

# The use and control of nitrate and nitrite for the processing of meat products

Karl-Otto Honikel

*Federal Research Centre for Nutrition and Food, E.C. Baumann Strasse 20, 95326 Kulmbach, Germany*

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## Abstract

Nitrate and nitrite are used for the purpose of curing meat products. In most countries the use of both substances, usually added as potassium or sodium salts, is limited. Either the ingoing or the residual amounts are regulated by laws.

The effective substance is nitrite acting primarily as an inhibitor for some microorganisms.

Nitrite added to a batter of meat is partially oxidized to nitrate by sequestering oxygen – thus it acts as an antioxidant – a part of nitrite is bound to myoglobin, forming the heat stable NO-myoglobin, a part is bound to proteins or other substances in meat. Nitrate may be reduced to nitrite in raw meat products by microorganisms.

As oxidation and reduction may occur the concentrations of nitrite plus nitrate in a product has to be controlled and measured especially if the residual amounts are regulated.

This sum of both compounds is important for the human body. Intake of nitrate with food leads to its absorption over the digestive tract into the blood. In the oral cavity nitrate appears again where it is reduced to nitrite. With the saliva the nitrite is mixed with food, having the same effect as nitrite in a batter (inhibiting growth of some pathogenic microorganisms) and swallowed. In the stomach nitrite can eventually form carcinogenic nitrosamines in the acidic environment.

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## 1. Introduction

The use of nitrite and nitrate in the manufacturing of meat products is commonly expressed as “curing”. The verb “to cure” means to correct, restore, treat (*Oxford Dictionary & Thesaurus III, 2001*). With other words curing of meat is meant originally as something positive one could do to meat. Curing preserved in ancient times the meat and fish from spoilage. It meant in the beginning the use of salt which lowered the water activity and inhibited the growth of spoiling microorganisms. But in the 19th century people realized that some salts were preserving better than others. Saltpetre ( $\text{KNO}_3$ ) was recognized as a contaminant of the salt which enhanced its preserving action and gave a red colour to the product.

Polenske (1891) from the German Imperial Health Office published experiments where he could prove that by adding nitrate (saltpetre) only to a pickling solution nitrite was formed apparently due to the action of some microorganisms in the brine. Lehmann (1899) and Kisskalt (1899) confirmed that nitrite was the agent producing the red colour of meat and its heat stability. Haldane (1901) brought light into the chemistry of the curing process by showing that red-ox reactions occurred in meat on curing. He also extracted the NO-myoglobin as the substance responsible of the bright red colour of cured meat.

Hoagland (1910, 1914) showed that not the nitrite anion was the reactant. It was the nitrous acid ( $\text{HNO}_2$ ) or a metabolite like NO which reacted with the myoglobin (Fig. 1).

So the colouring of meat by nitrogen compounds was understood at the turn to the 20th century. The antimicrobial

*E-mail address:* [karl-otto.honikel@t-online.de](mailto:karl-otto.honikel@t-online.de)



B together with nitrite and salt (Robinson, Gibson, & Roberts, 1982).

From the above it becomes clear that nitrite is a very reactive substance which undergoes in meat products many reactions and thus its use has to be controlled.

### 3. Legislation

After it was discovered that nitrite was the genuine curing agent it took only a few years until nitrite was introduced into meat products manufacturing. But nitrite itself is rather toxic in comparison to nitrate. As a rule of thumb nitrite is 10 times more toxic than nitrate. The lethal oral doses for human beings are established in 80–800 mg nitrate/kg body weight and 33–250 mg nitrite/kg body weight (Schuddeboom, 1993).

Nitrite was added to meat products sometimes in too high amounts and e.g. in Germany some people died in the 3rd decade of the 20th century due to intoxication by nitrite in meat products. Germany solved the problem in 1934 with the Nitrit-Pökelsalz-Gesetz (nitrite curing salt law). It enforced that the use of nitrite in meat products is only allowed in premixes with table salt; its content should be 0.5% and must not exceed 0.6%. Only nitrate could be added directly to meat batters. 0.5% means that with 20 g nitrite curing salt/kg of batter (2%) 100 mg nitrite/kg batter (100 ppm) would be added.

In the 1950s, the Fleischverordnung (meat regulation) limited the residual amount to 100 mg sodium nitrite/kg in ready-to-eat meat products. In raw hams 150 mg NaNO<sub>2</sub>/kg were permitted. Also nitrate restrictions were applied. In the Fleischverordnung of 1982 nitrate was limited to some non-heated products with ingoing amounts of 300, respectively, 600 mg/kg and residual amounts of 100–600 mg/kg product.

This regulation was followed by the European Parliament and Council Directive 95/2/EC on food additives other than colours and sweeteners (Directive, 1995) where indicative ingoing amount of 150 mg nitrite/kg and 300 mg nitrate/kg were permitted in more or less all meat products (Table 1). The residual amounts could be 50 mg nitrite/kg in non-heat treated meat products and 100 mg nitrite/kg in all other meat products except Wiltshire bacon and some other similar products to 175 mg nitrite/kg. With

nitrate the residual amounts were 250 mg nitrate/kg in all meat products.

Denmark opposed this and excluded the use of nitrate from all meat products except Wiltshire bacon and some other raw hams with ingoing 300 mg nitrate/kg. Nitrite up to 150 mg ingoing amounts of nitrite/kg were written down in the Danish regulation 1055/95 of 18 December 1995 (Danish Regulation, 1995).

The European Court in Luxembourg decided on March 20, 2003 that the European union must reconsider their regulation 95/2/EC.

This happened in 2006. With directive 2006/52/EC of 5 July 2006 (Directive, 2006) the use of nitrates is limited to non-heated meat products with 150 mg (ingoing amount must be calculated as sodium nitrite)/kg but with quite a number of exemptions and nitrite up to 100, respectively, 150 mg nitrite (ingoing amount)/kg in all meat products again with a number of exemptions (Table 2). Whereas in the directive of 1995 the maximum of ingoing amounts was recommended and the residual amounts were limited, in the directive of 2006 ingoing amount are in general recommended and a number of specified products have either residual amounts – some higher than the general ingoing amounts, some are permitted with recommended ingoing and residual amounts. All concentrations must be expressed as sodium nitrite equivalents.

That is, in general 150 mg nitrite/kg are allowed to add for all meat products plus 150 mg nitrate/kg for unheated meat products. That is a maximum of 300 mg nitrite + nitrate/kg may be in a batter or a piece of raw ham. The exemptions, e.g. Wiltshire or dry cured bacon may have 175 mg residual nitrite/kg plus 250 mg residual nitrate/kg, i.e. 425 mg residual nitrite + nitrate/kg. In non-specified raw products no more than 300 mg nitrite + nitrate/kg may be added. In heated products no more than 150 mg added nitrite/kg is permitted. Can this be understood by a health conscious consumer?

Many countries have similar regulations. As an example the regulations of the USA should be reported here. In their Code of Federal Regulations (USA, 2005) they state: “The food additive sodium nitrite may be safely used in or on specified foods in accordance with the following prescribed conditions:

Table 1  
Nitrate and nitrite in meat products; extract from Directive (1995) (valid until 2006)

E No.	Name	Foodstuff	Indicative ingoing amount during the manufacturing (mg/kg)	Residual amounts (mg/kg)
E249	Potassium nitrite	Non-heat-treated cured and dried Meat products	150	50
E 250	Sodium nitrite	Other cured meat products <i>Cured bacon</i>	150	100 175
E 251	Sodium nitrate	Cured meat products	300	250
E 252	Potassium nitrate			

Table 2  
Extract from Directive (2006) regarding nitrite and nitrate for meat products

E No.	Name	Foodstuff	Maximum amount that may be added during manufacturing (expressed as NaNO <sub>2</sub> ) (mg/kg)	Maximum residual level (expressed as NaNO <sub>2</sub> ) (mg/kg)
E249 <sup>a</sup>	Potassium nitrite	Meat products	150	–
E 250 <sup>a</sup>	Sodium nitrite	Sterilized meat products (F <sub>O</sub> > 3.00) <sup>b</sup>	100	–
		Traditional immersion cured meat products (number of products)	–	50–175
		Traditional dry cured meat products (number of products)	–	50–175
		Other traditionally cured meat products (number of products)	180	50
E 251 <sup>c</sup>	Potassium nitrate	Non-heat-treated meat products	150	–
E 252 <sup>c</sup>	Sodium nitrate	Traditional immersion cured meat products (number of products)	300	10–250 (some without added)
		Traditional dry cured meat products (number of products)	300	>50 (some without nitrite added)
		Other traditionally cured meat products	250–300 (without nitrite added)	10–250

<sup>a</sup> When labelled “for food use” nitrite may be sold only in a mixture with salt or a salt substitute.

<sup>b</sup> F<sub>O</sub>-value 3 is equivalent to 3 min heating at 121 °C.

<sup>c</sup> Nitrates may be present in some heat-treated meat products resulting from natural conversion of nitrites to nitrates in a low acid environment.

As a preservative and colour fixative, with sodium nitrate, in meat curing preparations for the home curing of meat and meat products (including poultry and wild game), with directions for use which limit the amount of sodium nitrite to not more than 200 parts per million in the finished meat product, and the amount of sodium nitrate to not more than 500 parts per million in the finished meat product.”

All regulations, directives and laws take into account that nitrite is a toxic substance and that contrary to other additives nitrite does not remain unchanged in the product during processing. Also the discoveries of the early 20th century are taken into consideration. Nitrate is only effective after being reduced to nitrite. This happens only in products which are not heat treated early after manufacturing that means in raw hams and raw sausages. The intake of nitrite and nitrate should be thus limited to the necessary minimal requirements.

#### 4. Nitrite and nitrate in meat products

##### 4.1. Nitrite and nitrate in meat

If nitrites are added to meat the salts are dissolved due to their good solubility in the aqueous solution of pH around 5.5 in the meat. Due to the pK of nitrous acid of 3.37 which means that a pH 3.37 around 50% of the acid is dissociated, it can be expected that about 99% of the nitrite is existing of pH 5.5 as an anion (NO<sub>2</sub><sup>-</sup>). The small

amount of undissociated nitrous acid is in equilibrium with its anhydride N<sub>2</sub>O<sub>3</sub> which again is in equilibrium with the two oxides nitric oxide and nitric dioxide (see Fig. 1).

The NO<sub>2</sub> can react with water (Fig. 4). In the sum it means that from two HNO<sub>2</sub> molecules one HNO<sub>2</sub> and one HNO<sub>3</sub> molecule are formed. Furthermore, the NO molecule itself can easily be oxidized to NO<sub>2</sub> in the presence of oxygen. This means an oxygen sequestering and thus the antioxidative action of nitrite in meat batters or hams. Due to the lack of oxygen the development of rancidity or a warmed over flavour are retarded. This reaction is rather important in meat batters as nitrite acts in this way as an antioxidant. This fact is also mentioned as a foot note in Directive (2006) (Table 2). Metal ions seem to accelerate the oxidation process. Nitrate added to meat will fully dissociate into Na<sup>+</sup>/K<sup>+</sup> + NO<sub>3</sub><sup>-</sup>. No detectable amount of undissociated HNO<sub>3</sub> will be found.

##### 4.2. Concentration in meat products

The oxidation of nitrite to nitrate in meat also explains why in meat products to which only nitrite has been added nitrate will be found in considerable concentrations. In Figs. 5 and 6, the nitrite and nitrate concentrations of German meat products are shown. The emulsion type and cooked sausages and cooked hams are manufactured with nitrite only but they contain a mean of 20–30 mg nitrate/kg as also shown in Table 3 in a very recent survey. Nitrite is in most cases lower than nitrate in the finished product

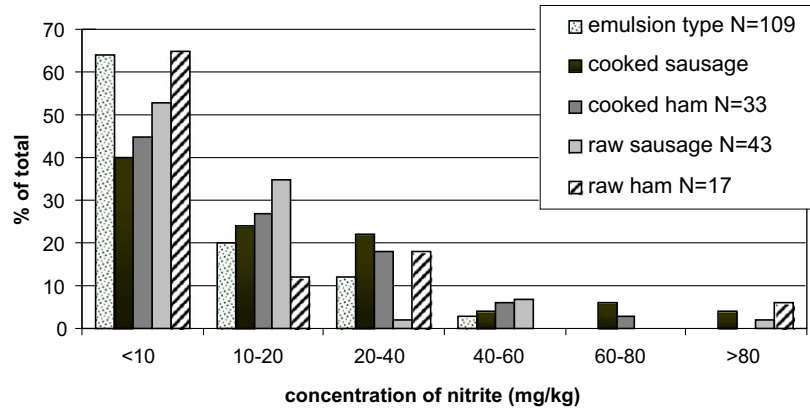


Fig. 5. Nitrite in German meat products (1996–2001; Dederer, 2006).

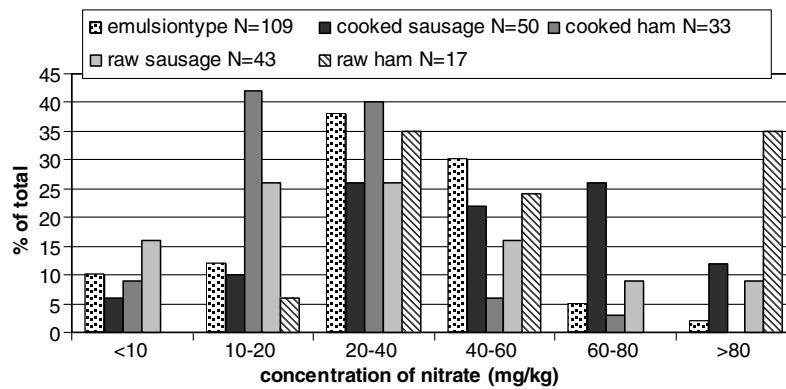


Fig. 6. Nitrate in German meat products (1996–2001; Dederer, 2006).

Table 3  
Nitrite and nitrate concentrations in meat products of Germany (2003–2005) (Dederer, 2006)

	Year	n	Median (mg/kg)	
			Nitrite	Nitrate
Emulsion type sausage	2003	30	13.2	23.4
	2004	32	12.65	20.5
	2005	29	19.9	30.0
Raw sausages	2003–2005	15	17.9	59.2
Raw ham	2003–2005	14	19.2	16.9
Liver/blood sausages (cooked sausages)	2003–2005	16	12.1	43.3

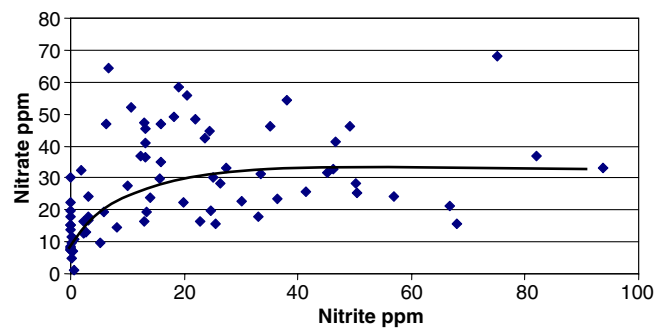


Fig. 7. Concentration of nitrite and nitrate in emulsion type sausages ( $N = 48$ ) (Dederer, 2006).

with concentration below 20 mg nitrite/kg in the median value. Only a few samples of cooked and raw sausages and raw ham exist above 60 mg nitrite/kg (Fig. 5) which also have higher nitrate concentrations. In the raw products nitrate may have been added.

It could be assumed that the concentration of nitrate in a sausage where only nitrite is added is related to the nitrite content. Fig. 7 shows that with emulsion type sausages (only nitrite curing salt is used) the residual amounts of nitrite and nitrate exhibit no relationship above 20 mg

residual nitrite/kg. There is no generally recognizable increase of nitrate with increasing residual amounts of nitrite. Without nitrite addition a residual amount of nitrate up to 30 mg/kg is probably due to the added drinking water and spices into the batter (0–50 mg nitrate/l).

#### 4.3. Changes with time of storage

When does the nitrite disappear in the product? Table 4 shows results from Russian colleagues (Kudryashov, 2003). The largest decrease is observed during the



Table 4  
Remaining nitrite (mg/kg) during storage at 2 °C of an emulsion type sausage (Kudryashov, 2003)

Storage	Concentration of nitrite added (mg/kg)			
	75	100	150	200
After heating	21.9	30.5	59.5	53.7
20 days	7.5	9.3	10.2	15.4
40 days	3.6	6.4	7.6	7.7
60 days	0.5	0.9	4.0	5.8

Table 5  
Nitrite breakdown and nitrate appearance after nitrite addition to meat of various pH values after heating and storage (Đorđević et al., 1980)

pH	Days of storage	Nitrite		Nitrate	
		100 mg/kg added	200 mg/kg added	100 mg/kg nitrite added	200 mg/kg nitrite added
5.3	After heating				
	0	28	70	20	50
	6	20	41	16	27
	12	5	18	9	20
5.8	After heating				
	0	45	120	30	64
	6	24	110	22	40
	12	13	21	8	17
6.3	After heating				
	0	58	135	18	40
	6	41	112	17	30
	12	31	90	10	22

manufacturing up to the end of the heating process. This early loss amounts usually to about 65% independent of the ingoing concentrations. Within 20 days of cold storage the concentrations drop further to a third of the concentration after heating.

The disappearance continues until 60 days of cold storage. Table 5 confirms the results. It furthermore shows that a higher pH value retards the disappearance of nitrite. It also confirms the results of Table 3 that nitrate concentrations are already high at day 0 after heating. Nitrate also falls in concentrations with time of storage. The reduction during storage is slower with increasing pH.

Table 6 shows the influence of different heat treatment in meat homogenates. To muscles with different pH, 100 mg nitrite/kg was added and the homogenate was either mildly heated (pasteurized) or sterilized. The nitrite and nitrate concentrations immediately after heating and 12 days of storage were measured. The results were: The higher the heating, the greater is the loss of nitrite. The formation of nitrate is also reduced. The residual nitrite plus nitrate added at no time of measurement to 100 mg/kg. Immediately after pasteurization both added to about 75 mg/kg. Sterilisation and storage reduced the added concentrations even further. Both compounds seem to react with other ingredients and are no longer analytically measured as inorganic nitrite or nitrate. The addition of ascorbate and polyphosphate show that the disappearance of nitrite is accelerated by ascorbate in the raw batter (Table 7). Heating for 7 min to 80 °C leads to a slower loss of nitrite. Heating for additionally 1 h at 70 °C retards the loss even longer. This is probably due to the inactivation of microorganisms and inactivation of enzymes by heating. With ascorbate and even more by polyphosphates the retarding by heating is also observed.

Nitrite in all cases described here is partially oxidized to nitrate. In many experiments (see Tables 5 and 6) about 10–40% of the nitrite is oxidized to nitrate. This is known

Table 7  
Approximate number of days for residual nitrite to fall below 10 mg/kg in a pork slurry of pH 5.5–6.2 at 2.5–4.5% NaCl at a storage temperature of 15 °C (N = 5); (Gibson et al., 1984)

Nitrite added (mg/kg)	Heat treatment		
	Unheated (days)	80 °C/7 min (days)	80 °C/7 min + 70 °C/1 h (days)
<i>(A) No further addition</i>			
100	5	12	63
200	10	12	68
300	21	21	>168
<i>(B) As in A + ascorbate (1000 mg/kg)</i>			
100	5	9	10
200	5	9	9
300	5	21	48
<i>(C) As A + ascorbate (1000 mg/kg) + polyphosphate 0.3% (w/v)</i>			
100	5	10	21
200	10	21	21
300	5	5	12

Table 6  
Mean values of nitrite and nitrate content in pasteurized and sterilized groups of homogenates (Đorđević et al., 1980)

Homogenate 100 mg/kg of NaNO <sub>2</sub> added	Way of heat treatment <sup>a</sup>	After heat treatment		After 12 days at 2–4 °C	
		NaNO <sub>2</sub> (mg/kg)	NaNO <sub>3</sub> (mg/kg)	NaNO <sub>2</sub> (mg/kg)	NaNO <sub>3</sub> (mg/kg)
M. longissimus dorsi	P	38.6	27.4	10.9	7.9
pH 5.8	S	12.9	13.1	3.5	6.0
M. quadriceps femoris	P	49.2	35.8	26.7	12.8
pH 6.15	S	15.6	19.4	7.4	9.8

<sup>a</sup> P = pasteurization (75 °C); S = sterilization (>110 °C).

Table 8  
Nitrite and metabolites in meat products

Bound to/or form	Percentage of total <sup>a</sup>	Percentage of total <sup>b</sup>
Nitrite	5–20	
Nitrate	1–10	10–40 <sup>a</sup>
Myoglobin	5–15	
Bound to –SH	1–15	
Bound to lipids	1–15	
Bound to proteins	20–30	
Gas	1–5	
Sum	~70	90

<sup>a</sup> Cassens et al. (1978), Cassens (1990).

<sup>b</sup> Assumption realized by the author (K.O. Honikel) according to results presented in Figs. 5 and 6.

since decades. Already in 1978 Cassens et al. postulated that nitrite is bound to various meat constituents (Table 8).

#### 4.4. Cured meat colour

The red colour of cured meat products is one of the important effects of nitrite in meat products. The red colour is developing in a number of complicated reaction steps until NO-myoglobin (Fe<sup>2+</sup>) is formed.

Myoglobin exists in a muscle in three states, in which the cofactor haem, a porphyrin ring with an iron ion in its centre binds different ligands or in which the iron exists in the Fe<sup>2+</sup> or Fe<sup>3+</sup> state. In the native myoglobin, the porphyrin moiety (Fig. 8) is supported in the ligand binding by amino acids of the protein in the neighbourhood.

In the “original” state myoglobin with Fe<sup>2+</sup> in the porphyrin cofactor does not bind any ligand maybe a water molecule. In the presence of oxygen the myoglobin can bind an O<sub>2</sub> molecule and it becomes bright red. The iron ion is in the Fe<sup>2+</sup> state. But oxygen and other oxidizing agents like nitrite can oxidize the Fe<sup>2+</sup> to Fe<sup>3+</sup>. The formed metmyoglobin (MetMb) is brown.

The “original” myoglobin (Mb), the oximyoglobin (MbO<sub>2</sub>) and the metmyoglobin are occurring together in meat. In a muscle in a live animal there is very little metmyoglobin which increases post-mortem with the disappear-

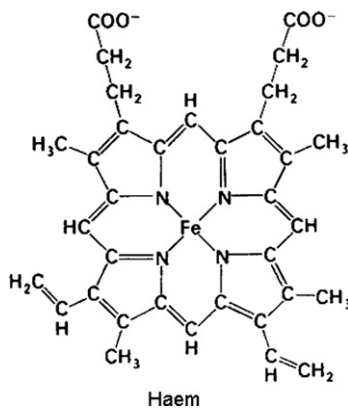


Fig. 8. Haem in the myoglobin molecule.

ance of oxygen except when meat is MAP-packed with high oxygen content.

The three states of myoglobin have three characteristics absorbance spectra between 400 and 700 nm. As the three are in a kind of equilibrium to each other, the spectra have an isosbestic point at 525 nm where all three absorption curve cross which each other. The absorbance of this wavelength can be used for detecting the percentage of each form in meat. Nitrosomyoglobin has a spectrum which has similar maximum wavelength like oximyoglobin (Fig. 9).

Oxygen and NO are biatomic molecules. A similar biatomic molecule CO also binds to myoglobin and also very tight. In some countries (e.g. USA and Norway) MAP packaging of meat with 1–2% CO is permitted.

By reducing enzymes or chemical reactions with reducing agent like ascorbate the Fe<sup>3+</sup> is reduced to Fe<sup>2+</sup>. The NO formed from N<sub>2</sub>O<sub>3</sub> can bind to the myoglobin (Fe<sup>2+</sup>) and forms a heat stable NO-myoglobin. Oximyoglobin is not heat stable and dissociates. The meat turns grey or brown.

On heating the NO-myoglobin the protein moiety is denatured but the red NO-porphyrin ring system (called often nitroso-myochromogen) still exists and is found in meat products heated to 120 °C.

This heat stable red colour will change on bacterial spoilage and it fades on UV light. The first one is advantageous as the consumer recognizes spoilage like in fresh meat which also changes colour on spoilage.

In most recent years the riddle about the red colour of cured raw hams like Parma ham without added nitrite or nitrate has been solved. Various authors could show and proof that the Fe<sup>2+</sup> in the porphyrin ring is exchanged with Zn<sup>2+</sup> which gives the products a pleasant red colour. Nitrite addition prevents the exchange (Adamsen, Møller, Laursen, Olsen, & Skibsted, 2006; Møller, Adamsen, & Skibsted, 2003; Parolari, Gabba, & Saccani, 2003;

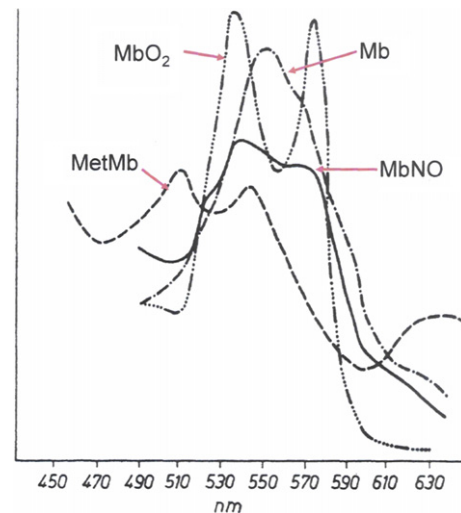


Fig. 9. Spectra of the various states of myoglobin.

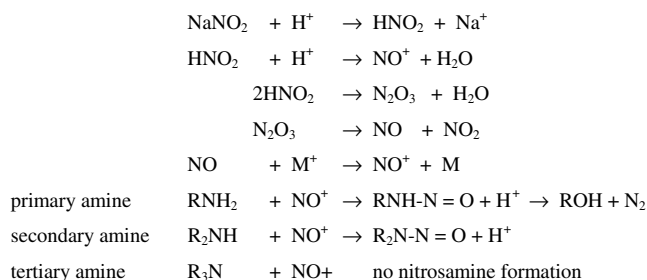


Fig. 10. Formation of nitrosamines, chemical reactions (M/M<sup>+</sup> are transition metal ions like Fe<sup>2+</sup>/Fe<sup>3+</sup> and others).

Wakamatsu, Nishimura, & Hattori, 2004; Wakamatsu, Okui, Ikeda, Nishimura, & Hattori, 2004).

#### 4.5. Nitrosamine formation

In the 1970s in USA a discussion started about the formation of nitrosamines in cured meat products, especially fried bacon. Fiddler et al. (1978) showed that bacon and its cookout on frying contained considerable amounts of nitrosopyrrolidine.

Nitrosamines are formed by amines with nitrite at higher temperatures according to the reactions in Fig. 10. But there are some prerequisites:

- Amines must be present. In fresh meat there are very minute amounts of amines present. These are creatine, creatinine and the free amino acids proline and hydroxyproline and some the decarboxylation products of other amino acids. During ageing and fermentation more amines will be formed.
- Only secondary amines form stable nitrosamines. Primary amines are immediately degraded to alcohol and nitrogen. Tertiary amines cannot react. Most amines in meat are primary amines derived from  $\alpha$ -amino acids.
- The pH must be low enough to produce NO<sup>+</sup> or metal ions must be engaged to form NO<sup>+</sup>.

As heated meat products are produced from fresh meat (chilled or frozen) no amines are available. In raw nitrate-cured meat products the nitrite concentration is rather low (see Fig. 5). Thus the formation of NO<sup>+</sup> is rather unlikely. In products heated above 130 °C nitrosamines can be formed. Bacon frying, cured sausage grilling or frying of cured meat products as pizza toppings may experience such conditions that nitrosamines are formed. Table 9 shows the results of an investigation by Deierling, Hemmrich, Groth, and und Taschan (1997). In German foods only beer and pizza exhibited dimethyl-nitrosamine in detectable amounts. Thus nitrosamines occur only in small amounts and they are easily avoidable by proper frying, grilling and pizza baking. A data base for nitrosamines in foods together with other processing related residues are published by Jakszyn et al. (2004). Besides amines also amides and unsaturated fatty acids or derivatives of the latter can

Table 9  
NO-dimethylamine in foods (µg/kg) (Deierling et al., 1997)

Food	Content			
	N	>0.5	Minimum	Maximum
Beer	195	3	0.5	1.2
Pizza	57	6	0.5	8.7
Meat products	17	0	0	0
Milk products	6	0	0	0

react with nitrite or its derivatives. Fatty acids or its derivatives can form alkyl nitrites. About their concentrations very little are known about their presence in meat products. To the database Jakszyn et al. (2004) is referred.

In this context, it should be mentioned that nitrosamines can be present in elastic rubber nettings for meat products which may contaminate the edible parts of e.g. cooked ham (Fiddler, Pensabene, Gates, & Adam, 1998; Helmick & Fiddler, 1994).

#### 5. Conclusions

Curing of meat is a process known since ancient times with the intention to prolong the shelf life of meat. The curing agents nitrite and nitrate react due to easily varying oxidation status of nitrogen into many derivatives with meat ingredients.

The curing agents give the products an esteemed and stable red colour, nitrite acts as an antioxidant, nitrite prevents or retards microbial growth and finally the curing agents give a pleasant flavour. The positive effects are overwhelming against the small possibility of the formation of nitrosamines. The intake of curing agents by meat products is small in comparison with other foods.

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