain low levels of lipid and protein degradation compounds, are used for the industrial preparation of heated meat products, the *N*-nitrosamine formation is restricted. However, in the interest of public health it may be opportune to investigate the possible formation of *N*-nitrosamines during the barbecuing and baking of foods containing processed meat products (i.e. pepperoni pizza). It can be expected that a combination of processed meats, which contain amine precursors, and an intense heating process, often in combination with uncontrolled smoking, may result in an increased risk of *N*-nitrosamine formation. In addition, secondary amines, like piperidine (from pepper) can be, although slowly, nitrosated without the necessity of heat treatments. Therefore, the use of alkaloid and nitrate containing spices and herbs can be considered as an additional source of *N*-nitrosamines in meat products.

Besides the presence of amine precursors, the highest risk on the formation of *N*-nitrosamines is caused by the use of nitrite in meat products. Due to the many technological advantages of nitrite, the main mitigation strategy in the past to combat the *N*-nitrosamine formation in meat products is the limitation of the added amount of nitrite to 150mg/kg and the use of anti-oxidants such as ascorbate as nitrite scavenger. Although this approach causes a significant reduction of *N*-nitrosamines, still traces and occasionally high levels can be detected. Therefore current research is challenged to the further reduction (e.g. 60 mg/kg as imposed by the Danish regulation) and even elimination of nitrite. Gamma irradiation can reduce *N*-nitrosamines, as well as both precursors (nitrite and biogenic amines). However, the main draw back in Europe is the consumer's resistance towards this technology. In order to meet the needs of consumers for more natural meat products, the current research is mainly focused on the application of natural antioxidants, preferably from sustainable sources such as fruit and vegetable by-products. Nevertheless, the ultimate approach to obtain healthier and more natural meat products implies the total elimination of nitrite. As a consequence, the future challenge is to develop appropriate meat processing technologies such as the natural reddening by zinc protoporphyrin. In this way safe and attractive meat products without the use of chemical additives can be obtained.

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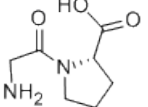
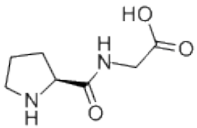
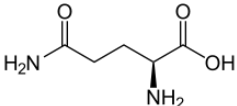
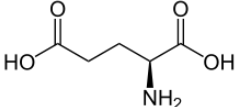
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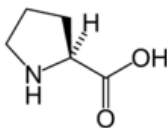
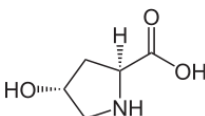
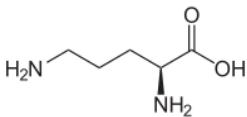
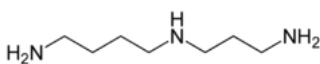

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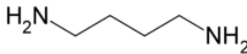
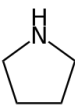
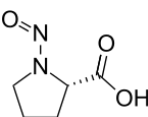


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Table 1. *N*-nitrosopyrrolidine formed at elevated temperatures in model food systems from potential precursors

Class <sup>1</sup>	Precursor <sup>2</sup>	Ratio <sup>2</sup>	NPYR	System <sup>3</sup>	Temp (°C)	Time	Reference
PP	Collagen	10:1	+	OW	180	10 min	Gray et al., 1977b
	Glycyl-L-proline	1:1	+	Dry	170	2 hrs	Huxel et al., 1974
			(0.8%)				
	L-Prolyl-glycine	1:1	+	Dry	170	2 hrs	Huxel et al., 1974
AA			(0.3%)				
							
	Glutamine	1:1	-	OW	170	10 min	Bills et al., 1973
							
	Glutamic acid	1:1	-	OW	170	10 min	Bills et al., 1973
							
	Proline	1:1	+	Dry	170	2 hrs	Huxel et al., 1974
			(3.7%)				
		1:1	+	OW	170	10	Bills et al.,

		1:1	(0.4%)	H <sub>2</sub> O (7)	170	min	1973
			+			2 hrs	Huxel et al.,
			(0.6%)				1974
	Hydroxyproline	1:1	-	Dry	170	2 hrs	Huxel et al.,
		1:1	-	OW	170	10	Bills et al.,
						min	1973
		1:1	-	H <sub>2</sub> O (7)	170	2 hrs	Huxel et al.,
							1974
	Ornithine	2:1	+	LM	160	2 hrs	Warthesen et
			(1.0%)				al., 1975
BA	Spermidine	1:1	+	OW	170	10	Bills et al.,
			(1.0%)			min	1973
		10:1	+	H <sub>2</sub> O (4)	25 -	1 hr	Hildrum,
					80		1975a
	Spermine	10:1	-	H <sub>2</sub> O (4)	25 -	1 hr	Hildrum,
					80		1975a
			+	LM	160	2 hrs	Warthesen et
	Putrescine <sup>c</sup>	2:1	(22%)				al., 1975

		1:1	+	OW	170	10	Bills et al.,
				(0.04%)		min	1973
CA	Pyrrolidine	1:1	+	Dry	170	2 hrs	Huxel et al.,
				(0.4%)			1974
		1:1	+	OW	170	10	Bills et al.,
				(1.0%)		min	1973
NA	Nitrosoproline	0:1	+	OW	170	10	Bills et al.,
				(2.6%)		min	1973

<sup>1</sup>Class: PP = protein and peptide, AA = amino acid, BA = biogenic amine, CA = cyclic amine,

NA = *N*-nitrosamine.

<sup>2</sup>Ratio nitrite/precursor,

<sup>3</sup>System: OW = reflux boiling in oil/water (100 ml + 1 ml); LM = Low moisture system (5% water), oven heating of closed test tubes; Dry = dry powder mixtures, oven heating of closed test tubes; H<sub>2</sub>O (7) = buffered aqueous solution (phosphate, pH 7.0); H<sub>2</sub>O (4) = buffered aqueous solution (acetate, pH 4.0).

Table 2. Volatile *N*-Nitrosamines formation in food systems enriched with potential precursors

Meat model <sup>1</sup>	Precursor <sup>2</sup>	NDMA	NPYR	NPIP	Reference
Cooked ham	Proline <sup>a</sup>	-	+	-	Drabik-Markiewicz et al., 2009, 2010
	Hydroxyproline <sup>a</sup>	-	-	-	Drabik-Markiewicz et al., 2009, 2010
	Spermidine <sup>a</sup>	+	-	+	Drabik-Markiewicz et al., 2011
	Spermine <sup>a</sup>	-	-	-	Drabik-Markiewicz et al., 2011
	Putrescine <sup>a</sup>	+	-	-	Drabik-Markiewicz et al., 2011
	Cadaverine <sup>a</sup>	-	-	+	Drabik-Markiewicz et al., 2011
	Pyrrolidine <sup>d</sup>	-	+	-	Drabik-Markiewicz et al., 2010
	Piperidine <sup>d</sup>	-	-	+	Drabik-Markiewicz et al., 2010
Dry fermented sausages	Cadaverine <sup>b</sup>	-	-	-	De Mey et al., 2014c
	Piperidine <sup>c,d</sup>	-	-	+	De Mey et al., 2014c

<sup>1</sup>Meat model: Cooked ham = heating (30 min, 85-220°C) of canned cured lean pork meat; Dry fermented sausages = pork sausages, fermented (3 days, 24°C) and subsequently dried (18 days, 15°C),

<sup>2</sup>Amount of added precursor:

<sup>a</sup>1000 mg/kg,

<sup>b</sup>500 mg/kg,

<sup>c</sup>100 mg/kg,

<sup>d</sup>10 mg/kg

Table 3 Antioxidants with potential *N*-nitrosamine blocking properties, authorised to be used in meat and meat products (EU 1129/2011).

		Processed meat products		Meat preparations <sup>1</sup>
E No.	Additive name	Non heat treated	Heat treated	
E 300	Ascorbic acid	qs	qs <sup>2</sup>	qs <sup>3</sup>
E 301	Sodium ascorbate			
E 302	Calcium ascorbate			
E 304	Fatty acid esters of ascorbic acid	qs	qs <sup>2</sup>	
	i) Ascorbyl palmitate			
	ii) Ascorbyl stearate			
E 306	Tocopherol-rich extract			
E 307	Alpha tocopherol			
E 308	Gamma tocopherol			
E 309	Delta tocopherol			
E 315	Erythorbic acid	500 mg/kg <sup>4</sup>		
E 316	Sodium erythorbate			

<sup>1</sup> as defined by Regulation (EC) No 853/2004,

<sup>2</sup> except for *foie gras*, *foie gras entier*, *blocs de foie gras*, only E 300 and E 301 are allowed

<sup>3</sup> only *gehakt* and prepacked preparations of fresh minced meat,

<sup>4</sup> E 315 and E 316 are authorised individually or in combination, expressed as erythorbic acid

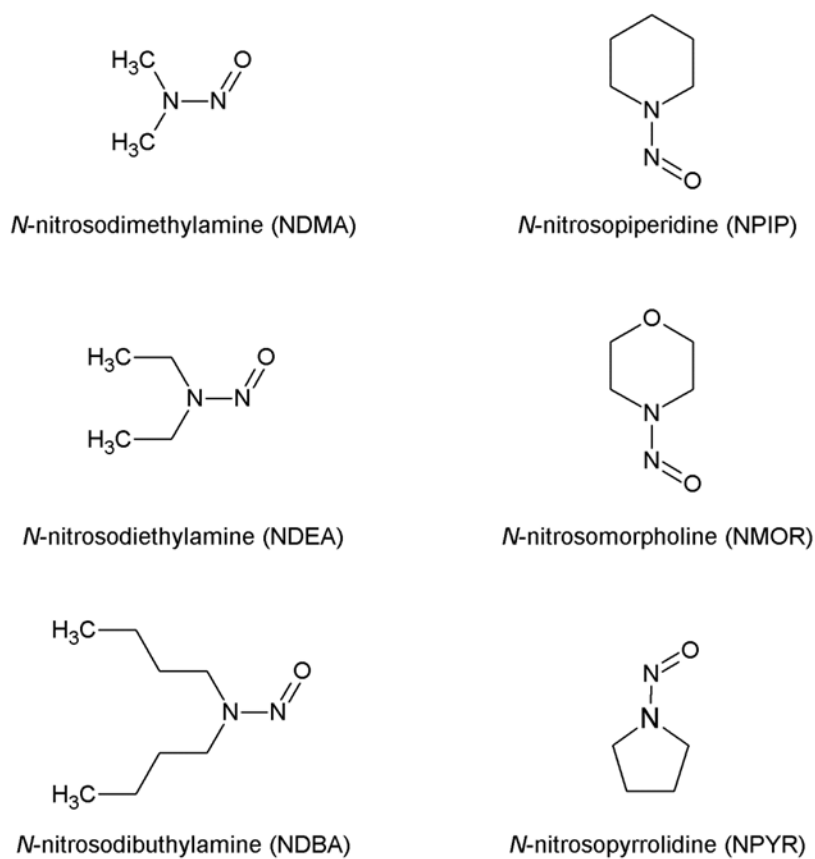


Figure 1. Chemical structure of the most common volatile *N*-nitrosamine in meat products.



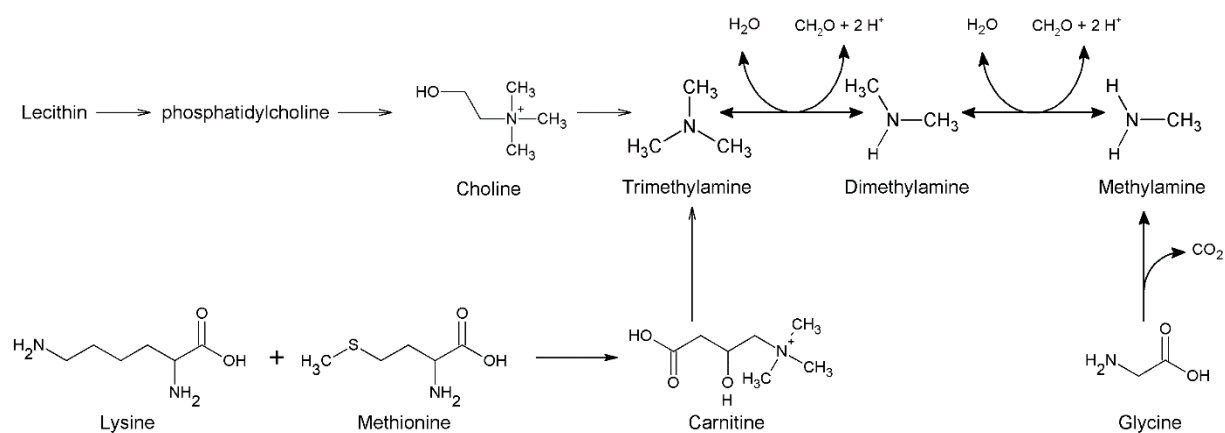


Figure 2. Possible routes for the formation of the precursor dimethylamine.

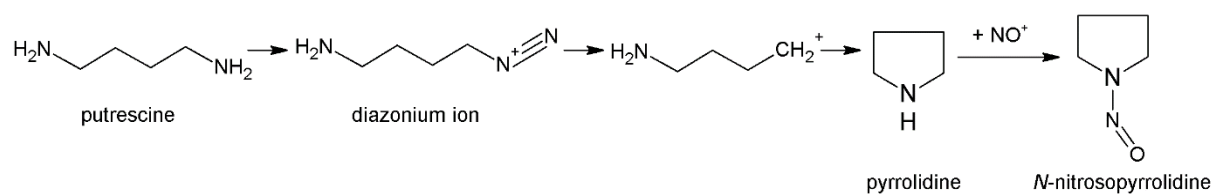


Figure 3. Formation pathway of NPYR from the biogenic amine putrescine (redrafted from Gray & Collins, 1977a).

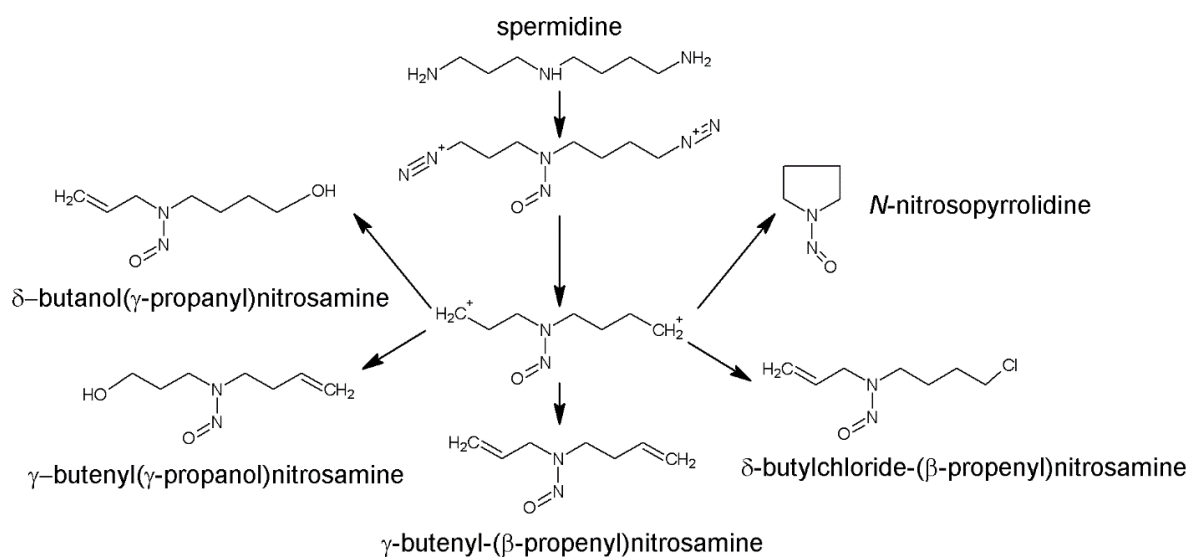


Figure 4. Pathway of the formation of several nitrosocompounds from the nitrosation of spermidine (redrafted from Hildrum, 1975a).

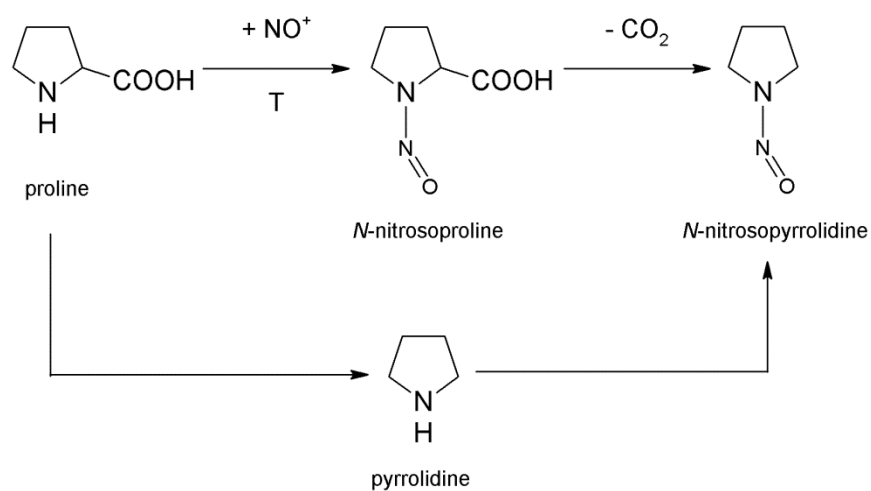


Figure 5. Formation mechanism of NPYR from the amino acid proline in fried bacon (redrafted from Kikugawa & Kato, 1991).

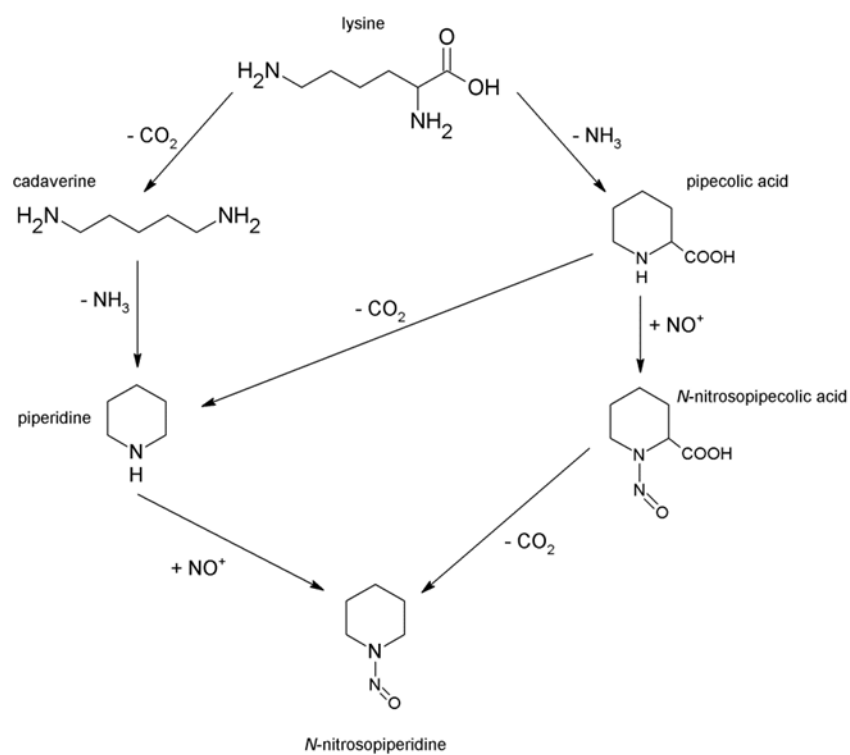


Figure 6. Possible routes for the formation of NPIP from lysine and CAD (redrafted from Warthesen et al., 1975).

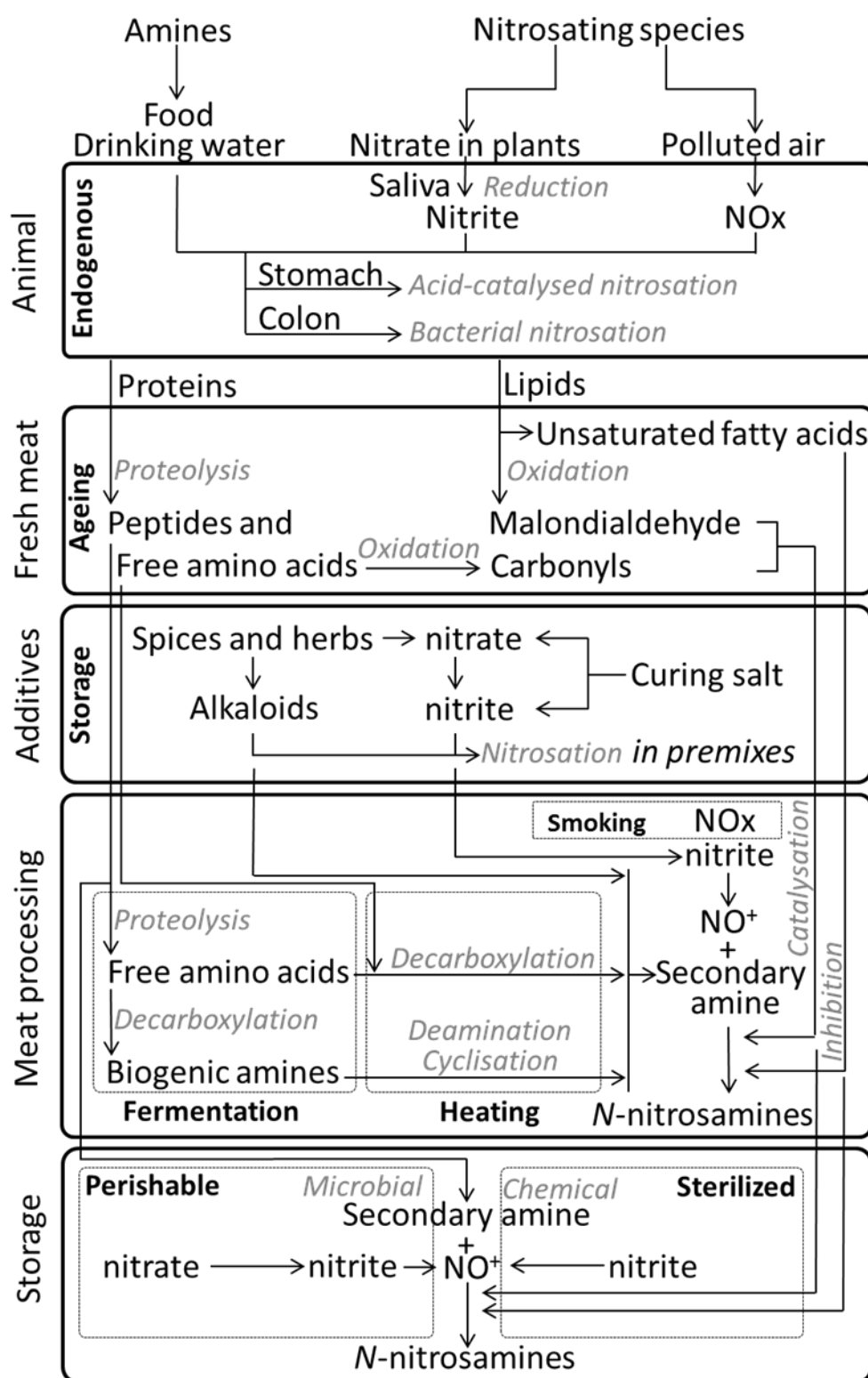


Figure 7. Factors influencing the formation of *N*-nitrosamines and its precursors in meat and meat products.