

# 4

## Principles of Curing

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### 4.1 Definition of curing

Curing of meat indicates the addition of nitrite and/or nitrate salt together with table salt (NaCl) to meat in various degrees of comminution and at different processing steps. The most basic method of curing is the covering of meat cuts (e.g., hind legs of hogs) with a mixture of salt and curing agents. These solids diffuse/penetrate slowly into the muscle tissue. Such a dry-curing process lasts up to several weeks. The process can be accelerated by wet curing, in which the meat cuts are inserted into a brine of salt and curing agents and/or brine is injected directly into the meat by multiple needles (i.e., stitch pumping) or via blood vessels (i.e., arterial injection). In small pieces, and in minced or comminuted meat such as sausages, NaCl and the curing agents are mixed or comminuted with the meat in a bowl chopper. This means of curing is fast, and within a short time (1–2 days, depending on the processing technique) leads to the characteristic appearance of a cured meat product with a pink/red color and a “cured” flavor.

### 4.2 History of curing

Salting as a means of preserving meat and fish was essential in ancient times, providing nutrient-dense foods during times of scarcity. This process was usually combined with drying and/or smoking of the products. Salt addition and drying result in a reduction of the free water in which microorganisms can grow; that is, by lowering the water activity ( $a_w$ ), spoilage is prevented. Smoking covers the exterior surface of meat with bacteriostatic and mycostatic compounds. Hence, smoking also aids in preservation.

Salt in its purest form is sodium chloride. Many sources of salt, such as rock salt and marine/sea salt, contain small amounts of natural contaminants, such as sodium or potassium nitrate/nitrite, that contribute to the curing process, although these were unrecognized in ancient times. Not until the late nineteenth century was it discovered that saltpeter (potassium and sodium nitrate) was a constituent of many preserving salts.

\* Karl O. Honikel has regrettably passed away prior to the publication of this chapter.

The formation of the red curing color depends on the presence of nitrate in the salt. In 1891, Polenske, of the German Imperial Health Office, published a paper in which he tried to explain what the red compound in meat products actually was (Polenske, 1891). He had observed that cured meat and pickling solutions contained nitrite, even though only saltpeter (potassium nitrate) had been employed in the fresh solution of the salt. A heat-sterilized solution did not produce nitrite, but an unheated solution did, after a few days of storage. The answer was simple but revolutionary: nitrate ( $\text{NO}_3^-$ ) was reduced to nitrite ( $\text{NO}_2^-$ ) by endogenous microorganisms. This observation was followed by that of Lehmann (1899), who noted that an acidified nitrite solution turned meat a bright red color, but that nitrate and nitric acid did not. In the same year, Kisskalt (1899) observed that cooking meat in a nitrite solution turned it red, but not if nitrate was applied. Nitrate only turned meat bright red after the meat was left in a nitrate solution at ambient temperatures for a few days.

Thus, three scientists confirmed, within a decade, that nitrite was responsible for the curing color of meat, and not nitrate. Haldane (1901) brought the chemistry of the process to light. He discovered that the change in color caused by cutting the surfaces of fresh meat (with its formation of oxymyoglobin,  $\text{MbO}_2$ ) returned to the usual, less red state on covering with a glass plate. Salt-treated (nitrate/nitrite-containing) meat in the raw state was bright red after a fresh cut, but turned brown upon exposure to air. Covering this meat surface with a glass plate then turned it bright red again. Extracting the colored pigments, Haldane found that the bright red salted, cured color was a nitrogen oxide–myoglobin (NOMb) complex. NOMb was found to exist in both raw cured and cooked cured meat.

Hoagland (1910, 1914) reported that only an acidified solution led to the red cured color. He concluded that nitrous acid ( $\text{HNO}_2$ ), or a metabolite such as NO, yielded the bright red cured meat color. Figure 4.1 shows the sequence of events involved in meat curing as known in the second decade of the 20th century.

So, during the early part of the 20th century, the mechanistic principle behind the red color of cured meat was understood, but the antimicrobial action was still thought to be caused by the salt, and nitrite/nitrate were not considered responsible for the preservation itself. Research on nitrite's role in meat products still had a long way to go.

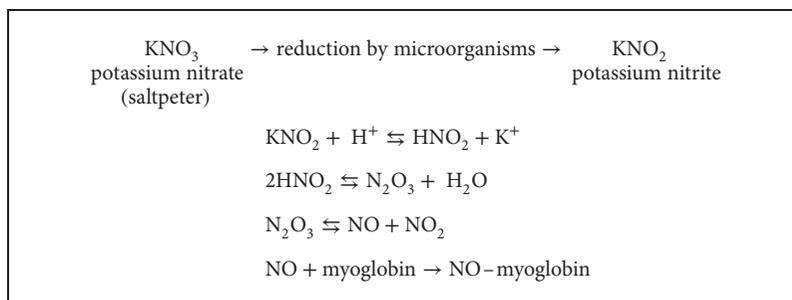


Figure 4.1 Scheme of Hoagland's (1910, 1914) proposal concerning the action of nitrate in cured meat products.

### 4.3 Legislation

After it was discovered that nitrite was the genuine curing agent, it took only a few years before it was introduced into the manufacture of meat products. Yet, as a rule of thumb, nitrite is 10 times more toxic than nitrate. The lethal oral doses for humans are 80–800 mg nitrate/kg body weight and only 33–250 mg nitrite/kg body weight (Schuddeboom, 1993).

Nitrite was thus sometimes added to meat products in too-high amounts. For instance, in the 1920s in Germany, people died due to intoxication by nitrite in meat products. In 1934, Germany solved the problem with the Nitrit-Pökelsalz-Gesetz (nitrite curing salt law), which stated that the use of nitrite in meat products was only to be 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. With 20 g nitrite curing salt/kg of batter (2%), this 0.5% meant that 100 mg nitrite/kg batter (100 ppm) would be incorporated.

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  $\text{NaNO}_2$ /kg was permitted.

Nitrate restrictions were also enforced. In the Fleischverordnung of 1982, nitrate was limited to some nonheated products, with ingoing quantities of 300–600 mg/kg and residual amounts of 100–600 mg/kg product (Das Bundesgesetzblatt, 1982).

This regulation was followed by the European Parliament and Council Directive 95/2/EC on “food additives other than colours and sweeteners” (European Parliament and Council, 1995), in which indicative ingoing amounts of 150 mg nitrite/kg and 300 mg nitrate/kg were permitted in more or less all meat products. The residual levels could go up to 50 mg nitrite/kg in non heat-treated meat products and 100 mg nitrite/kg in all other meat products, except for Wiltshire bacon and some other products, which could go to 175 mg nitrite/kg. With nitrate, the residual quantities were 250 mg/kg in all meat products.

Denmark opposed this regulation and eliminated the use of nitrate from all meat products except for Wiltshire bacon and some other raw hams, at a level of 300 mg nitrate/kg meat. On December 18, 1995, ingoing quantities of nitrite up to 150 mg/kg were restricted in meat products according to regulation 1055/95 (Lovtidende, 1995). On March 20, 2003, the European Court in Luxembourg decided that the European Union must reconsider its regulation of 95/2/EC. It was not until 2006 that this finally happened. With

Directive 2006/52/EC of July 5, 2006 (European Parliament and Council, 2006), it was decided that the use of nitrates should be limited to nonheated meat products at 150 mg (ingoing amount must be calculated as sodium nitrite equivalents/kg meat, but with quite a number of exemptions) and nitrite up to 100–150 mg nitrite (ingoing quantity)/kg in all other meat products, again with a number of exemptions; Table 4.1). In the directive of 1995, a maximum level for ingoing amounts was recommended, while residual amounts were limited. In the directive of 2006, ingoing levels were generally recommended and a number of specified products had residual amounts—some were higher than the general ingoing amounts, and some were permitted with recommended ingoing and residual amounts. All concentrations, however, had to be expressed as sodium nitrite equivalents.

Many countries have similar regulations. As an example, the US Food and Drug Administration Code of Federal Regulations, Title 21, CFR § 172.175 (FDA, 2012) states:

The food additive sodium nitrite may be safely used in or on specified foods in accordance with the following prescribed conditions:

(a) It is used or intended for use as follows:

(3) As a preservative and color 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, unlike other additives, does not remain unchanged in the product during processing. For example, some is converted to nitrate. Furthermore, the discoveries of the early 20th century are also taken into consideration: nitrate is only effective after first being reduced to nitrite. This occurs only in products that are not heat-treated early after manufacturing; that is, in raw hams and raw sausages. Intake of nitrite and nitrate is therefore limited to the necessary minimal requirements.

### 4.4 Chemistry of nitrite and nitrate

#### 4.4.1 Chemical and physical characteristics of nitrites, nitrates, and related compounds

Nitrite and nitrate are employed in cured meat products in the form of their sodium and potassium salts:

**Table 4.1** Nitrate and nitrite in meat products.

E no.	Name	Foodstuff	Maximum level added during manufacture (expressed as NaNO <sub>2</sub> ) (mg/kg)	Maximum residual level (expressed as NaNO <sub>2</sub> ) (mg/kg)
E 249 <sup>a</sup>	Potassium nitrite	Meat products	150	—
E 250 <sup>a</sup>	Sodium nitrite	Sterilized meat products (F <sub>0</sub> > 3.00) <sup>b</sup>	—	50–175
		Traditional immersion-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 NO <sub>2</sub> added)
		Traditional dry-cured meat products (number of products)	300	> 50 (some without NO <sub>2</sub> added)
		Other traditionally cured meat products	250–300	10–250

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

<sup>b</sup>F<sub>0</sub> value of 3.00 is equivalent to 3 minutes’ 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.

Source: Extract from EU Directive 2006.

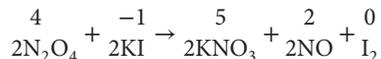
- Sodium nitrite, NaNO<sub>2</sub>, sodium salt of nitrous acid, CAS no. 7632-00-0, molecular weight 69 Dalton, white or yellowish white crystals, odorless, density 2.17 g/cm<sup>3</sup>, solubility 85.2 g/100 g water at 20 °C and 163 g/100 g water at 100 °C, pH in aqueous solution ~9, melting point 271 °C, hygroscopic in air, oxidizes in air slowly to nitrate, strong oxidizing agent with organic matter and inorganic material, especially ammonia compounds, toxicity oral rat LD<sub>50</sub> 85 mg/kg, human LD<sub>50</sub> 50 mg/kg body weight, irritating to skin and other body surfaces.
- Potassium nitrite, KNO<sub>2</sub>, potassium salt of nitrous acid, CAS no. 7758-09-0, molecular weight 85.1 Dalton, white or yellowish white crystals, odorless, density 1.92 g/cm<sup>3</sup>, solubility ~300 g/100 g water at 20 °C and 413 g/100 g water at 100 °C, pH in aqueous solution ~9, melting point 387 °C, instable above 350 °C, strong oxidizing agent with organic matter, toxicity as NaNO<sub>2</sub>.
- Sodium nitrate (saltpeter), NaNO<sub>3</sub>, sodium salt of nitric acid, CAS no. 7631-99-4, used as gun powder, molecular weight 84.99 Dalton, white crystals, odorless, hygroscopic in moist air, density 2.16 g/cm<sup>3</sup>, solubility 73 g/100 g water at 0 °C and 180 g/100 g water at 100 °C, pH in aqueous solution ~7, melting point 307 °C, stable under normal conditions, may oxidize reduced organic matter.
- Potassium nitrate (saltpeter), KNO<sub>3</sub>, potassium salt of nitric acid, CAS no. 7757-79-1, molecular weight 101.1 Dalton, white crystals,

odorless, salty taste, density 2.11 g/cm<sup>3</sup>, solubility 31.6 g/100 g water at 20 °C and 247 g/100 g water at 100 °C, pH in aqueous solution ~7, melting point 334 °C, stable under normal conditions, may oxidize organic matter and reacts vigorously with ammonia salts.

- Nitrous acid, HNO<sub>2</sub>, known only in solution, molecular weight 47.02 Dalton, anhydride N<sub>2</sub>O<sub>3</sub>, which exists only in the solid state at temperatures below –102 °C; above this the liquid is a mixture of N<sub>2</sub>O<sub>3</sub> ⇌ NO + NO<sub>2</sub>, middle strong acid, pK<sub>a</sub> 3.37.
- Nitric acid, HNO<sub>3</sub>, CAS no. 7697-37-2, molecular weight 63.01 Dalton, colorless liquid in closed containers, melting point –42 °C, boiling point 86 °C, density 1.52 g/g at 20 °C, mixable in any quantity with water, strong acid, totally dissociates in water.
- Nitric oxide/nitrogen oxide, NO, CAS no. 10102-43-9, molecular weight 30 Dalton, colorless gas, melting point –163.6 °C, boiling point –151.8 °C, oxidizes in air 2NO + O<sub>2</sub> → N<sub>2</sub>O<sub>4</sub> → 2 NO<sub>2</sub>, can bind to myoglobin to form NOMb, can easily lose an electron to form an NO<sup>+</sup> cation, which may react with amines to form N-nitrosamines, formed in the human body and functions as a physiological messenger, playing a role in the cardiovascular, neurologic, and immune systems through pathogen suppression, vasodilation, and neurotransmission (Ignarro, 1990; Lowenstein & Snyder, 1992; Lüscher, 1990, 1992; Marletta, 1989; Moncada *et al.*, 1991; Nathan, 1992; Parthasarathy & Bryan, 2012; Stamler *et al.*, 1992).

• Nitrogen dioxide,  $\text{NO}_2$  (or its dimer,  $\text{N}_2\text{O}_4$ ), molecular weight ( $\text{NO}_2$ ) 46.01 Dalton,  $\text{N}_2\text{O}_4$  is a colorless solid, turning brown by dissociation into  $\text{NO}_2$  upon warming up, liquid at ambient temperature, melting point  $-11.2^\circ\text{C}$ , boiling point  $21.15^\circ\text{C}$ ,  $\text{N}_2\text{O}_4$  formed in the solid state only; at melting point and higher, more and more  $\text{NO}_2$  is formed.

$\text{N}_2\text{O}_4$  reacts with potassium iodide (KI) in the following manner:



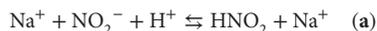
Iodide is oxidized to  $\text{I}_2$ ,  $\text{NO}_2$  is oxidized to nitrate and then reduced to NO. For this reason, the addition of iodide as an iodine-fortifying substance to salt is not possible; Iodate ( $\text{IO}_3^-$ ) must be used instead.

#### 4.4.2 Nitrite and nitrate in meat

If nitrite is added directly to meat, the salt dissolves easily, due to its good solubility in the aqueous portion of the meat matrix at  $\sim\text{pH}$  5.5. Because the  $\text{pK}_a$  of  $\text{HNO}_2$  is 3.37, at a pH of 3.37  $\sim$ 50% of the acid is dissociated. So, at pH 5.5, it can be expected that about 99% of the nitrite exists as its anion,  $\text{NO}_2^-$  (Figure 4.2). The small quantity of undissociated  $\text{HNO}_2$  is in equilibrium with its anhydride, dinitrogen trioxide ( $\text{N}_2\text{O}_3$ ), which again is in equilibrium with the two oxides of NO and  $\text{NO}_2$  (Figure 4.3).

$\text{NO}_2$  can readily react with water. In sum, this means that one  $\text{HNO}_2$  and one  $\text{HNO}_3$  molecule are generated from two  $\text{HNO}_2$  molecules (Figure 4.3). Furthermore, the NO molecule itself can easily be oxidized to  $\text{NO}_2$  in the presence of molecular oxygen. This means there is an oxygen-sequestering and thus antioxidative action of nitrite in meat batters or hams. Interestingly, this fact is mentioned as a footnote in European Parliament and Council (2006). Metal ions act as catalysts and accelerate the oxidation process. Nitrate added to meat will fully dissociate into  $\text{Na}^+/\text{K}^+ + \text{NO}_3^-$ . No detectable quantity of undissociated  $\text{HNO}_3$  will be found.

Meat with 100 ppm (mg/kg) of nitrite added =  $1.45 \times 10^{-3}$  M



pH of meat at 5.5 =  $3 \times 10^{-6}$  M of  $\text{H}^+$

$\text{pK}_a$  of nitrous acid = 3.37;  $K = 4.27 \times 10^{-4}$

So, reaction (a) lies  $>$  99% to the left of the equation at pH 5.5

Figure 4.2 Chemical state of nitrite in meat homogenates.

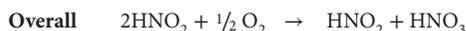
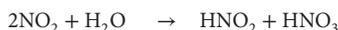


Figure 4.3 Reaction of nitrous acid and its derivatives.

## 4.5 Nitrite and nitrate in meat products

### 4.5.1 Concentration in meat products

The oxidation of nitrite to nitrate in meat also explains why nitrate will be present at considerable concentrations in meat products to which only nitrite has been added. Figures 4.4 and 4.5 show the nitrite and nitrate concentrations of German meat products. Emulsion-type and cooked sausages and cooked hams are manufactured only with sodium nitrite, but contain a mean 20–30 mg nitrate/kg, as noted in Table 4.2. In most cases, the level of nitrite in the finished product is lower than that of nitrate, at a median concentration of  $<$ 20 mg nitrite/kg. Only a few samples of cooked and raw sausages and raw ham exist with  $>$ 60 mg nitrite/kg (Figure 4.4), and these possess higher nitrate concentrations. In the raw products, nitrate may have been added; in cooked (e.g., liver and blood sausages), the ingoing nitrite is apparently strongly oxidized to nitrate by the meat's enzyme systems or metal ions, due to their high pH values of between 6.0 and 6.8.

For roughly 50 years, the employment of ascorbic acid, ascorbate, or isoascorbate (erythorbate) in cured meat formulations has been common practice in industry. Ascorbic acid and its derivatives are cure accelerators that facilitate the conversion of nitrite to NO. They are added to meat batters at  $\sim$ 500 mg/kg. Ascorbates and erythorbates are reducing agents with antioxidative activity, and they may also sequester oxygen (Figure 4.6). In this way, they retard the oxidation of NO to  $\text{NO}_2$  and the formation of nitrate. Furthermore, erythorbate seems to react with nitrite or one of its metabolites and helps maintain cured color during product storage. Dahn *et al.* (1960), Fox & Ackerman (1968), and Izumi *et al.* (1989) showed that ascorbate reacts with nitrite and binds the resulting NO. Nitrite is reduced to NO, and the ascorbate is oxidized to dehydroascorbate. The bound NO seems to be able to react as NO with other meat ingredients. Ascorbate is also added, to reduce the formation of *N*-nitrosamines; this will be discussed later.

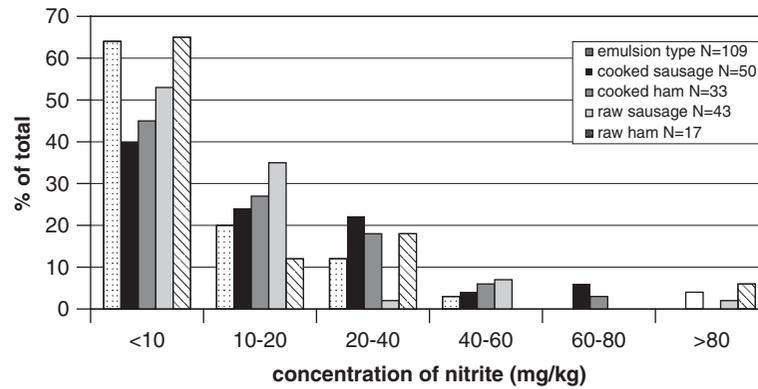


Figure 4.4 Residual nitrite levels (mg/kg) in selected German meat products from 1996 to 2001. Source: Adapted from Dederer, pers. comm.

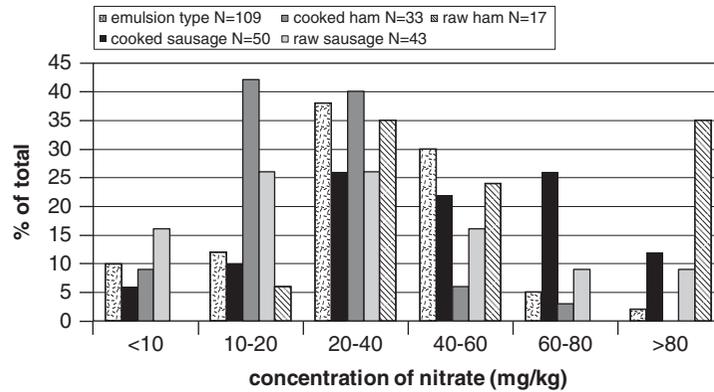


Figure 4.5 Nitrate levels (mg/kg) in selected German meat products from 1996 to 2001. Source: Adapted from Dederer, pers. comm.

Table 4.2 Nitrite and nitrate concentrations in selected German meat products (2003–2005).

	Year	n	Median concentration (mg/kg)	
			Nitrite	Nitrate
Emulsion-type sausage	2003	30	13.2	23.4
	2004	32	12.7	20.5
	2005	29	19.9	30.0
Raw sausage	2003–2005	15	17.9	59.2
Raw ham	2003–2005	14	19.2	16.9
Liver/blood sausage (cooked sausage)	2003–2005	16	12.1	43.3

Source: Adapted from Dederer, pers. comm.

Ascorbate and myoglobin are not the only chemicals to react with nitrite derivatives; amino acids (Figure 4.7) and unsaturated fatty acids can do so as well. These products are rather unstable and either release NO or are oxidized to NO<sub>2</sub> and/or nitrocompounds. Such reactions of NO with other substances are another reason for the low residual amounts of nitrite detected in ready-to-eat meat products. Following the reported reactions, it can be assumed that the concentration of nitrate in a sausage to which only nitrite was added is related to the ingoing nitrite content.

Figure 4.8 shows that with emulsion-type sausages (only nitrite curing salt was used), the residual levels 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 probably results from the water or ice added to the formulation (0–50 mg nitrate/L).

#### 4.5.2 Changes with time of storage

When does the nitrite in the product disappear? Table 4.3 provides some data from Kudryashov (pers. comm.) of the V.M. Gorbатов All-Russian Meat Research Institute. The largest decrease is observed during manufacture up to the end of the heating process. This early

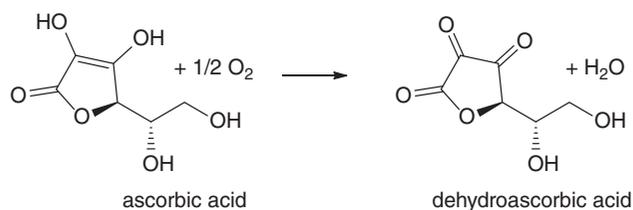


Figure 4.6 Oxygen sequestration by ascorbate (ascorbic acid).

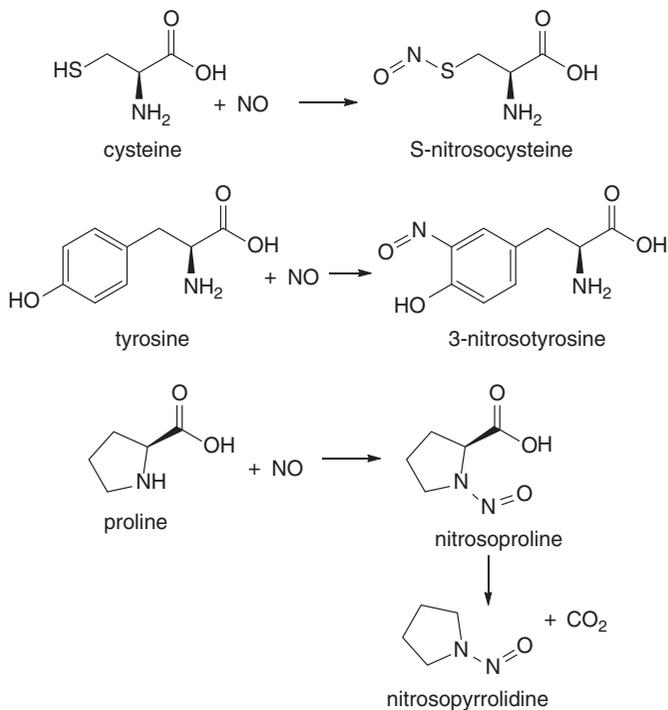


Figure 4.7 Reactions of nitric oxide (NO) with selected  $\alpha$ -amino and imino acids.

loss usually exceeds 65%, and is independent of the ingoing nitrite concentrations. Within 20 days of cold storage, the level drops further to a third of the concentration detected after heating.

The disappearance continues up to 60 days of cold storage. Table 4.4 confirms the findings. It also shows that a higher pH value helps to retard the disappearance of nitrite. Moreover, it confirms

the results of Table 4.3, which show that the nitrate level is already high at day 0 after heating. Nitrate concentrations also fall with time of storage; however, this reduction is slower with increasing pH.

Table 4.5 reports the influence of different heat treatments in meat homogenates; 100 mg nitrite/kg was added to muscles with varying pH, and the homogenate was either mildly heated (pasteurized) or sterilized. The nitrite and nitrate levels were measured immediately after heating and then 12 days into storage. The results show that the higher the heating, the greater the loss of nitrite. The formation of nitrate is also reduced. A higher pH value and/or a different muscle type show less nitrite loss and a higher nitrate concentration. The addition of ascorbate and polyphosphate indicates that the disappearance of nitrite is accelerated by the presence of ascorbate in the raw batter (Table 4.6). Thermal processing to 80 °C for 7 minutes leads to a slower loss of nitrite. Heating for an additional 1 hour at 70 °C retards the loss even longer. This is likely due to the inactivation of microorganisms and of enzymes by heat. With added ascorbate, and even more so with polyphosphates, retardation by heating is also observed.

The main loss of nitrite in meat products occurs during the heating step. The application of high pressure up to 800 MPa to cured meats can be employed to inactivate microorganisms, and by this process, the temperature never exceeds 35 °C. After ultra-high pressure (UHP) treatment, residual nitrite analyses reveal that ~20% of the ingoing nitrite is oxidized to nitrate, but the sum of the residual nitrite and nitrate levels totals nearly the amount of nitrite originally added (Table 4.7). During storage, the nitrite level decreases to ~50% of the ingoing quantity, while the nitrate level increases to ~40%. Even at 21 days of storage, the sum of nitrite plus nitrate is ~90% of the ingoing quantity of nitrite. In comparison to the results of Tables 4.3–4.5, these findings indicate that only a small amount of nitrite reacts with other substances besides oxygen to form nitrate on application of high pressure. The reason for this observation is unknown.

In all cases described here, nitrite is partially oxidized to nitrate. In many experiments (see Tables 4.4, 4.5, and 4.7), roughly 10–40% of the ingoing nitrite is so oxidized. As already mentioned, this fact has been known for decades. Only for the results of Table 4.7 does the sum of nitrite plus nitrate add up to ~90% or more of the added nitrite. Cassens *et al.* (1978) postulated that nitrite is bound to various constituents in the meat matrix, and provided some percentages based on <sup>15</sup>NO<sub>2</sub> tracer studies (Table 4.8).

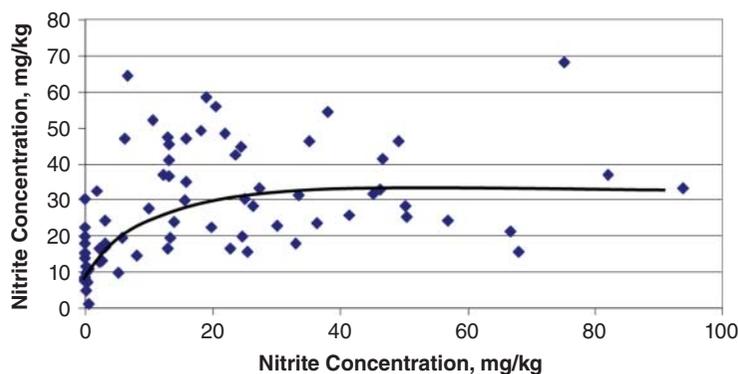


Figure 4.8 Concentration of nitrite and nitrate in emulsion-type sausages ( $n = 48$ ). Source: Adapted from Dederer, pers. comm.

**Table 4.3** Residual nitrite levels (mg/kg) determined during storage at 2 °C for an emulsion-type sausage.

Storage period	Concentration of sodium nitrite added (mg/kg)			
	75	100	150	200
Immediately 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

Source: Adapted from Kudryashov, pers. comm.

**Table 4.4** Nitrite decomposition and nitrate formation after sodium nitrite addition to meat at various pH values following heating and storage.

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

Source: Adapted from Đorđević *et al.* (1980).

**Table 4.5** Mean values of nitrite and nitrate content in pasteurized and sterilized groups of homogenates.

Homogenate, 100 mg/kg NaNO <sub>2</sub> added	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
<i>M. longissimus dorsi</i>	P	38.6	27.4	10.9	7.9
pH 5.8	S	12.9	13.1	3.5	6.0
<i>M. quadriceps femoris</i>	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 at 75 °C; S, sterilization at >110 °C.

Source: Adapted from Đorđević *et al.* (1980).

#### 4.5.3 Natural, “nitrate/nitrite-free” cured meat products

Due to the negative perceptions of nitrite-cured meats held by some consumers, there has been an interest of late in so-called “nitrate/nitrite-free” natural meat products. Any traditionally cured product produced in the United States that does not include the addition of a “chemically-derived” form of nitrite is labeled as uncured. The addition of nitrate/nitrite to meat products is responsible for many of the distinctive properties of cured meats: it develops a characteristic color and flavor, for which there is no

known substitute; without it, natural processed meat products appear brown and their flavor is less desirable. The USDA permits the manufacture of uncured versions of typical cured meats according to the Code of Federal Regulations Title 9, CFR § 317.17 and 319.2, but the statement “no nitrate or nitrite added” must be placed adjacent to the product name. Such products do exist in the marketplace, but are not that popular.

In order to circumvent nitrite regulation and labeling issues, in the late 1990s a group of products with traditional cured-meat characteristics were prepared through the use of sea salt and

**Table 4.6** Approximate number of days required for the residual nitrite level to fall below 10 mg/kg in a pork slurry of pH 5.5–6.2 with 2.5–4.5% sodium chloride at a storage temperature of 15 °C ( $n = 5$ ).

Nitrite added (mg/kg)	Heat treatment		
	Unheated days	80 °C/7 minutes days	80 °C/7 minutes + 70 °C/1 hour days
<i>(i) No further addition</i>			
100	5	12	63
200	10	12	68
300	21	21	>168
<i>(ii) Ascorbate (1000 mg/kg)</i>			
100	5	9	10
200	5	9	9
300	5	21	48
<i>(iii) Ascorbate (1000 mg/kg) + polyphosphate 0.3% (w/v)</i>			
100	5	10	21
200	10	21	21
300	5	5	12

Source: Adapted from Gibson *et al.* (1984). Reproduced with permission of Wiley.

**Table 4.7** Nitrite and nitrate concentrations in a Lyoner (an emulsion-type sausage, 72 ppm nitrite added) after UHP application and storage of the unheated batter.

Days	0				7				14				21			
	Nitrite level (mg/kg)				Nitrate level (mg/kg)				Nitrite + nitrate level (mg/kg)							
Control	54.3	47.1	42.8	39.3	15.15	19.5	23.8	26.2	69.45	66.55	66.6	65.5				
400 MPa	53.3	46.4	41.5	37.7	15.8	22	25.9	26.95	69.05	68.4	67.4	64.6				
600 MPa	53.0	44.8	40.7	37.2	16.15	23.85	27.35	29.1	69.1	68.65	68.0	66.25				
800 MPa	52.3	44.7	41.6	37.7	17.5	23.95	26.55	26.6	69.75	68.65	68.15	64.25				

Source: Adapted from Ziegenhals, pers. comm.

vegetable juice/concentrate/powder high in naturally-occurring nitrates (e.g., celery has nitrate levels typically ranging from 1500 to 2800 ppm, while celery juice powder has been reported to contain ~27 500 ppm or 2.75% nitrate), in combination with a nitrate-reducing starter culture (e.g., *Kocuria varians*, *Staphylococcus xylosum*, *Staphylococcus carnosus*). The nitrate-reducing bacteria convert nitrate to nitrite and thereby “indirectly” cure the meat product. The labeling of these products as uncured is viewed by some consumers as incorrect at best and deceptive at worst (Sebranek *et al.*, 2012); such labeling is required for most naturally cured items, in order to meet USDA requirements.

Even though modern technology has developed concentrated vegetable extracts from celery (*Apium graveolens* var. *dulce*) with nitrate concentrations of ~3% and efficient strains of nitrate-reducing bacteria, the process still requires an incubation step at 38–42 °C to allow adequate formation of nitrite by the culture in the meat matrix prior to thermal processing. In order to overcome the incubation period in real-time processing, processors began to incubate the celery juice with the culture prior to its addition to the

meat product (Krause *et al.*, 2011; Terns *et al.*, 2011). This practice was extended to suppliers of the celery concentrate, who began to market preconverted nitrite from nitrate.

Processors today use both preconverted nitrates (already converted to nitrite) from celery powder and unconverted celery powder with a starter culture containing a nitrate-reducing enzyme. This practice has resulted in a category of processed meats in the United States that is labeled confusingly and perhaps even misleadingly to the consumer. Moreover, the protection against spore germination of *Clostridium botulinum* afforded by the addition of nitrite to meat products is potentially compromised in these uncured products, because the conversion of the nitrate present in celery to nitrite is not a well-controlled reaction. Sebranek *et al.* (2012) quite correctly point out that the addition of a celery concentration or powder containing 3.0% (30 000 ppm) nitrate to meat at a typical use concentration of 0.3% will result in 90 ppm of nitrite, at most, in the meat product (assuming a 100% conversion of nitrate to nitrite, which is not realistic). Furthermore, because preconverted celery products contain roughly 15 000–20 000 ppm nitrite, the

**Table 4.8** Forms of nitrite and its reaction products detected in meat products.

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

<sup>a</sup>Assumption by Honikel according to results presented in Figures 4.4, 4.5, and 4.6.

Source: Adapted from Cassens *et al.* (1978). Reproduced by permission of Oxford University Press.

addition of 0.3% of this to a meat formulation is equivalent to only 45–60 ppm of nitrite. This raises questions over the product's microbiological safety in vacuum-packed bags. For this reason, the USDA is very concerned about chilling rates for finished packaged products. A processor manufacturing a “naturally” cured product must follow the restrictive chilling regime outlined in Appendix B of the USDA meat regulations.

For the most part, the processing procedures involved in natural curing are similar to those using sodium nitrite. Nitrate is more stable than nitrite, so sufficient time to allow the starter culture to reduce exogenous nitrate to nitrite is required. If preconverted nitrite is not being used, the time required depends on a number of factors, including temperature, pH, the growth cycle of the starter culture (i.e., number of microorganisms), and the level of nitrate in the added celery powder. A good distribution of the ingredients is essential to ensure a uniform cure in such products. If dry, the vegetable powder (i.e., the natural nitrate source) is typically blended into the dry seasonings for comminuted products or added directly to curing brines. The starter culture is often first mixed with water before being added to comminuted products, and then dispersed via agitation for optimal distribution in the meat product. The USDA allows a maximum 0.5% combined water and starter culture without the need to include the added water on the label. The finished product, however, must bear a label disclaimer such as “no nitrates or nitrites added, except for that which occurs naturally in celery juice powder.”

Finally, to maintain the image of “naturally cured” products, processors often include a clean-label alternative to sodium erythorbate as a cure accelerator in their formulations. As described earlier, ascorbic acid/sodium ascorbate/sodium erythorbate facilitates the conversion of nitrite to NO for cured color development. Typical natural sources of ascorbic acid employed by the industry include cherry powder and acerola (*Malpighia emarginata* DC). Acerola, also known as Barbados cherry and West Indian cherry, contains markedly high levels of ascorbic acid, typically 4–5%, as well as a number of phenolics and polyphenolics; these provide potent antioxidants in the fruit extracts/powders (Mezadri *et al.*, 2008).

## 4.6 Nitrosomyoglobin (NOMB)

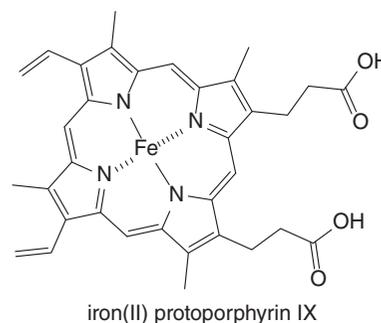
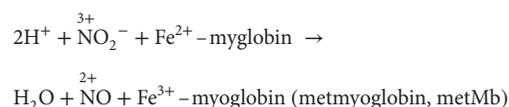
The pink to red color of thermally-processed cured meat products is one of the important effects stemming from the addition of nitrite to meat. This color is developed through a number of complicated reaction steps, culminating in the formation of nitrosomyoglobin (Fe<sup>2+</sup>) (Pegg & Shahidi, 2000).

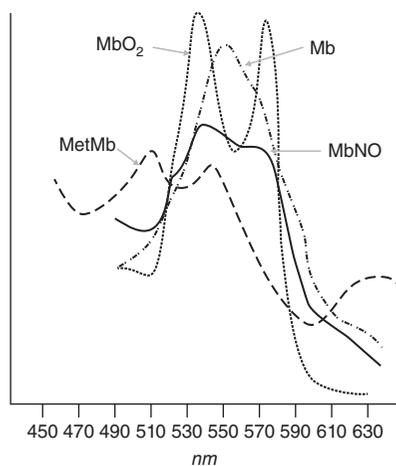
Myoglobin is a globular protein that is made up of a single polypeptide chain consisting of ~153 amino acids and a prosthetic heme group, an iron(II) protoporphyrin-IX complex (Figure 4.9). Myoglobin exists in muscle tissue in three states: (i) one in which the cofactor heme binds different ligands; (ii) one in which the iron exists in the ferrous (Fe<sup>2+</sup>) state; and (iii) one in which the iron exists in the ferric (Fe<sup>3+</sup>) state. In native myoglobin, the heme moiety is held in a cleft of the globin protein by a coordinate bond between the imidazole nitrogen of the proximal histidine residue and the Fe(II) ion, and by several nonpolar and hydrogen-bonding interactions at the porphyrin periphery.

In the purple–red deoxy form, the Fe<sup>2+</sup> in the porphyrin cofactor of myoglobin does not bind any ligand, except perhaps a water molecule. In the presence of air, oxygenation takes place and the porphyrin binds with O<sub>2</sub> and becomes a bright cherry red. The iron ion of oxymyoglobin (MbO<sub>2</sub>) exists in the reduced Fe<sup>2+</sup> state, but over time oxygen itself and other oxidizing agents (such as nitrite) oxidize the Fe<sup>2+</sup> to Fe<sup>3+</sup> (Figure 4.10), generating metmyoglobin (metMb), which is brown in appearance.

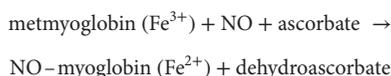
The pigments deoxy-myoglobin (Mb), MbO<sub>2</sub>, and metMb can all be found in fresh meat. In the muscle of a live animal, there is very little metMb, but its content increases post mortem with the disappearance of O<sub>2</sub>, except when the meat product is modified atmosphere packaging (MAP)-packed with high O<sub>2</sub>.

The three states of myoglobin each have distinctive absorbance spectra in the visible region of the electromagnetic spectrum between 400 and 700 nm. Because the three exist in a kind of equilibrium with one another, the spectra have an isobestic point at λ = 525 nm, where all three absorption curves cross each other.

**Figure 4.9** Iron(II) protoporphyrin IX—the heme moiety in myoglobin.**Figure 4.10** Reaction of nitrite with myoglobin in meat at pH 5.5.



**Figure 4.11** Visible spectra of various chemical forms of myoglobin. Mb, deoxy-myoglobin; MbO<sub>2</sub>, oxymyoglobin; MetMb, metmyoglobin; MbNO, nitrosomyoglobin.



**Figure 4.12** Formation of nitrosomyoglobin (NOMb).

Based on developed equations, the absorbance at this wavelength can be used to determine the percentage of each form of myoglobin in the meat at a given point. Nitrosomyoglobin (NOMb) has a spectrum with similar maxima to those of MbO<sub>2</sub> (Figure 4.11).

Both O<sub>2</sub> and NO are diatomic and paramagnetic molecules. Carbon monoxide, CO, a similar diatomic molecule, also binds tightly to myoglobin. In some countries, MAP packaging of ready-case meat products with 1–2% CO is permitted.

Through the action of endogenous reducing enzymes of the meat matrix or chemical reactions with a reductant like erythorbate, the Fe<sup>3+</sup> is reduced to Fe<sup>2+</sup> (Figure 4.12). The NO generated from N<sub>2</sub>O<sub>3</sub> can bind to myoglobin (Fe<sup>2+</sup>), which forms a heat-stable NOMb. MbO<sub>2</sub> is not heat-stable and dissociates, allowing the meat to turn gray or brown. On heating, the protein moiety of NOMb denatures,

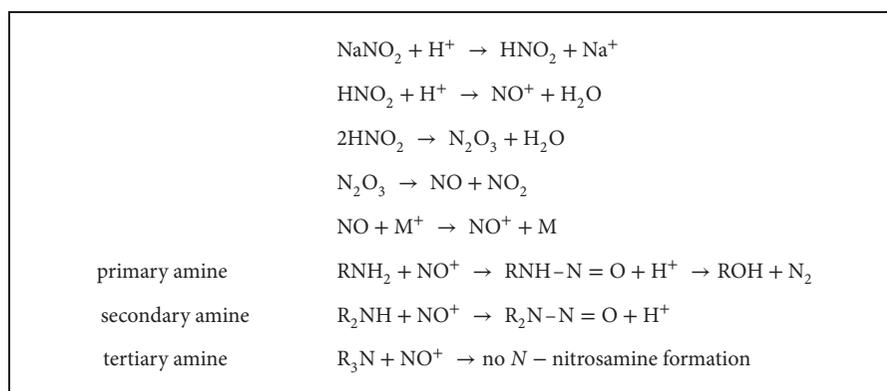
but the red NO–porphyrin ring system (often called mononitrosyl hemochromogen or nitrosohemochromogen) remains intact, and it is found in meat products heated to 120 °C. The heat-stable red color will change in appearance as a result of bacterial spoilage and will fade upon exposure to UV light.

Nearly 10 years ago, the riddle concerning the red color of non-nitrite/nitrate-cured raw hams such as Parma ham and jamón ibérico was solved by Japanese researchers using electrospray ionization high-resolution mass spectrometry (Wakamatsu *et al.*, 2004a). Since then, various authors have confirmed the finding and shown that the ferrous ion in the porphyrin ring of heme is exchanged with Zn<sup>2+</sup> during the long ripening period, which gives the products a stable bright red color. Nitrite addition prevents this exchange from happening (Adamsen *et al.*, 2006; Møller *et al.*, 2003; Parolari *et al.*, 2003; Wakamatsu *et al.*, 2004b).

#### 4.7 N-nitrosamine formation

During the 1970s, discussions arose in the United States concerning the formation of N-nitrosamines in nitrite-cured meat products, especially fried bacon. Fiddler *et al.* (1978) showed that bacon and its cookout on frying contained considerable amounts of N-nitrosopyrrolidine and some N-nitrosodimethylamine.

N-nitrosamines constitute a family of potent carcinogens that are formed readily from a diverse set of nitrogen-containing compounds and from nitrite and its derivatives. In meat, they are produced by the reaction of secondary amines with nitrite at high temperatures, according to the reaction schemes depicted in Figure 4.13. A number of prerequisites must be met in order for N-nitrosamines to be generated: (i) amines must be present—in fresh meat, trivial quantities of amines can be found, including creatine, creatinine, proline, hydroxyproline, and decarboxylated amino acids; by and large, aging and the fermentation of meat products will generate more; (ii) secondary amines must exist to form stable N-nitrosamines (Figure 4.13)—primary amines are immediately degraded to alcohol and nitrogen, while tertiary amines cannot react with nitrite at all; unlike in fish, most amines in meat are primary amines, derived from α-amino acids; and (iii) the pH must be sufficiently low to produce NO<sup>+</sup>, or else metal ions must be engaged to form NO<sup>+</sup> (Figure 4.13).



**Figure 4.13** Reaction pathways for the formation of N-nitrosamines in cured meat products. M and M<sup>+</sup> represent transition-metal ions such as Fe<sup>2+</sup> and Fe<sup>3+</sup>.

**Table 4.9** *N*-nitrosodimethylamine content ( $\mu\text{g}/\text{kg}$ ) in selected food products.

Food	<i>n</i>	Content		
		>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

Source: Adapted from Deierling *et al.* (1997). Reproduced with permission of WILEY-VCH Verlag GmbH & Co. KGaA.

When heated meat products are prepared from fresh meat (chilled or frozen), no amines are available. In raw nitrate-cured meat products, the nitrite concentration is rather low (see Figure 4.4), so the formation of  $\text{NO}^+$  is quite unlikely. In products heated above  $130^\circ\text{C}$ , *N*-nitrosamines can form. Frying of bacon, grilling of cured sausages, and frying of cured meat products such as pizza toppings may all provide the conditions leading to *N*-nitrosamines formation. Table 4.9 shows the results of an investigation into the *N*-nitrosamine content of such foods by Deierling *et al.* (1997); in German foods, only beer and pizza exhibit *N*-nitrosodimethylamine at detectable  $\mu\text{g}/\text{kg}$  levels. Thus, *N*-nitrosamines occur only in minute quantities, and they are easily avoidable by proper frying, grilling, and pizza baking. Jakszyn *et al.* (2004) prepared a database of *N*-nitrosamine levels in foods, together with levels of heterocyclic amines and polycyclic aromatic hydrocarbons.

#### 4.8 Conclusion

The curing of meat is a process that predates written history. The main curing agents, nitrite and nitrate, react with components of the meat matrix and added adjuncts because of the varying oxidation status of nitrogen in these additives. Nitrite is multifunctional, in that it is responsible for the typical color and flavor associated with cooked cured meat, it acts as an antioxidant, preventing the development of warmed-over flavor, and it retards the formation of the *Clostridium botulinum* toxin in combination with NaCl in vacuum-packaged products. The positive effects attributed to nitrite and nitrate addition in meat products overwhelm the low possibility of *N*-nitrosamine formation. Moreover, the intake of curing agents (i.e., nitrate and nitrite) from meat products in the daily diet is minor (only a few per cent) in comparison with other foods.

#### References

- Adamsen CE, Møller JKS, Laursen K, Olsen K, Skibsted LH. 2006. Zn-porphyrin formation in cured meat products: effect of added salt and nitrite. *Meat Science*, 72, 672–679.
- Das Bundesgesetzblatt. 1982. Verordnung über Fleisch und Fleischerzeugnis (Fleischverordnung) in der Fassung der Bekanntmachung vom 21. Bundesgesetzblatt I, 3, 89–101.
- Cassens RG, Ito T, Lee M, Buege D. 1978. The use of nitrite in meat. *Bio-science*, 28, 633–637.
- Dahn H, Loewe L, Bunton CA. 1960. Über die Oxydation von Ascorbinsäure durch saltpetrig Säure. Teil VI: Übersicht und Diskussion der Ergebnisse. 18. Mitteilung über Reduktone und 1,2,3-Tricarbonsäureverbindungen. *Helvetica Chimica Acta*, 43, 320–333.
- Deierling H, Hemmrich U, Groth N, Taschan H. 1997. Nitrosamine in Lebensmitteln. *Lebensmittelchemie*, 51, 53–61.
- Dorđević V, Vuksan B, Radetić P, Đurđić H, Mitković M. 1980. Prilog ispitivanju uticaja pojedinih faktora na promene sadržaja nitrita u mesu. *Tehnologija Mesa* 21, 10, 287–290.
- European Parliament and Council. 1995. European Parliament and Council Directive 95/2/EC. Official Journal of the EU, L61, 18.3.1995, Food Additives Other than Colours and Sweeteners, p. 1–40.
- European Parliament and Council. 2006. Directive 2006/52/EC of the European Parliament and of the Council of July 5, 2006, amending Directive 95/2/EC on Food Additives Other than Colours and Sweeteners and Directive 95/35/EC on Sweeteners for Use in Foodstuffs, Official Journal of the EU, L204, 26.7.2006.
- FDA. 2012. United States Food and Drug Administration, Code of Federal Regulations (USA). Title 21, Volume 3, Revised April 1, Food and Drugs. Food Preservatives, 21CFR § 172.175.
- Fiddler W, Pensabene JW, Piotrowski EG, Phillips JG, Keating J, Mergens WJ, Newmark HL. 1978. Inhibition of formation of volatile nitrosamines in fried bacon by the use of cure-solubilized  $\alpha$ -tocopherol. *Journal of Agricultural and Food Chemistry*, 26, 653–656.
- Fox JB Jr., Ackerman SA. 1968. Formation of nitric oxide myoglobin: mechanisms of the reaction with various reductants. *Journal of Food Science*, 33, 364–370.
- Gibson AM, Roberts TA, Robinson A. 1984. Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized cured meats. VI. Nitrite monitoring during storage of pasteurized pork curries. *International Journal of Food Science & Technology*, 19, 29–44.
- Haldane J. 1901. The red colour of salted meat. *Journal of Hygiene*, 1901, 115–122.
- Hoagland R. 1910. The action of saltpeter upon the color of meat. In: 25th Annual Report of the Bureau of Animal Industry, US Department of Agriculture. Government Printing Office, Washington, DC, pp. 301–316.
- Hoagland R. 1914. Coloring matter of raw and cooked salted meats. *Journal of Agricultural Research*, 3, 211–225.
- Ignarro LJ. 1990. Biosynthesis and metabolism of endothelium-derived nitric oxide. *Annual Review of Pharmacology and Toxicology*, 30, 535–560.
- Izumi K, Cassens RG, Greaser ML. 1989. Reaction of nitrite with ascorbic acid and its significant role in nitrite-cured food. *Meat Science*, 26, 141–153.
- Jakszyn P, Agudo A, Ibáñez R, García-Closas R, Pera G, Amiano P, González CA. 2004. Development of a food database of nitrosamines, heterocyclic amines, and polycyclic aromatic hydrocarbons. *Journal of Nutrition*, 134, 2011–2014.
- Kisskalt K. 1899. Beiträge zur Kenntnis der Ursachen des Rothwerdens des Fleisches beim Kochen, nebst einigen Versuchen über die Wirkung der schwefligen Säure auf die Fleischfarbe. *Archiv für Hygiene*, 35, 11–18.
- Krause BL, Sebranek JG, Rust RE, Mendonca A. 2011. Incubation of curing brines for the production of ready-to-eat, uncured, non-nitrite-or-nitrate-added, ground, cooked and sliced ham. *Meat Science*, 89, 507–513.
- Lehmann KB. 1899. Über das Haemorrhodin, ein neues weitverbreitetes Blutfarbstoffderivat. *Sitzungs-Berichte der Physikalisch-medicinischen Gesellschaft zu Würzburg*, 48, 57–61.
- Lovtidende. 1995. Danish regulation (1995 A), p. 5571 of 18.12.1995.
- Lowenstein CJ, Snyder SH. 1992. Nitric oxide, a novel biologic messenger. *Cell*, 70, 705–707.
- Lüscher TF. 1990. Endothelial control of vascular tone and growth. *Clinical and Experimental Hypertension. Part A, Theory and Practice* 1990, 12, 897–902.

- Lüscher TF. 1992. Endogenous and exogenous nitrates and their role in myocardial ischaemia. *British Journal of Clinical Pharmacology*, 34, 29S–35S.
- Marletta MA. 1989. Nitric oxide: biosynthesis and biological significance. *Trends in Biochemical Sciences*, 14, 488–492.
- Mezadri T, Villaño D, Fernández-Pachón MS, García-Parrilla MC, Troncoso AM. 2008. Antioxidant compounds and antioxidant activity in acerola (*Malpighia emarginata* DC.) fruits and derivatives. *Journal of Food Composition and Analysis*, 21, 282–290.
- Møller JKS, Adamsen CE, Skibsted LH. 2003. Spectral characterisation of red pigment in Italian-type dry-cured ham. Increasing lipophilicity during processing and maturation. *European Food Research and Technology*, 216, 290–296.
- Moncada S, Palmer RMJ, Higgs EA. 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacological Reviews*, 43, 109–142.
- Nathan C. 1992. Nitric oxide as a secretory product of mammalian cells. *FASEB Journal*, 6, 3051–3064.
- Parolari G, Gabba L, Saccani G. 2003. Extraction properties and absorption spectra of dry cured hams made with and without nitrite. *Meat Science*, 64, 483–490.
- Parthasarathy DK, Bryan NS. 2012. Sodium nitrite: the “cure” for nitric oxide insufficiency. *Meat Science*, 92, 274–279.
- Pegg, RB, Shahidi F. 2000. Nitrite Curing of Meat. The N-Nitrosamine Problem and Nitrite Alternatives. Trumbull, CT: Food & Nutrition Press.
- Polenske E. 1891. Über den Verlust, welchen das Rindfleisch und Nährwert durch das Pökeln erleidet, sowie über die Veränderungen Salpeter-haltiger Pökellaken. *Arbeiten aus dem Kaiserlichen Gesundheitsamte*, 7, 471–474.
- Schuddeboom LJ. 1993. Nitrates and Nitrites in Foodstuffs. Council of Europe Press, Publishing and Documentation Service.
- Sebranek JG, Jackson-Davis AL, Myers KL, Lavieri NA. 2012. Beyond celery and starter culture: advances in natural/organic curing processes in the United States. *Meat Science*, 92, 267–273.
- Stamler JS, Singel DJ, Loscalzo J. 1992. Biochemistry of nitric oxide and its redox-activated forms. *Science*, 258, 1898–1902.
- Terns MJ, Milkowski AL, Claus JR, Sindelar JJ. 2011. Investigating the effect of incubation time and starter culture addition level on quality attributes of indirectly cured, emulsified cooked sausages. *Meat Science*, 88, 454–461.
- Wakamatsu J, Nishimura T, Hattori A. 2004a. A Zn-porphyrin complex contributes to bright red color in Parma ham. *Meat Science*, 67, 95–100.
- Wakamatsu J, Okui J, Ikeda Y, Nishimura T, Hattori A. 2004b. Establishment of a model experiment system to elucidate the mechanism by which Zn-protoporphyrin IX is formed in nitrite-free dry-cured ham. *Meat Science*, 68, 313–317.