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The occurrence of *N*-nitrosamines, residual nitrite and biogenic amines in commercial dry fermented sausages and evaluation of their occasional relation



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ABSTRACT

Regarding food borne intoxications, the accumulation of biogenic amines must be avoided in all kinds of food products. Moreover, biogenic amines can function as precursors for the formation of carcinogenic *N*-nitrosamines when nitrite is present. To estimate the food safety of the dry fermented sausages available on the Belgian market, a screening of the residual sodium nitrite and nitrate contents, biogenic amines and volatile *N*-nitrosamine concentrations was performed on 101 samples. The median concentrations of residual NaNO₂ and NaNO₃ were each individually lower than 20 mg/kg. In general, the biogenic amine accumulation remained low at the end of shelf life. Only in one product the amounts of cadaverine and putrescine reached intoxicating levels. Concerning the occurrence of *N*-nitrosamines, only *N*-nitrosopiperidine and *N*-nitrosomorpholine were detected in a high number of samples (resp. 22% and 28%). No correlation between the presence of *N*-nitrosamines and the biogenic amines content was observed. Although the *N*-nitrosamines could not be linked to specific product categories, the occurrence of *N*-nitrosopiperidine could probably be attributed to the use of pepper.

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1. Introduction

Fermentation and drying of meat have been used for centuries to prevent the spoilage at room temperature. In fact, the manufacturing of sapid and shelf stable dry fermented sausages became an art and provided the market with a very diverse range of delicacies. The variety in process parameters and meat batter formulations, such as the fresh meat origin and used spices, is endless. In Europe a distinction can roughly be made between Northern and Southern types of fermented sausages. On the one hand, Mediterranean products (Southern types) are characterized by a long ripening time, which results in a low water activity ($a_w < 0.90$) and a rich aromatic profile. These products, e.g. Italian Milano Salami and French Rosette, are often covered with molds which

contribute to the specific flavor, due to proteolysis. On the other hand, Northern dry fermented sausages have a distinctive acidification step and a shorter drying period, resulting in a final pH between 4.5 and 5.0, and higher water activity ($a_w > 0.90$). Instead of covering the surface with molds, the surface of these sausages is smoked, e.g. Dutch Boerenmetworst and German Cervelatwurst, to prevent undesired yeast growth. In most cases pork meat is used, while combinations of pork and beef can be found in types like Salami d'Ardennes and horse meat in Boulogne. Furthermore, for religious considerations turkey is used to provide halal products. Besides the application of different process parameters and/or meats to obtain a characteristic taste, extraordinary seasonings, for instance, sweet paprika in Spanish Chorizo, or excessive amounts of spices, like black pepper in pepper salami, are often added (Toldrá, 2007).

Regardless all differences in formulations, preservatives, like nitrite (KNO₂/E249 and NaNO₂/E250) and indirectly nitrate (NaNO₃/E251 and KNO₃/E252), are generally used to avoid the growth of putrefactive and pathogenic bacteria, like *Clostridium botulinum*. Moreover, nitrite will serve as processing adjuvant in the sense that it promotes the color formation, delays lipid oxidation and gives the product a typical cured meat flavor. Despite the given advantages, excessive intake of nitrite can induce methaemoglobinemia. Therefore the addition of nitrite and

Abbreviations: CAD, cadaverine; HIS, histamine; LOD, limit of detection; LOQ, limit of quantitation; MAOI, monoaminoxidase inhibitor; NDBA, *N*-nitrosodibutylamine; NDEA, *N*-nitrosodiethylamine; NDMA, *N*-nitrosodimethylamine; NMOR, *N*-nitrosomorpholine; NPIP, *N*-nitrosopiperidine; NPYR, *N*-nitrosopyrrolidine; PUT, putrescine; SPD, spermidine; SPM, spermine; TEA, thermal energy analyzer; TYR, tyramine.

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nitrate in meat products is restricted to 150 mg/kg for each additive (Official Journal of the EU, 2006). Nitrite can also be converted to the nitrosating agent NO^+ which can react with biogenic amines to form carcinogenic *N*-nitrosamines (Honikel, 2008).

Biogenic amines are basic nitrogenous compounds, formed by decarboxylation of free amino acids. They can occur in dry fermented products in high concentrations since their accumulation is mainly related to the action of decarboxylase-positive bacteria and meat enzymes during the fermentation and ripening. Thereby, during the sausage processing, other intrinsic and extrinsic factors, such as salt content and drying parameters, are influencing the biogenic amine formation rate (Suzzi & Gardini, 2003). The wide range in product formulations and hygienic practices is reflected in a great variety of types and amounts of biogenic amines. In view of food safety, elevated concentrations of biogenic amines must be avoided. For instance, histamine (HIS) and tyramine (TYR) may induce migraines and hypertensive crises in sensitive individuals (Latorre-Moratella et al., 2008). Moreover, putrescine (PUT) and cadaverine (CAD) are known to intensify the adverse effects of HIS and TYR as they compete for some of the mechanisms involved in their detoxification (Bardocz, 1995). In addition to the direct toxicological effects, it was proven that the biogenic amines (PUT and CAD) and the natural polyamines spermine (SPM) and spermidine (SPD) can be precursors of the carcinogenic *N*-nitrosamines during the heating of meat products (Drabik-Markiewicz et al., 2011). These amines successively undergo deamination and cyclization to secondary amines before reacting with the nitrosating agent (NO^+). In this way CAD can be converted to *N*-nitrosopiperidine (NPIP), while PUT, SPM and SPD can form *N*-nitrosopyrrolidine (NPYR) (Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués, Mariné-Font, & Vidal-Carou, 1997).

The concern about the presence of *N*-nitrosamines in meat products finds its origin in the late 1960s, early 1970s, when high concentrations of *N*-nitrosodimethylamine (NDMA) and NPYR were detected in fried bacon. To control this contamination, the amount of added nitrite was limited and the use of nitrite-scavenging anti-oxidants like ascorbate was introduced in the meat production (Gray & Dugan, 1975). However, in the period 1980–1986, meat and meat products contributed for approximately 80% to the total volatile *N*-nitrosamine intake of Swedish food products (Österdahl, 1988). In the same period, a survey of several foods present on the Italian market showed that all of the four analyzed salami samples, were contaminated with various amounts of *N*-nitrosamines, e.g. NDMA (0.59–7.76 $\mu\text{g}/\text{kg}$), *N*-nitrosodimethylamine (NDEA, nd – 4.04 $\mu\text{g}/\text{kg}$), *N*-nitrosodibutylamine (NDBA, 0.73–50.12 $\mu\text{g}/\text{kg}$) and NPIP (nd – 0.38 $\mu\text{g}/\text{kg}$) (Gavinelli, Fanelli, Bonfanti, Davoli, & Airoidi, 1988). More recently lower, *N*-nitrosamine levels in several meat products have been reported (Domanska & Kowalski, 2003; Yurchenko & Mölder, 2007).

The aim of this study was to examine the safety of dry fermented sausages available on the Belgian market, regarding the occurrence of nitrite, nitrate, biogenic amines and volatile *N*-nitrosamines. In addition, the data was explored by multivariate methods, more specifically principal component analysis (PCA) and hierarchical cluster analysis (HCA) in order to search for patterns in the occurrence of biogenic amines and *N*-nitrosamines in the commercial meat products, and their relation with physical and chemical characteristics.

2. Materials and methods

2.1. Samples

A total of 101 dry fermented sausages were collected in spring 2011 representing the current market supply of 4 major food stores in Belgium. In stores 1 and 2, classified as supermarkets, promoting A-brands, 38 and 33 samples, respectively, were purchased. In stores 3 and 4, two discount stores selling low-budget products, the range was restricted to 22 and 8 products, respectively. The dry fermented sausages were categorized by label information. The products were stored at 7 °C until the end of

shelf life. After the storage period, the whole sample was minced and homogenized in a 2096 Homogenizer (FOSS, Hillerød, Denmark) and divided into portions to perform the analyses.

2.2. Physicochemical analyses

The pH of the minced sausage samples was measured by inserting the glass pH electrode (KnickPortamess, Elscolab, Terschuur, The Netherlands). The water activity (a_w) was determined using an electronic hygrometer (Aqualab, Decagon Devices, Pullman, USA). Moisture content was determined by drying a homogenized test portion to constant mass at 103 °C (ISO 1442, 1997). Subsequently the dried test portion was extracted with *n*-hexane in order to determine the free fat content (ISO 1444, 1996). The protein content was estimated from the amount of nitrogen measured by the Kjeldahl method (ISO 937, 1978). Salt percentage, expressed as % NaCl, was determined by the argentometric method (Gros, Bruttel, & von Kloeden, 2005) using an automatic titrator (Titrimo, Metrohm, Herisau, Switzerland). All reported values represent the mean of three random measurements of the sausage sample.

2.3. Residual nitrite and nitrate determination

Aliquots (1 g) of the dry fermented sausage samples were extracted by adding 10 ml hot ultra-pure water followed by incubation for 15 min in a hot water bath (ED-19, Julabo, Allentown, USA) at 55 °C. Subsequently, the mixture was centrifuged (Heraeus Labofuge 200, Thermo Fisher Scientific, Waltham, USA) for 10 min at 1000 g. The supernatant was deproteinized with 5 ml acetonitrile (HPLC grade, Thermo Fisher Scientific) and incubated at 4 °C to solidify the fat for easy removal. The extracts were filtered through an Acrodisc syringe filter Gx/F/Glass (\varnothing 25 mm, Pall, Zaventem, Belgium) and the volume adjusted to 25.0 ml with ultra-pure water. After filtration through a 0.2 μm filter (Acrodisc GHP), 20 μl was injected onto the HPLC-UV (La Chrom Elite, VWR-Hitachi, Darmstadt, Germany). Nitrite and nitrate were separated by ion chromatography using a Hamilton PRP-X100 column (150 mm, 4.6 mm i.d., Grace Davison Discovery Sciences, Lokeren, Belgium) and isocratic elution with $\text{NaCl}/\text{NaH}_2\text{PO}_4$ (40 mM/2 mM) at a flow rate of 2.5 ml/min. The detection of the ions was carried out at 210 nm. The nitrite and nitrate levels were expressed as residual NaNO_2 and NaNO_3 , respectively.

2.4. Biogenic amine determination

The biogenic amines were analyzed as described earlier (De Mey et al., 2012). Summarized, a 2-g aliquot of minced meat sample was spiked with 0.8 mg/kg 1,7-diaminoheptane as internal standard (Sigma Aldrich, Schnelldorf, Germany) and mixed with 25 ml 0.4 M HClO_4 (VWR International). After homogenization (Ultra-Turrax T18 homogeniser, IKA, Staufen, Germany) the extract was in succession incubated at 4 °C, centrifuged and filtered. Subsequently, a 2-ml portion of the supernatants was alkalized and buffered before derivatization with 4 ml dabsyl-chloride solution (5 mg dabsyl-chloride per 1 ml acetonitrile, Sigma Aldrich, Schnelldorf, Germany). After incubation for 20 min at 70 °C, a solid phase extraction on a C18 cartridge (Grace Davison Discovery Sciences) was performed where 0.4 M HClO_4 and acetonitrile were used as washing and elution solvents, respectively. The biogenic amines, i.e. tryptamine (TRYP), phenethylamine (PHE), putrescine (PUT), cadaverine (CAD), histamine (HIS), serotonin (SER), tyramine (TYR), spermine (SPM) and spermidine (SPD), were separated and detected by RP-HPLC-UV. Two Chromolith C18e columns (each 100 \times 3 mm i.d., VWR International) were coupled and gradient elution was performed at a flow rate of 1 ml/min. The gradient profile was the following: mobile phase B (methanol-acetonitrile, 25/75, v/v) was increased from 0% to 40% in 8 min. Subsequently, an increase to 60% in 5 min, raise to 100% in 7 min, 100% for 5 min, decrease to 0% in 2 min, and finally

equilibration at 100% mobile phase A (water–methanol–acetonitrile; 50/12.5/37.5, v/v/v) for 3 min. The detection was carried out at 450 nm.

2.5. *N*-nitrosamine determination

The volatile *N*-nitrosamines were analyzed according to the method of Drabik-Markiewicz et al. (2009). Meat samples (50 g) were spiked with 10 µg/kg *N*-nitrosodipropylamine (NDPA, Sigma Aldrich) and mixed with 200 ml 3 N KOH (VWR International). The volatile *N*-nitrosamines were then extracted from the meat samples by means of vacuum distillation (Heidolph Laborota 4010-digital, Schwabach, Germany). After addition of 4 ml 37% HCl (VWR International), the distillate (150 ml) was extracted three times with 50 ml of dichloromethane (DCM). Subsequently the extract was concentrated to 100 µl in a Kuderna–Danish apparatus (Sigma Aldrich). For the detection and quantification of *N*-nitrosamines, i.e. *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosopiperidine (NPIP), *N*-nitrosopyrrolidine (NPYR) and *N*-nitrosomorpholine (NMOR), a gas chromatograph coupled to a thermal energy analyzer (GC-TEA, Thermo Electron Cooperation) was used. The extracts (5 µl) were injected on a packed column (10% Carbowax 20 M + 2% KOH on Chromosorb WAW, 80/100 mesh, 1.8 m, 2 mm i.d. Varian, Middelburg, The Netherlands) and a chromatographic separation was carried out by using argon as carrier gas (25 ml/min). The injection port was set at 175 °C and the oven temperature was increased from 110 °C to 180 °C at 5 °C/min. The temperatures of the interface and pyrolyzer of the TEA were set at 250 °C and 500 °C, respectively.

2.6. Statistical analysis

Statistical calculations (median, minima and maxima, ...) were done by PASW Statistics 18.0.0 (SPSS, Chicago, USA). For the exploratory data analysis, subroutines developed under Matlab 5.3 software (The Mathworks, Natick, MA, USA) were used to perform principal components analysis (PCA) and hierarchical cluster analysis (HCA). Prior to PCA and HCA, the data matrix variables were pretreated with autoscaling.

3. Results and discussion

3.1. Physicochemical composition of the retail products

The dry fermented sausages available on the Belgian market were divided in different categories. Based on the label information, the products were categorized according to the regional origin and the use of certain meat species or dominant spices in the recipes. In this way, 15 categories of dry fermented sausage products were obtained. Of the 101 samples, 52 can be considered as South European types while the other 49 were produced as Northern types. In stores 1 and 2, the A-brand stores, the largest range of products (38 and 33, resp.) can be found and almost every sausage category is represented by several trademarks. In the discount stores, the available assortment is smaller, 22 and 8 products for stores 3 and 4, respectively. As a consequence, not all categories were present. Mainly northern products, e.g. Boulogne, Boerenring and Garlic Salami, were not available in the discount stores.

The results of the physicochemical analyses of the sausages are reported in Table 1. The lowest pH (pH 4.4) was recorded for Rosette salami (French type). The ready-to-eat Snack sausages showed the highest

Table 1

The physicochemical composition of the commercial dry fermented sausages, the results are presented as median (min–max) values.

Type	n	Physical analysis		Chemical analysis (g/100 g)			
		pH	a _w	Moisture	Free fat	Protein	NaCl
<i>North European types</i>							
Boerenring	3	5.0 (4.8–5.0)	0.92 (0.88–0.93)	38.8 (32.4–40.6)	31.5 (26.3–36.6)	20.2 (19.8–21.0)	3.4 (3.0–3.7)
Boulogne	3	4.7 (4.5–4.7)	0.93 (0.88–0.94)	43.2 (40.0–43.7)	28.7 (28.7–38.8)	20.7 (18.8–20.7)	3.5 (2.9–3.6)
Garlic salami	6	4.8 (4.7–4.9)	0.94 (0.92–0.95)	42.3 (38.8–46.3)	31.2 (28.8–35.6)	19.4 (16.3–19.5)	3.0 (2.6–3.4)
German types	2	4.8 (4.6–5.0)	0.94 (0.94–0.94)	44.8 (43.0–46.7)	30.7 (27.3–34.2)	16.5 (15.6–17.4)	3.0 (2.9–3.0)
Light salami	5	4.9 (4.8–5.0)	0.94 (0.87–0.92)	48.3 (45.6–55.3)	17.3 (13.6–22.3)	23.1 (20.4–28.1)	3.2 (2.6–3.6)
Pepper salami	4	4.9 (4.7–5.0)	0.93 (0.91–0.93)	39.7 (33.5–49.7)	29.7 (23.3–35.6)	19.9 (18.5–22.2)	3.3 (2.8–3.5)
Salami	10	5.0 (4.6–5.6)	0.91 (0.88–0.94)	41.7 (37.1–48.5)	30.3 (22.6–40.0)	21.7 (16.8–24.7)	3.7 (2.5–4.3)
Smoked salami	10	4.9 (4.5–5.1)	0.94 (0.91–0.95)	46.0 (40.0–49.3)	28.9 (23.6–36.1)	18.5 (17.2–25.1)	3.2 (2.5–4.0)
Turkey salami	6	4.7 (4.6–5.1)	0.93 (0.91–0.95)	50.1 (42.2–57.2)	21.7 (14.7–28.2)	22.1 (19.5–23.4)	3.7 (3.4–4.1)
<i>South European types</i>							
Chorizo	10	4.8 (4.6–5.6)	0.90 (0.91–0.94)	41.0 (24.3–50.6)	29.5 (19.7–39.0)	23.9 (18.5–27.6)	3.7 (3.1–4.5)
Dry sausage	6	5.3 (5.2–6.2)	0.88 (0.83–0.89)	34.0 (30.4–39.0)	29.4 (25.8–32.9)	26.0 (23.4–31.1)	4.3 (4.0–5.0)
French types	6	5.0 (4.4–5.5)	0.90 (0.89–0.96)	41.4 (37.6–53.8)	24.8 (18.5–31.8)	23.7 (21.3–27.5)	3.9 (2.6–4.3)
Italian types	9	5.5 (5.1–5.9)	0.91 (0.87–0.92)	41.0 (32.5–45.3)	27.9 (20.2–30.9)	24.9 (22.1–27.0)	3.7 (3.5–4.2)
Saucisson d'Ardennes	6	4.9 (4.8–5.6)	0.91 (0.84–0.93)	40.4 (36.5–46.5)	31.5 (17.9–34.2)	22.1 (19.7–29.8)	3.5 (3.1–5.3)
Snack	15	5.6 (5.0–6.7)	0.71 (0.66–0.86)	18.0 (11.6–29.2)	36.7 (25.2–44.1)	30.9 (23.7–36.0)	4.5 (3.5–5.1)
Total	101	5.0 (4.4–6.7)	0.91 (0.66–0.96)	40.7 (11.6–57.2)	29.6 (13.6–44.1)	22.4 (15.6–36.0)	3.6 (2.5–5.3)

value (pH 6.7). Dry sausages and Italian types, like Milano, did not have a clear acidification due to the missing lactic acid producing starter culture. In shelf stable products, the pH must be lower than 5.2 when the water activity is 0.95 or higher (Toldrá, 2007). To reduce the spoilage of mildly fermented sausages (pH > 5.2), the water activity in the commercial products is lower than 0.91. The small sized Snack sausages are strongly dried (a_w 0.71) resulting in a higher salt concentration (4.5 g/100 g) and decreased moisture protein ratio. In this study, the median salt concentration is 3.6 g/100 g. The current trend is to reduce the salt content (more specific the sodium content) to obtain healthier meat products. As a result, salt concentrations as low as 2.5 g NaCl/100 g, can be found in the products available on the Belgian market.

The median moisture content of the investigated sausages is 40.7 g/100 g with a large range, depending on the drying rate. Moisture contents of 11.6 g/100 g in Snack sausage up to 55.3 g/100 g in Turkey salami were found. Due to the partial substitution of fat by water in the Light salami, the final fat content of this kind of products is only 17.3 g/100 g. The median fat content of the Belgian retail products was 29.6 g/100 g. The maximum fat (44.1 g/100 g) and protein contents (36.0 g/100 g) were found in Snack sausages.

3.2. Residual nitrite and nitrate content

The screening of the commercial sausages at the end of shelf life revealed median residual sodium nitrite and sodium nitrate levels of 6.3 and 11.8 mg/kg, respectively (Table 2). A large variance in nitrite and nitrate levels is seen; ranging from not detected to 147.5 mg NaNO₂/kg and 167.8 mg NaNO₃/kg. The most recent directive 2006/52/EC (Official

Journal of the EU, 2006) imposes that the initial addition of sodium nitrite and nitrate in non-heated meat products is restricted to 150 mg/kg each. It is known that a strong reduction of nitrite and nitrate takes place during the processing. As such, the measured residual amounts in the end products do not necessarily reflect the initially added amounts. Consequently, it is impossible to verify the correct application of the current directive by analyzing the end products. The former directive 95/2/EC (Official Journal of the EU, 1995) was more enforceable, since a limit for the residual sodium nitrite was also included. In this study 87% of the analyzed samples contained residual sodium nitrite levels under that limit of 50 mg NaNO₂/kg.

3.3. Biogenic amines accumulation

In general the accumulation of biogenic amines at the end of shelf life was low and a large variability in the nature and quantity of the available biogenic amines was found in the different types of dry fermented sausages (Table 2). Traces of SER were only detected in Snack sausage samples which contain walnuts, a rich source of SER (Feldman & Lee, 1985). In 77% of the samples no HIS was detected. In a few cases the concentrations were notably higher. For example, in some Turkey salami samples the HIS content exceeded the maximum concentration of 100 mg/kg as allowed in scombroid fish (Official Journal of the EU, 2005). No specific legislation exists in Europe about the HIS content in meat products. Besides SER and HIS, low levels of TRYP and PHE were measured in the dry fermented sausage samples. These are probably due to the non-specific activity of phenylalanine decarboxylase in micrococci and

Table 2
The residual sodium nitrite and sodium nitrate concentrations and biogenic amine contents in commercial dry fermented sausages, the results are presented as the median (min–max) concentration (mg/kg).

Type	n	NaNO ₂ ^{a,b}	NaNO ₃ ^{a,b}	TRYP ^{a,b}	PHE ^{a,b}	PUT ^{a,b}	CAD ^{a,b}	HIS ^{a,b}	SER ^{a,b}	TYR ^{a,b}	SPM ^{a,b}	SPD ^{a,b}
<i>North European type</i>												
Boerenring	3	nd	38.2 (25.1–100.7)	7.7 (nd–13.8)	nd (nd–6.5)	0.6 (0.5–1.2)	Nd	nd	nd	7.1 (1.9–29.0)	9.1 (5.5–12.0)	3.3 (2.5–6.0)
Boulogne	3	11.4 (nd–62.5)	47.6 (23.0–159.5)	45.8 (20.3–109.7)	3.4 (3.0–30.6)	97.5 (29.2–182.4)	3.9 (1.44–7.7)	nd	nd	140.7 (102.5–302.9)	5.5 (2.5–5.6)	2.0 (1.1–2.2)
Garlic salami	6	9.5 (nd–52.9)	20.9 (nd–130.7)	4.3 (nd–8.1)	1.4 (nd–5.2)	20.3 (0.4–143.2)	1.0 (nd–2.31)	nd	nd	59.8 (1.3–123.6)	6.5 (4.3–9.6)	3.2 (1.5–5.8)
German types	2	20.9 (5.9–35.9)	16.5 (12.4–20.6)	26.2 (5.3–47.2)	10.0 (nd–20.0)	73.5 (7.0–139.9)	27.7 (nd–55.4)	6.2 (nd–12.4)	nd	107.2 (15.7–198.6)	6.7 (4.3–9.2)	3.7 (1.2–6.2)
Light salami	5	6.4 (nd–13.9)	nd (nd–107.3)	6.5 (nd–29.4)	nd (nd–1.6)	3.8 (1.0–49.4)	0.4 (nd–2.5)	nd (nd–0.7)	nd	26.3 (22.0–113.9)	8.4 (4.6–9.1)	2.3 (1.7–6.1)
Pepper salami	4	nd (nd–10.5)	70.2 (4.2–158.9)	3.6 (nd–7.2)	nd (nd–1.6)	8.4 (0.5–41.7)	0.3 (nd–1.9)	nd	nd	3.7 (1.6–85.4)	6.6 (4.6–10.6)	3.7 (0.8–5.1)
Salami	10	5.5 (nd–81.8)	13.7 (nd–167.8)	5.0 (nd–22.7)	0.9 (nd–10.8)	67.0 (0.8–229.0)	1.9 (0.3–246.8)	nd (nd–21.9)	nd	27.3 (1.6–174.1)	6.3 (nd–9.28)	1.6 (nd–3.7)
Smoked salami	10	nd (nd–9.2)	11.5 (nd–130.7)	5.3 (nd–32.2)	nd (nd–4.5)	1.5 (0.3–99.3)	0.4 (nd–2.2)	nd (nd–20.4)	nd	5.2 (nd–99.1)	8.4 (4.2–11.4)	5.0 (1.3–6.3)
Turkey salami	6	6.5 (nd–12.3)	20.6 (nd–94.0)	13.1 (5.4–56.2)	0.7 (nd–54.4)	4.2 (0.4–90.5)	1.1 (0.5–10.3)	nd (nd–131.0)	nd	19.1 (1.6–250.9)	5.6 (2.8–9.8)	4.1 (1.0–6.6)
<i>South European type</i>												
Chorizo	10	8.7 (nd–147.5)	11.3 (nd–73.8)	11.4 (1.4–105.4)	4.3 (1.3–38.1)	27.6 (0.3–183.6)	1.3 (0.2–380.1)	nd (nd–32.86)	nd	78.8 (1.9–242.7)	7.4 (3.5–21.1)	2.2 (nd–5.8)
Dry sausage	6	nd (nd–7.7)	9.4 (nd–20.4)	5.6 (4.4–7.0)	nd (nd–2.9)	6.7 (0.6–62.2)	3.5 (0.7–6.1)	nd	nd	10.0 (1.2–25.1)	6.1 (2.6–8.4)	0.3 (nd–2.3)
French types	6	2.4 (nd–108.0)	9.1 (nd–105.3)	11.7 (4.0–92.4)	4.2 (1.6–21.0)	114.6 (0.7–316.4)	18.9 (1.6–641.4)	2.3 (nd–54.1)	nd	113.4 (46.2–410.8)	5.3 (3.5–6.7)	1.2 (0.5–5.2)
Italian types	9	32.4 (nd–82.2)	nd (nd–24.3)	47.0 (3.4–99.8)	24.9 (2.2–57.1)	170.4 (1.9–236.8)	7.8 (0.3–68.7)	1.4 (nd–65.9)	nd	149.9 (126.2–201.2)	5.4 (2.3–10.3)	0.7 (nd–6.6)
Saucisson d'Ardenne	6	5.5 (nd–74.5)	20.8 (nd–55.2)	7.3 (2.6–54.0)	1.1 (nd–42.8)	57.1 (0.3–182.0)	0.8 (nd–2.9)	nd (nd–80.6)	nd	68.0 (nd–210.7)	5.3 (3.6–9.6)	3.5 (0.3–7.4)
Snack	15	16.5 (nd–95.9) ^b	11.8 (nd–60.3)	4.6 (nd–24.4)	nd (nd–26.3)	7.1 (0.5–124.1)	0.3 (nd–31.2)	nd (nd–51.1)	nd	3.6 (nd–10.1)	4.5 (nd–146.0)	2.1 (nd–14.9)
Total	101	6.3 (nd–147.5)	11.8 (nd–167.8)	6.2 (nd–109.7)	1.3 (nd–57.1)	9.3 (0.3–316.4)	1.1 (nd–641.4)	nd (nd–131.0)	nd	26.3 (nd–410.8)	6.1 (nd–21.1)	2.3 (nd–13.3)

^a nd = not detected, values below the limit of detection (LOD): 1.0 mg/kg for NaNO₂ and NaNO₃, 0.1 mg/kg for CAD, 0.2 mg/kg for PHE, PUT, SER and SPD, 0.9 mg/kg for TRYP, HIS and SPM, 1.0 mg/kg for TYR.

^b values estimated below the limit of quantification (LOQ) are printed in italics: 5.0 mg/kg for NaNO₂ and NaNO₃, 0.3 mg/kg for CAD, 0.4 mg/kg for PHE, PUT, 0.5 mg/kg for SER, 0.6 mg/kg for SPD, 2.9 mg/kg for SPM, 3.1 mg/kg for HIS, 3.2 mg/kg for TRYP and TYR.

staphylococci, which are able to grow during the beginning of the fermentation process (Nakazawa, Sano, Kumagai, & Yamada, 1977).

This study confirms that TYR is the major biogenic amine in dry fermented sausages (Latorre-Moratella et al., 2008). Concentrations ranged from not detected to a maximum of 411 mg/kg. It is recommended to susceptible individuals to avoid TYR rich foods, as it can cause migraine, mainly when drugs e.g. monoaminooxidase inhibitors (MAOI) are used. The risk of food migraines by the consumption of fermented sausages is limited. The threshold of 6 mg TYR for patients, treated with the classical MAOI drugs (McCabe, 1986), is only exceeded when eating, for example, more than 68 g of the most heavily contaminated sausages. Products such as Dry sausage, Boerenring and Snacks (in exception of one outlier of 146.0 mg/kg) showed TYR values below 50 mg/kg. The small diameter of these sausages resulted in a low moisture content and a largely aerobic meat environment. Hence, the optimal conditions for the synthesis of decarboxylases were not met and therefore biogenic amine production and especially TYR levels remained low (Bover-Cid, Schoppen, Izquierdo-Pulido, & Vidal-Carou, 1999). Sausage samples with an increased TYR level often seems to show also the presence of low concentrations of PHE, probably attributed to the non-specific tyrosine decarboxylase activity of enterococci (Nakazawa et al., 1977).

As described in the literature (Latorre-Moratella et al., 2008), after TYR the most important biogenic amines in dry fermented sausages are PUT and CAD. In this study all samples were contaminated with PUT. Nevertheless, the CAD contamination rate of the samples collected on the Belgian market remained low. Due to the complex interaction between processing parameters, fresh meat quality and microbial flora, a large variation in PUT and CAD concentrations is seen among the different analyzed products. Boerenring has the lowest contamination rate for PUT and even no CAD was detected. A Gaumais sample, categorized as French type, showed the highest levels for PUT (316 mg/kg) and CAD (614 mg/kg). The high total biogenic amine content, (1234 mg/kg) was in big contrast to the other samples (median = 57 mg/kg). Of all investigated products, this is the only sample that – at the end of shelf life – exceeded the level of 1000 mg/kg, which has been suggested to be dangerous for human health (Santos, 1996).

The concentrations of the SPM and SPD were limited with maximum levels of 21 mg/kg and 13 mg/kg, respectively. The small variability is explained by the natural origin of SPD and SPM in the meat. Almost no microbial synthesis takes place during the fermentation. During the storage, a small decline in SPD and SPM can occur, most likely due to deamination by microbial polyamine oxidase enzymes (Razin, Gery, & Bachrach, 1959). This explains that at the end of shelf life the values are slightly lower than in other studies where measurements were performed earlier (Hernández-Jover et al., 1997; Komprda et al., 2004; Ruiz-Capillas & Jimenez-Colmenero, 2004).

3.4. Occurrence of volatile N-nitrosamines

In 54 of the 101 samples, collected on the Belgian market, no volatile N-nitrosamines were detected at the end of shelf life. With 50% of N-nitrosamine contaminated sausages, the South-European products scored just slightly higher than the North-European (43%). When N-nitrosamines were detected, their total amount remained below 5.5 µg/kg, except for one sample, which showed an extreme total N-nitrosamine concentration of 14.0 µg/kg. This particular sample, categorized as a Pepper salami, was mainly contaminated by NPIP (12.3 µg/kg). Moreover, NPIP is also the most dominant N-nitrosamine in this study. NPIP is present above LOD in 28% (LOD = 0.8 µg/kg) of all sausages samples, but only 3% of the investigated sausages contained a concentration higher than the LOQ-value of 2.5 µg/kg. On the one hand biogenic amines, especially CAD and SPD are considered to be precursors for the NPIP formation (Shalaby, 1996). On the other hand, the use of piperine-containing spices such as black pepper, can supply the piperidine ring necessary for the formation of NPIP (Scotter & Castle, 2004).

With a contamination rate of 22% (above LOD of 0.6 µg/kg), NMOR is the second most common N-nitrosamine found in the dry fermented sausages. Except for one particular Chorizo sample (1.6 µg/kg), the detected NMOR concentrations remained below the LOQ-value of 1.5 µg/kg. In contrast to NPIP, no relationship between the investigated biogenic amines and NMOR is expected, since the direct precursor is morpholine. It can be questioned how this presumed precursor could contaminate the dry fermented sausages, since the presence of morpholine is mainly due to the use of waxes in packaging materials or the use of anticorrosion agents in the meat factories (Domanska & Kowalski, 2003).

To a lesser extent (12%), the samples could also contain detectable amounts of NDMA (above LOD of 0.2 µg/kg). Two samples – a Turkey sample and a Light product – contained NDMA levels above the LOQ value of 0.8 µg/kg. The occurrence of NPYR was more rare in this study and only three samples – a Chorizo sample (0.8 µg/kg), a sausage with Garlic (0.3 µg/kg) and a Pepper salami (1.5 µg/kg) – were found to be contaminated with trace amounts of NPYR (between LOD of 0.5 µg/kg and LOQ of 1.6 µg/kg). The presence of NPYR in the Chorizo sample can be explained by the use of a large quantity of paprika, which is known to contain pyrrolidine (Huxel, Scanlan, & Libbey, 1974). The rare occurrence of NPYR in the dry fermented sausage samples can be explained by the lack of a heating step, needed for the efficient formation of NPYR from its precursors, like pyrrolidine (derived from PUT), proline and spermidine (Drabik-Markiewicz et al., 2010; Drabik-Markiewicz et al., 2011). In none of the dry fermented sausage samples NDEA was detected (all below LOD of 0.3 µg/kg), while only two samples, classified as Saucisson d'Ardennes and Chorizo, showed NDBA contamination above LOD of 0.6 µg/kg. The concentrations remained below the LOQ value of 2.0 µg/kg. Since the presence of NDEA and NDBA in meat products is related to the contact with rubber nettings and plastics (Sen, Baddoo, & Seaman, 1993), it is obvious that the low contamination rate on the Belgian market proves the suitable quality of the used packaging materials.

3.5. Exploratory data analysis

To obtain a better understanding of the relationships of the physico-chemical factors and the chemical composition of the samples in the different product categories, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed. For the data treatment, only the variables were considered which contained a considerable percentage of detectable values. As a consequence, the following 18 variables were considered: pH (AN_pH), a_w (AN_AW), salt (AN_SALT), moisture (AN_WATER), free fat (AN_FAT), protein (AN_PROT), moisture-protein ratio (CAL_MPR), residual sodium nitrite (AN_NO₂), residual sodium nitrate (AN_NO₃), TRYP, PHE, PUT, CAD, TYR, SPD, SPM, total biogenic amine (TOT_BA) and the total N-nitrosamine (TOT_NA) content. The individual N-nitrosamines were disregarded since the percentages of detectable values were too small.

In a first instance PCA was performed to reveal patterns among the different product categories. Three principal components (PC) were extracted which explained 63.6% (PC1: 29.4%, PC2: 25.8% and PC3: 8.4%) of the total variance. On the PC1–PC2 score plot (Fig. 1) a distinction of regional origin of the sausages can be made along the PC1. The large majority of the North-European types (product categories 1, 2, 6, 7, 9, 10, 11, 13 and 15) are located on the positive part of the PC1-axis, while the South-European types (categories 3, 4, 5, 8, 12, and 14) are situated on the negative part. Based on the PC1–PC2 loading plot (Fig. 2), the differentiation seemed, in a first instance, to be attributed to the SPD, PHE and PUT concentrations. In the boxplot of PHE (Fig. 3A), the Southern products have higher PHE concentrations, while the Northern products scarcely contain PHE. However, for the SPD contents, no clear differences between North and South-Europe could be observed in the boxplot (Fig. 3B). For PUT (Fig. 3C), the median concentrations of both groups are low but in the Southern group greater numbers of high concentrations are seen.

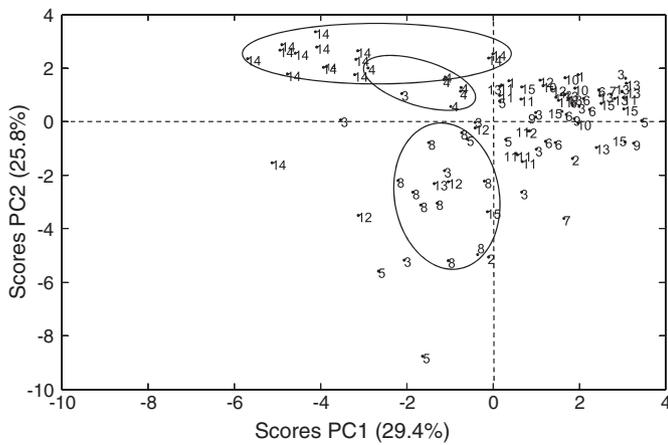


Fig. 1. Score plot of the first two principal components, samples labeled according to the product category: Boerenring (1), Boulogne (2), Chorizo (3), Dry sausage (4), French type (5), Garlic salami (6), German type (7), Italian type (8), Light salami (9), Pepper salami (10), Salami (11), Saucisson d'Ardennes (12), Smoked salami (13), Snack (14) and Turkey salami (15).

Considering the Southern products, the Snack sausages (nr. 14) are mainly clustered in the upper left quadrant of Fig. 1 (negative PC1, positive PC2), while the Italian Style sausages (nr. 8) are situated at a negative PC2. The products of the category Dry sausages (nr. 4) are located in between, but have also positive PC2 scores. The location of these three groups on the PC2-axis is mainly dominated by the concentrations of SPM (positive PC2 loading) and to a lesser extent of TRYP (negative PC2 loading) and of the variables AN_WATER and AN_SALT (Fig. 2). However, when considering the categories 4 and 14 (Dry sausage and Snacks) versus category 8 (Italian types), SPM does not show a clear difference (Table 2), while the three other variables can be considered different. The TRYP concentration (Table 2) and AN-WATER (moisture content; Table 1) are clearly lower in the categories 4 and 14 than in the category 8. For AN_SALT the opposite is seen (NaCl; Table 1).

A distinction between the different Northern classes (right side in Fig. 1) could not be made. This indicates that only limited intrinsic differences in the physicochemical properties and the biogenic amine contents among the Northern product categories could be observed.

In summary, the principal component analysis was able to discriminate some of the product categories. However, this clustering was mainly attributed to variance of some specific biogenic amine concentrations.

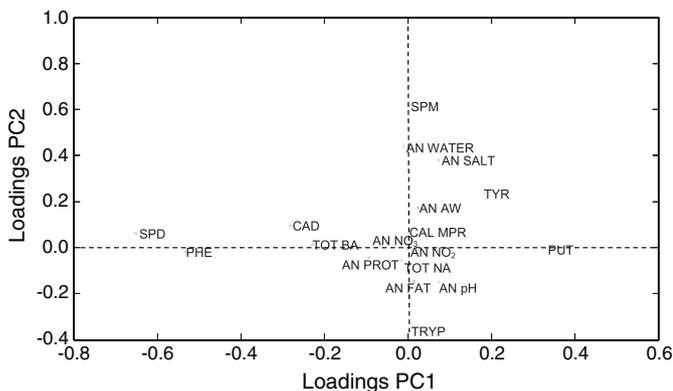


Fig. 2. Loading plot of the first two principal components, variables: pH (AN_pH), a_w (AN_AW), salt (AN_SALT), moisture (AN_WATER), free fat (AN_FAT), protein (AN_PROT), moisture–protein ratio (CAL_MPR), residual sodium nitrite (AN_NO₂), residual sodium nitrate (AN_NO₃), TRYP, PHE, PUT, CAD, TYR, SPD, SPM, total biogenic amine (TOT_BA) and the total N-nitrosamine (TOT_NA) content.

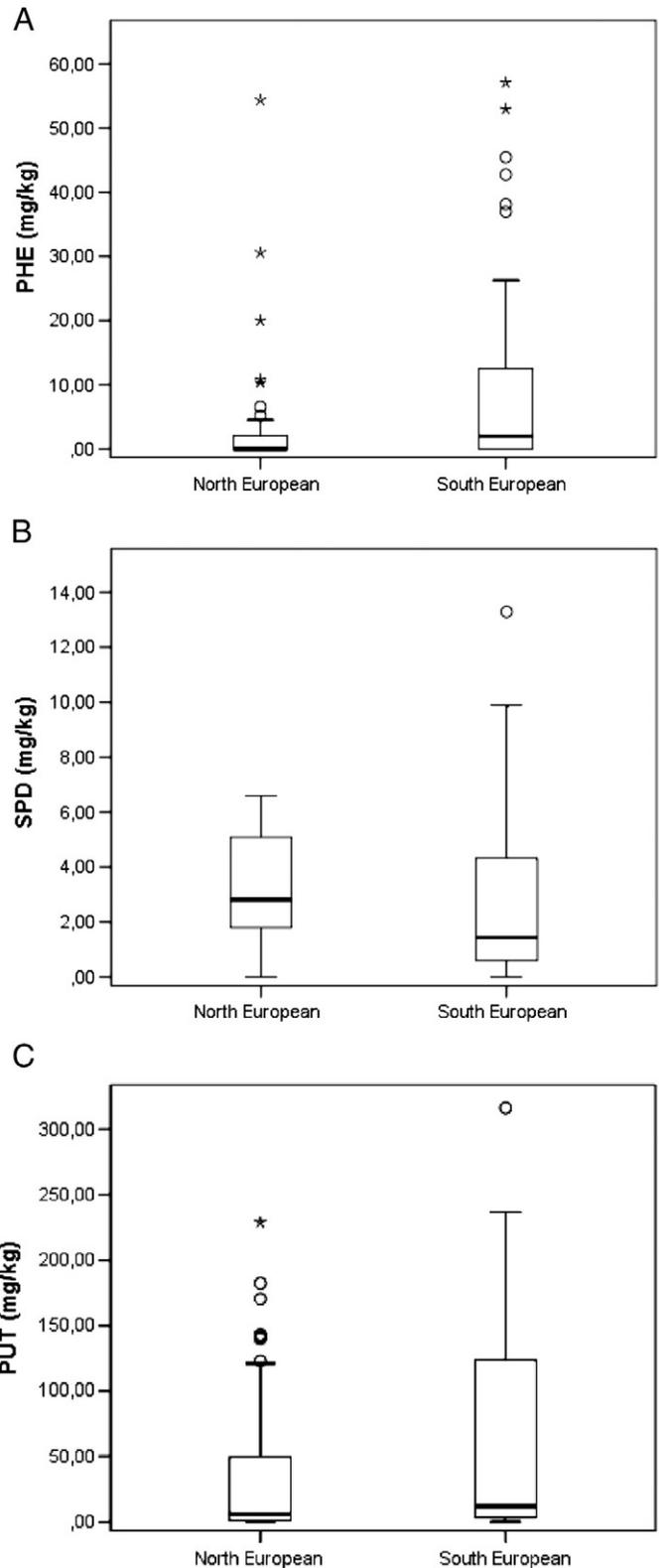


Fig. 3. The boxplots of PHE (A), SPD (B) and PUT (C) for each regional origin.

None of the product categories could be related to an increased N-nitrosamine risk since the variable TOT_NA was situated in the center of the loading plot (low PC1 and PC2 loading). The HCA of the samples confirmed the observations seen in the PCA plots but did not provide additional information (dendrograms not shown).

A HCA of the variables is given in the dendrogram in Fig. 4. All the biogenic amines, except the natural polyamines SPD and SPM can be

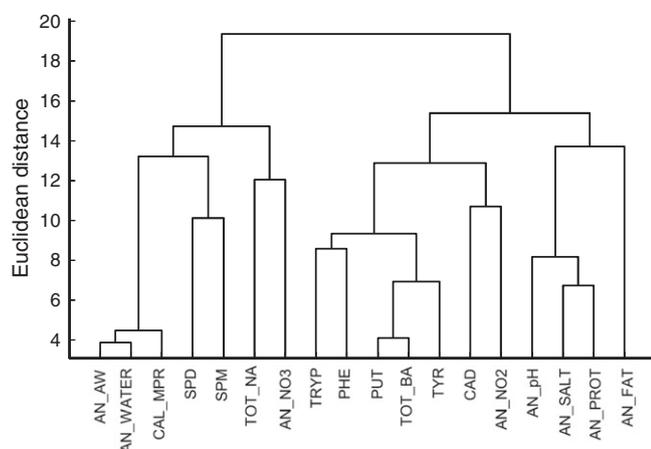


Fig. 4. Dendrogram for the variables, using Euclidean distances as similarity measure with complete linkage to merge clusters.

found in one cluster. The variables PUT and TYR showed most similarity with the total biogenic amine content. In accordance to many other studies concerning the biogenic amines content in dry sausages (Komprda et al., 2004; Suzzi & Gardini, 2003), it can be concluded that PUT and TYR are dominating the biogenic amine profile of dry fermented sausages.

The natural polyamines (SPD and SPM) are clustered to a lesser extent with the physicochemical variables related to the water content of the product (AN_AW, AN_WATER and CAL_MPR). This relationship can be explained by the fact that SPM and SPD are decreasing during the ripening (Parente & Suzzi, 2001). As a consequence, lower amounts of these polyamines will be related to an increased extent of drying which occurs during an extended ripening period.

For the formation of *N*-nitrosamines, the availability of secondary amines and a nitrosating agent is required (Drabik-Markiewicz et al., 2011; Honikel, 2008). From the latter variables, investigated in this study, only the residual NaNO₃ concentration could be linked to a given extent to the total *N*-nitrosamine content (Fig. 4). No clustering is seen with the investigated biogenic amines.

4. Conclusion

The biogenic amine accumulation in dry fermented sausages at the end of shelf life remains low. Only in one sample the total biogenic amine content reached critical levels (1000 mg/kg), mainly caused by the accumulation of PUT, CAD and TYR. Although it is assumed that biogenic amines, such as CAD, PUT and the natural polyamines SPD and SPM, are potential precursors of *N*-nitrosamines, no relationships were observed.

In this study, only a limited relation of the *N*-nitrosamine content was observed with the residual NaNO₃ level and no relationship with NaNO₂ level. It can be assumed that the amounts of carcinogenic *N*-nitrosamines remained low because the median concentrations of residual NaNO₂ and NaNO₃ levels were lower than 20 mg/kg in the screened products.

Only NMOR and NPIP were detected in a relatively high number of samples (22% and 28%, respectively), but quantifiable levels were rarely measured. The incidence of *N*-nitrosamine contamination could not be linked to specific product categories or physicochemical variables. Given the higher prevalence of NPIP and the absent link with tested biogenic amines, another secondary amine may be involved. It can be assumed that the NPIP formation is attributed to the presence of piperidine, originating from the use of black pepper.

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References

- Bardocz, S. (1995). Polyamines in food and their consequences for food quality and human health. *Trends in Food Science and Technology*, 6, 341–346.
- Bover-Cid, S., Schoppen, S., Izquierdo-Pulido, M., & Vidal-Carou, M. -C. (1999). Relationship between biogenic amine contents and the size of dry fermented sausages. *Meat Science*, 55, 305–311.
- De Mey, E., Drabik-Markiewicz, G., De Maere, H., Peeters, M. -C., Derdelinckx, G., Paelinck, H., & Kowalska, T. (2012). Dabsyl derivatization as an alternative for dansylation in the detection of biogenic amines in fermented meat products by reversed phase high performance liquid chromatography. *Food Chemistry*, 130, 1017–1023.
- Domanska, K., & Kowalski, B. (2003). Occurrence of volatile *N*-nitrosamines in polish processed meat products. *Bulletin of the Veterinary Institute in Pulawy*, 47, 507–514.
- Drabik-Markiewicz, G., Dejaegher, B., De Mey, E., Impens, S., Kowalska, T., Paelinck, H., & Vander Heyden, Y. (2010). Evaluation of the influence of proline, hydroxyproline or pyrrolidine in the presence of sodium nitrite on *N*-nitrosamine formation when heating cured meat. *Analytica Chimica Acta*, 657, 123–130.
- Drabik-Markiewicz, G., Dejaegher, B., De Mey, E., Kowalska, T., Paelinck, H., & Vander Heyden, Y. (2011). Influence of putrescine, cadaverine, spermidine or spermine on the formation of *N*-nitrosamines in heated cured pork meat. *Food Chemistry*, 126, 1539–1545.
- Drabik-Markiewicz, G., Van den Maagdenbergh, K., De Mey, E., Deprez, S., Kowalska, T., & Paelinck, H. (2009). Role of proline and hydroxyproline in *N*-nitrosamine formation when heating cured meat. *Meat Science*, 81, 479–486.
- Feldman, M., & Lee, M. (1985). Serotonin excretion content of foods: Effect of 5-hydroxyindoleacetic. *The American Journal of Clinical Nutrition*, 42, 639–643.
- Gavinelli, M., Fanelli, R., Bonfanti, M., Davoli, E., & Airolidi, L. (1988). Volatile nitrosamines in foods and beverages: Preliminary survey of the Italian market. *Bulletin of Environmental Contamination and Toxicology*, 40, 41–46.
- Gray, J. L., & Dugan, L. R. (1975). Inhibition of *N*-nitrosamine formation in model food systems. *Journal of Food Science*, 40(5), 981–984.
- Gros, L., Bruttel, P., & von Kloeden, M. (2005). *Monograph: Practical titration – Training manual for titrimetric volumetric analysis*. Herisau, Switzerland: Metrohm Ltd.
- Hernández-Jover, T., Izquierdo-Pulido, M., Veciana-Nogués, M. T., Mariné-Font, A., & Vidal-Carou, M. (1997). Biogenic amines and polyamines contents in meat and meat products. *Journal of Agricultural and Food Chemistry*, 45, 2098–2101.
- Honikel, K. (2008). The use and control of nitrate and nitrite for the processing of meat products. *Meat Science*, 78, 68–76.
- Huxel, E., Scanlan, R., & Libbey, L. (1974). Formation of nitrosopyrrolidine from pyrrolidine ring containing compounds at elevated temperatures. *Journal of Agricultural and Food Chemistry*, 22, 698–700.
- ISO (International Organization for Standardization) (1978). Determination of nitrogen content, ISO 937:1978 standard. *International standards meat and meat products*. Geneva, Switzerland: International Organization of Standardization.
- ISO (International Organization for Standardization) (1996). Determination of free fat content, ISO 1444:1996 standard. *International standards meat and meat products*. Geneva, Switzerland: International Organization of Standardization.
- ISO (International Organization for Standardization) (1997). Determination of moisture content, ISO 1442:1997 standard. *International standards meat and meat products*. Geneva, Switzerland: International Organization of Standardization.
- Komprda, T., Smělá, D., Pechová, P., Kalhotka, L., Stencl, J., & Klejduš, B. (2004). Effect of starter culture, spice mix and storage time and temperature on biogenic amine content of dry fermented sausages. *Meat Science*, 67(4), 607–616.
- Latorre-Moratalla, M., Veciana-Nogués, T., Bover-Cid, S., Garriga, M., Aymerich, T., Zanardi, E., Ianieri, A., Fraqueza, M., Patarata, L., Drosinos, E., Lauková, A., Talon, R., & Vidal-Carou, M. (2008). Biogenic amines in traditional fermented sausages produced in selected European countries. *Food Chemistry*, 107, 912–921.
- McCabe, B. J. (1986). Dietary tyramine and other pressor amines in MAOI regimes: a review. *Journal of the American Dietetic Association*, 86(8), 1059–1064.
- Nakazawa, H., Sano, K., Kumagai, H., & Yamada, H. (1977). Distribution and formation of aromatic L-amino acid decarboxylase in bacteria. *Agricultural and Biological Chemistry*, 41, 2241–2247.
- Official Journal of the EU (). Directive 95/52/EC. L61 food additives other than colours and sweeteners. *Official Journal of the European Union*, 1–40.
- Official Journal of the EU (). Commission regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union*, 1–26.
- Official Journal of the EU (). Directive 2006/52/EC food additives other than colours and sweeteners. *Official Journal of the European Union*, 10–22.

- Österdahl, B. -G. (1988). Volatile nitrosamines in foods on the Swedish market and estimation of their daily intake. *Food Additives and Contaminants*, 5, 587–595.
- Parente, E. M., & Suzzi, G. (2001). Evolution of microbial populations and biogenic amine production in dry sausages produced in Southern Italy. *Journal of Applied Microbiology*, 90(6), 882–891.
- Razin, S., Gery, I., & Bachrach, U. (1959). The degradation of natural polyamines and diamines by bacteria. *The Biochemical Journal*, 71, 551–558.
- Ruiz-Capillas, C., & Jimenez-Colmenero, F. (2004). Biogenic amines in meat and meat products. *Critical Reviews in Food Science and Nutrition*, 44, 489–499.
- Santos, S. M. (1996). Biogenic amines: Their importance in foods. *International Journal of Food Microbiology*, 29, 213–231.
- Scotter, M., & Castle, L. (2004). *Chemical interactions between additives in foodstuffs: A review, food additives and contaminants*. York, UK: Taylor & Francis.
- Sen, N., Baddoo, P., & Seaman, S. (1993). Nitrosamines in cured pork products packaged in elastic rubber nettings: An update. *Food Chemistry*, 47, 387–390.
- Shalaby, A. (1996). Significance of biogenic amines to food safety and human health. *Food Research International*, 29, 675–690.
- Suzzi, G., & Gardini, F. (2003). Biogenic amines in dry fermented sausages: A review. *International Journal of Food Microbiology*, 88, 41–54.
- Toldrà, F. (2007). *Handbook of fermented meat and poultry*. Iowa, USA: Blackwell Publishing.
- Yurchenko, S., & Mölder, U. (2007). The occurrence of volatile N-nitrosamines in Estonian meat products. *Food Chemistry*, 100, 1713–1721.