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Analytical Methods

Biogenic amines in traditional fermented sausages produced in selected European countries

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Abstract

Aminogenesis in traditional fermented sausages produced in Europe was studied during manufacturing process taking into account technological, physico-chemical and microbial factors. Tyramine was the major amine, followed by putrescine and cadaverine, although the occurrence of di-amines was much more variable. By principal component analysis, relationships between aminogenesis and the country of origin, physico-chemical parameters, processing conditions and microbial counts, were not found, probably due to the high dispersion observed in those variables. Therefore, biogenic amines occurred irrespectively of physico-chemical changes and technological conditions applied for sausage manufacture. By cluster analysis, five groups of fermented sausages were identified on the basis of their quantitative and qualitative profile of total biogenic amine content. Group A included products from very low to low total amine content (from not detected to 150 mg/kg); group B, products with moderate levels (from 150 to 350 mg/kg) tyramine being the major amine; group C, also with moderate amine contents but cadaverine being the major amine; and groups D and E, comprising products with high (from 350 to 550 mg/kg) and very high (higher than 550 mg/kg) amine content, respectively. Samples with moderate, high or very high levels of biogenic amines could be considered as products of less quality, and their consumption could be unhealthy for sensitive individuals or for those under classical monoamine oxidase inhibitor drug therapy.

Keywords: Traditional fermented sausage; Biogenic amines; Tyramine; Histamine; Cadaverine; Hygienic quality

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1. Introduction

Fermentation is a traditional preservation technique, which provides relatively stable meat products with typical sensorial characteristics. This is the case of a certain type of

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sausage, for which minced meat, together with several ingredients, such as salt, sugar, spices and curing agents, is fermented and stuffed into a casing. Although the type and manufacture of fermented sausages differ depending on the region, industrial manufacturing processes tend to standardize procedures. Artisanal sausages are increasingly appreciated by consumers because of their sensory properties and their authenticity. Traditional products are usually manufactured in small-scale plants following spontaneous fermentation. Given the size of these operations, traditional producers may encounter difficulties (technical and financial) to comply with food safety standards established for industrial processes, such as for example, the introduction of the hazard analysis and critical control point (HACCP) plan.

The European project "Tradisausage" (QLK1 CT-2002-02240) aimed to evaluate and eventually improve the safety of fermented sausages produced via traditional methods in Europe while preserving their authenticity. In the frame of this project, biogenic amines (tyramine, putrescine, cadaverine, histamine, β-phenylethylamine, tryptamine), microbial metabolites resulting from the decarboxylation of precursor amino acids (tyrosine, ornithine, lysine, histidine, phenylalanine, tryptophan) (Brink, Damink, Joosten, & Huis in't Veld, 1990), have been evaluated. Polyamines, spermidine and spermine, are considered natural amines, since they are not related to inducible microbial decarboxylase activity.

Interest in biogenic amine content of food, in particular fermented sausages, lies in safety and quality issues. From a toxicological point of view, the vasoactive and psychoactive effects, of mainly tyramine and histamine, are related to the occurrence of histaminic intoxication, food-induced migraines and hypertensive crises in sensitive individuals. The risk of health implications may be increased when the efficiency of enzymatic systems is blocked by monoamine oxidase inhibitors (MAOI drugs), gastrointestinal diseases, genetic deficiencies, or potentiating factors such as alcohol or other biogenic amines (Brink et al., 1990; Mariné-Font, Vidal-Carou, Izquierdo-Pulido, Veciana-Nogués, & Hernández-Jover, 1995). Furthermore, some biogenic amines (mainly cadaverine and histamine) have been proposed as chemical indicators of the hygienic conditions of raw material and/or manufacturing practices since their accumulation is associated with the activity of contaminant bacteria (Bover-Cid, Hernández-Jover, Miguélez-Arrizado, & Vidal-Carou, 2003; Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994; Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués, Marine-Font, & Vidal-Carou, 1997).

Among the numerous studies on biogenic amines in fermented sausages, few have focused specifically on traditional manufacture (Miguélez-Arrizado, Bover-Cid, Latorre-Moratalla, & Vidal-Carou, 2006; Montel, Masson, & Talon, 1999; Parente et al., 2001). In this kind of sausage, the occurrence of biogenic amines could be considerable and higher than industrial ones. Amine content and profiles may vary depending on several extrinsic and intrinsic factors during the manufacturing process, such as the ripening conditions, formulation, physico-chemical and proteolytic parameters, as well as microflora development and its decarboxylase activity.

In the frame of Tradisausage project, biogenic amine accumulation in sausages from several regions throughout the Mediterranean area and Slovakia was studied. In particular, the objective of the present work was to study the aminogenesis during the manufacturing of traditional fermented sausages taking into account several factors such as origin, technological conditions, physico-chemical parameters and microbial counts. Moreover, biogenic amine contents in the final product were assessed to determine their hygienic implications as well as their potential risk for health.

2. Materials and methods

2.1. Samples and sampling

The different types of European fermented sausages examined in this study were manufactured by a total of 54 traditional processing units (PUs), which were selected by each country participating in the project to include different typologies according to the guidelines of the European Project Tradisausage: France (10 PUs), Spain (10), Italy (10), Portugal (11), Greece (10) and Slovakia (3).

Table 1 shows the range of length, temperature and relative humidity, both in fermentation and ripening steps used for the sample elaboration following the traditional customs of each country. All types of sausages were manufactured with pork (without beef); however, distinct types and/or amounts of ingredients, spices and additives were included. Fermentation was mediated by spontaneous flora, except in 5 PUs (1 in France, 2 in Spain, 1 in Greece and 1 in Slovakia), which used a starter culture. Products were smoked in Portugal, Greece and Slovakia.

For each PUs ($n = 54$), sausages were sampled at three points during the manufacturing process, point zero (Z): meat batter just before stuffing, mid point (M): after fermentation (at the end of microbial exponential growth), and final point (F): product after ripening when the product was ready for consumption. Moreover, to study the effect of batch, 13 additional batches from some selected PUs were studied. Three sausages at each sampling point were sampled. Samples were wrapped in aluminium foil, packed under vacuum, frozen at −20 °C and sent in dry ice to our laboratory. There the samples were stored at −20 °C until analysis. A total of 201 samples were examined for biogenic amine contents, nitrogen fractions (total nitrogen, α-amino nitrogen and non-protein nitrogen), and physico-chemical parameters (pH, water activity and moisture). Analyzes were performed in triplicate.

Table 1
Composition and technological conditions of fermented sausages from several countries (minimum and maximum of 10 processing units/country)

	France	Spain	Italy	Portugal	Greece	Slovakia
Formulation						
Meat species	Pork	Pork	Pork	Pork	Pork	Pork
Meat/fat ratio (%)	80/20	75–90/25–10	50–90/50–10	60–90/40–10	50–90/50–10	40–70/60–30
Salt (NaCl) (g/kg)	14–30	13–23	Unknown	Empirically	20–30	Empirically
Nitrates/Nitrites (g/kg)	0–0.08/ 0–0.3	0–0.5	Unknown	0.6	0.15/0.2	Unknown
Glucides (g/kg)	0–8	0–33	Unknown	None	0–3	0–6
Other	Pepper, wine, garlic	Pepper, wine	Pepper, wine, garlic	Red pepper, wine, garlic	Black and red pepper, paprika	Chilly pepper, garlic
Process conditions						
Temp (°C)/HR (%) smoking	None	None	None	2–21/50–90[a]	18–20/85–89	>26/30–95
Time (days) smoking	None	None	None	5–45[a]	2	Empirically
Temp (°C)/HR (%) fermentation	10–22/76–99	2–24/49–94	4–26/70–84	2–12	12–24/93–80	15–16/80
Time (days) fermentation	2–8	<1–5	1–10	1–3	1–7	5–12
Temp (°C)/HR (%) maturation	8–14/70–90	10–18/58–85	6–22/58–83	2–21/50–90[a]	12–17/76–78	15–25/82–90
Time (days) maturation	31–82	15–60	15–90	5–45[a]	14–60	12–21

[a] In nine out of ten PUs the smoking and maturation are not independent phases; it occurs simultaneously.

2.2. Analytical determinations

Biogenic amines were detected and quantified by ion-pair reverse-phase high performance liquid chromatography, as described in Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués, and Vidal-Carou (1996). This method is based on the formation of ion-pairs between biogenic amine, previously extracted with 0.6 N perchloric acid from 5 to 10 g of sample without casings, and octanesulphonic acid present in the mobile phase. Amine separation is performed through a C18 reverse phase column, followed by a post-column derivatization with *o*-phthalaldehyde (OPA) with spectrofluorimetric detection (λ ex: 340 nm and λ em: 445 nm).

Total nitrogen (TN) and non-protein nitrogen (NPN) contents were determined by the Kjeldahl method (AOAC, 1995). The NPN fraction was previously extracted from 5 to 10 g of sample with 0.6 N perchloric acid (Dierick, Vandekerckhove, & Demeyer, 1974). The proteolysis index (PI) was calculated through the quotient between NPN and TN multiplied by 100. The Sörensen method, by titration with formaldehyde (AOAC, 1995), was used to determine the free amino acid fraction as α-amino nitrogen (AAN). The pH was measured using a microcomputerized pH meter Crison 2001 (Crison Barcelona, Spain). The electrode was inserted in a mixture of 5 g of homogenised sample and 5 ml of distilled water. Water activity values were obtained at 25 °C by means of Aqualab® equipment (Decagon Devices Inc. Pullman, Washington). Moisture was determined by drying the sample at 100–105 °C until constant weight (AOAC, 1995).

2.3. Microbial enumeration

Twenty five g of product were aseptically transferred to 225 ml of sterile buffered peptone water solution (BPW, AES Laboratory) and homogenised with a stomacher. Serial dilutions in BPW were performed before plating as described in Talon et al. (2007).

Occurrence or enumeration of the following bacteria – yeasts and moulds, lactic acid bacteria (LAB), *Staphylococcus* and *Kocuria, Enterococcus, Enterobacteriaceae* and *Pseudomonas* were performed according to the methods presented by Talon et al. (2007) and according to ISOs.

2.4. Statistical analysis

ANOVA, Principal Component and Cluster Analysis were performed using the software package SPSS v.11.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Aminogenesis during manufacturing process

Biogenic amine contents during sausage manufacture at the three sampling points studied (Z, just stuffed, M, after fermentation and F, final product after ripening) are referred to dry matter (dm) to make up for the concentration effect of the drying process. Thus, products with distinct moisture content can be compared.

Physiological polyamines, spermidine and spermine, were the only biogenic amines always present, remaining constant throughout the manufacturing process, with an average value of approximately 4 mg/kg dm for spermidine and 49 mg/kg dm for spermine.

Just before stuffing (Z), with the exception of the physiological natural polyamines, most sausage samples did not show biogenic amines or these were detected only at very low levels (Fig. 1). Only nine samples showed total biogenic amine contents higher than 30 mg/kg, specially tyramine and cadaverine. Most of these samples showed

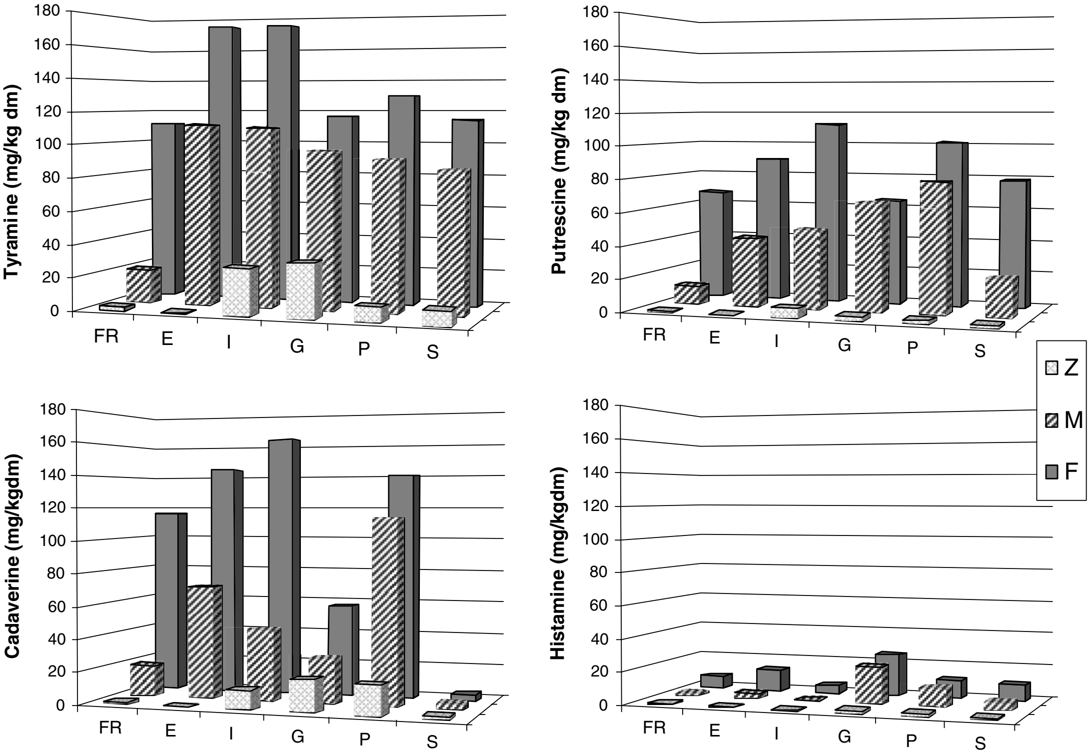

Fig. 1. Average contents (mg/kg dry matter) of biogenic amine contents in samples of just before stuffed products (Z), intermediate product after fermentation (M) and final product after ripening (F) for each country (FR: France; E: Spain; I: Italy; P: Portugal; G: Greece; S: Slovakia).

high counts of enterococci (higher than four log cfu/g in average) and/or enterobacteria (higher than four log cfu/g in average). These microbial groups are frequently reported to be strong producers of tyramine and cadaverine, respectively (Suzzi & Gardini, 2003).

Regarding aminogenesis throughout manufacturing (Fig. 1), biogenic amines were detected during fermentation (the increase between Z and M samples) as well as during ripening (the increase between M and F samples). However the intensity of amine formation in these two steps differed depending on the country. Thus, in most of the French products, aminogenesis occurred during ripening. In contrast, the greatest biogenic amine accumulation in Greek products was registered during fermentation.

In the final products (Table 2), tyramine was the dominant amine, accounting for one third to one half of the total amine content. Tyramine content followed a normal distribution and the mean value was 145 mg/kg dm. Putrescine was the second amine followed by cadaverine. These di-amines showed higher variability, with a non normal distribution. Most of the samples showed relatively low di-amine contents (median values of 51 mg/kg dm for putrescine and 41 mg/kg dm for cadaverine). Occasionally di-amine, especially cadaverine, surpassed the levels of tyramine. The occurrence of histamine was less frequent (31 out of 54 final products) and at much lower concentrations (median of 0.66 mg/kg dm) than those of the amines mentioned above. Histamine was always associated with the presence of di-amines, especially cadaverine, which is consistent with the fact that these two amines are produced mainly by enterobacteria (Bover-Cid, Izquierdo-Pulido, & Vidal-Carou, 2000; Roig-Sagués, Hernández-Herrero, López-Sabater, Rodriguez Jerez, & Mora-Ventura, 1996). The aromatic amine phenylethylamine was found in few samples and always when high contents of tyramine were present. This observation could be attributed to the fact that microorganisms with strong tyrosine-decarboxylase activity also have moderate capacity for decarboxylate phenylalanine (Bover-Cid, Hugas, Izquierdo-Pulido, & Vidal-Carou, 2001; Joosten, 1987). However, high cadaverine or tyramine content does not necessarily imply histamine and phenylethylamine accumulation respectively. In general, biogenic amine contents of the sausages did not differ clearly from those usually reported for fermented sausages, whether industrial or traditional (Hernández-Jover et al., 1997; Montel et al., 1999).

To evaluate the effect of manufacturing customs of the country of origin on the biogenic amine content a principal component analysis (PCA) was performed. The 67% of the total variance was explained by the three principal components. The first component explain the 38% of the total variance and was referred to biogenic amines of microbial origin corresponded to tyramine (correlation coefficient = 0.87), putrescine (0.85), cadaverine (0.55), histamine (0.61), phenylethylamine (0.73) and tryptamine (0.80). Second and the third principal component were influenced by the physiological polyamines, spermidine (0.78) and spermine (0.76), together with agmatine (0.66)

Table 2
Biogenic amine content (mg/kg dry matter) in the ripened fermented sausages for all processing units (PUs) evaluated

Country	PUs	TY[a]	PU[a]	CA[a]	HI[a]	PHE[a]	TRP[a]	Cluster group[b]
France	1	186.91	107.27	107.43	0.38	7.07	2.99	B
	2	5.25	0.43	0.55	0.39	0.01	0.01	A
	3	173.68	124.20	85.16	1.40	3.64	18.34	B
	4	113.32	121.75	16.20	7.33	0.01	0.01	B
	5	226.62	362.06	389.82	41.71	53.98	18.87	E
	6	130.05	15.49	115.47	5.46	1.41	10.22	C
	7	147.98	10.05	0.01	0.01	1.86	0.01	A
	8	133.21	42.20	259.79	10.47	4.30	19.13	D
	9	104.60	8.91	315.54	0.01	0.16	4.55	D
	10	7.08	0.22	0.01	0.01	0.01	0.01	A
Spain	1	204.40	252.51	4.38	0.01	17.82	44.94	B
	2	86.82	43.94	0.01	0.01	0.01	0.01	A
	3	174.72	17.56	610.96	0.01	3.69	0.01	D
	4	86.88	5.82	0.01	0.01	0.01	0.01	A
	5	190.53	98.42	79.75	1.93	11.39	25.78	B
	6	215.95	45.81	17.51	0.01	25.66	37.55	B
	7	473.47	448.85	302.63	133.39	30.76	78.51	E
	8	38.38	1.28	1.04	0.58	0.01	3.02	A
	9	49.93	5.49	0.40	0.01	0.01	0.01	A
	10	272.20	95.58	257.74	26.06	5.77	4.93	D
Italy	1	276.93	324.20	205.66	0.01	43.41	2.34	E
	2	229.19	209.87	29.28	4.88	10.21	9.56	B
	3	129.80	3.65	0.36	0.01	0.01	0.01	A
	4	214.40	46.77	352.32	0.01	0.01	0.01	D
	5	231.53	322.14	449.44	0.01	0.01	6.28	E
	6	168.87	139.04	20.23	8.84	0.01	12.50	B
	7	70.30	15.39	195.47	0.01	0.01	3.76	C
	8	117.86	104.58	40.45	0.74	4.36	0.01	B
	9	302.97	72.72	71.46	0.01	4.84	17.18	B
	10	60.24	10.34	139.58	0.01	0.01	0.01	C
Greece	1	14.29	0.01	0.01	0.01	0.01	0.01	A
	2	11.84	0.01	0.01	0.01	0.01	0.01	A
	3	157.67	95.90	0.01	15.54	3.63	0.50	B
	4	271.69	126.37	242.58	0.01	3.73	26.31	C
	5	86.37	65.32	20.32	6.86	0.01	2.62	A
	6	210.53	25.86	88.90	47.92	20.65	48.24	C
	7	119.01	122.48	7.30	41.79	2.15	0.01	B
	8	135.70	96.60	199.68	0.01	0.01	10.51	C
	9	85.60	2.02	106.80	0.01	0.01	0.01	C
	10	142.35	113.73	19.41	105.81	0.01	0.01	B
Portugal	1	12.95	4.26	2.74	3.11	0.01	0.01	A
	2	9.71	7.28	4.91	6.22	0.01	0.01	A
	3	259.04	257.40	484.65	0.01	45.47	33.74	E
	4	8.60	10.26	10.76	3.60	0.01	0.01	A
	5	104.83	117.09	262.46	14.34	2.18	6.38	D
	6	266.83	53.51	41.41	3.42	5.58	2.26	B
	7	154.76	20.19	198.41	0.01	0.01	6.91	C
	8	138.65	36.35	244.76	6.54	0.01	0.01	C
	9	208.64	180.42	25.43	2.19	0.01	0.01	B
	10	268.61	352.18	342.67	94.66	20.01	36.10	E
	11	82.03	83.69	121.35	4.13	0.01	0.68	C
Slovakia	1	117.94	49.29	2.51	2.19	4.52	1.89	A
	2	110.98	61.71	1.40	15.29	1.05	0.90	A
	3	0.01	0.01	0.01	0.01	0.01	0.01	A

TY: tyramine, PU: putrescine, CA: cadaverine, HI: histamine, PHE: phenylethylamine, TRP: tryptamine. Cluster group: (A) very low and low total contents; (B) moderate total contents, tyramine as the major amine; (C) moderate total contents, cadaverine as the major amine; (D) high total contents; (E) very high total contents.

[a] Biogenic amines expressed in dry matter to compensate differences attributed to different water content.

[b] Cluster groups were obtained taking the biogenic amine contents referred to fresh weight to better reproduce the conditions of consumption.

explaining the 17% and 12% of the total variance, respectively. This PCA did not let a separation of the samples by countries which indicated that the overall amine content was irrespective of the country of origin. The only comparative study about biogenic amines in fermented sausages produced in several European countries (Ansorena et al., 2002) published to date reports quantitative and qualitative differences between those manufactured in Southern and the Northern Europe. Southern Belgium and Italy showed greater levels than Northern Belgium and Norway. These differences were attributed to distinct processing methods and microbial characteristics.

3.2. Relationships between biogenic amines and processing parameters

Table 3 shows the results of physico-chemical (pH, aw, moisture) and proteolysis-related parameters (AAN content and PI) during manufacturing. Sausages produced following traditional methods generally have final pH values that are higher than those produced industrially (Aymerich, Martín, Garriga, & Hugas, 2003; Miguélez-Arrizado et al., 2006). The pH values of the final products were quite variable but two statistically different ($p < 0.005$) groups were distinguished: those with relatively high pH values, Italy (average pH of 6.07), Spain (5.98) and France (5.76); and those that were more acidic, corresponding to Slovakia (mean 5.23), Greece (5.37) and Portugal (5.46). In contrast, moisture and water activity parameters were not statistically different ($p > 0.05$) between samples from the countries studied. This observation can probably be explained by variable degrees of drying. Similarly, statistical differences were not found in the proteolysis parameters, ANN and proteolysis index, either between countries or between sampling points.

Biogenic amine accumulation is affected by a wide variety of factors, including technological and physico-chemical ones, which in turn, interact and change throughout the process, thereby influencing the growth and the activity of aminogenic microorganisms (Suzzi & Gardini, 2003). A PCA was performed to establish whether or not there was a relationship between technological and physico-chemical factors and biogenic amine accumulation during sausage manufacture. For this statistical treatment, the variables considered were the changes in biogenic amines, pH, water activity, AAN and PI during fermentation and ripening, and the technological conditions (time, temperature and relative humidity) applied in these two steps of the process. Changes during fermentation were defined as the difference between analytical data of the sampling points M and Z, whereas those during ripening were the difference between F and M. The factorial analysis extracted 3 principal components explaining 56% of the total variance. The first principal component (PC1) was described by the increase in biogenic amines and free amino acids (AAN), which is in agreement with the role of free amino acids as precursors of decarboxylation. The second principal component (PC2) included the length of the process (time) and the changes of intrinsic factors: water activity, proteolysis index, and pH values. Finally, the third principal component (PC3) was described by the technological conditions: relative humidity and temperature. Thus, the accumulation of biogenic amines occurred irrespectively of physico-chemical changes (except for AAN) and the technological conditions applied for the sausage manufacture. In the bidimensional plot representation for the first two principal components (Fig. 2, Graph a), two steps of the manufacturing process, fermentation and ripening, were distinguished from PC2.

The relationship between the changes in microbial counts and the biogenic amine accumulation was also studied through a PCA (Fig. 2, Graph b). The factorial analysis extracted two principal components, which explained 60% of the total variance. The first principal component (PC1) included the changes in biogenic amines and in the opposite direction those of spoilage microorganisms (enterobacteria and pseudomonads). The second component (PC2), in contrast, included the technological flora (LAB and staphylococci), enterococci as well as yeasts and moulds. A relationship between technological flora and the increase in biogenic amines could not be demonstrated. The PC2, related mainly to technological flora, was less efficient in distinguishing the two parts of the manufacturing process than the PC2 related to physico-chemical parameters.

The lack of correlation between biogenic amine increase and both microbial change and temperature and relative humidity is consistent with the data from Parente et al. (2001). Processing conditions are technological factors that depend on the country as well as the PU. These factors determine the selection and competitiveness of microbial communities and modulate their metabolic activity (including decarboxylase) as well as the biochemical and physico-chemical changes that occur in the sausage during ripening. The occurrence of several possible interactions among all the factors involved in amine production could be a reason for the lack of any statistical relationship between most of the individual factors and aminogenesis. Therefore, no general rule can be concluded to describe aminogenesis during the manufacture of traditional fermented sausages depending on the country, nor among the PUs within the same country.

3.3. Biogenic amines in final products: hygienic and technological interest

Taking into account that biogenic amine contents in final products could be considered hygienic and technological quality indicators, a cluster analysis was carried out using the total content of biogenic amines of microbial origin (tyramine, phenylethylamine, tryptamine, histamine, putrescine and cadaverine). These results are referred to fresh matter (mg/kg) to better reflect the conditions of consumption.

Table 3
Physico-chemical parameters determined in sausages for all processing units (PUs): pH, water activity (aw) and water content

Country	Step	pH	aw	Moisture (%)	AAN (mg/g)	IP (NNP/NT%)
France (*n* = 10)	Z	5.7 ± 0.2[a]	0.95 ± 0.35	61.0 ± 4.6	1.2 ± 0.3	7.5 ± 3.1
		(5.4–6.0)[b]	(0.85–0.97)	(53.0–66.0)	(0.9–1.7)	(3.0–12.4)
	M	5.5 ± 0.3	0.95 ± 0.04	58.0 ± 3.4	1.6 ± 0.4	9.6 ± 5.8
		(5.1–6.0)	(0.82–0.97)	(53.7–61.6)	(1.1–2.2)	(0.8–18.5)
	F	5.8 ± 0.4	0.87 ± 0.02	32.0 ± 4.4	2.1 ± 0.5	6.8 ± 3.4
		(5.2–6.5)	(0.84–0.93)	(26.2–38.9)	(1.2–2.9)	(0.6–11.2)
Spain (*n* = 10)	Z	6.1 ± 0.2	0.97 ± 0.01	62.1 ± 2.7	1.5 ± 0.5	6.6 ± 4.2
		(5.86–6.43)	(0.96–0.98)	(56.9–65.9)	(1.0–2.2)	(2.4–14.8)
	M	5.7 ± 0.5	0.94 ± 0.02	47.1 ± 6.1	2.2 ± 0.7	10.5 ± 6.2
		(4.8–6.5)	(0.91–0.97)	(36.6–55.5)	(1.4–3.4)	(3.8–21.5)
	F	6.0 ± 0.4	0.88 ± 0.03	32.9 ± 5.0	2.3 ± 0.9	8.8 ± 4.1
		(5.5–6.5)	(0.83–0.92)	(26.2–40.6)	(1.3–4.4)	(1.9–16.4)
Italy (*n* = 10)	Z	5.9 ± 0.2	0.94 ± 0.06	58.2 ± 5.7	1.35 ± 0.38	11.1 ± 5.9
		(5.6–6.3)	(0.77–0.97)	(48.4–65.4)	(0.86–2.24)	(5.6–25.1)
	M	5.7 ± 0.3	0.96 ± 0.01	55.0 ± 4.9	2.0 ± 0.5	13.6 ± 3.9
		(5.3–6.1)	(0.95–0.97)	(48.3–62.2)	(1.6–3.2)	(8.8–20.8)
	F	6.1 ± 0.5	0.89 ± 0.05	33.0 ± 4.7	2.3 ± 0.5	10.7 ± 3.2
		(5.5–6.9)	(0.78–0.94)	(25.2–39.1)	(1.7–2.9)	(6.0–15.8)
Greece (*n* = 10)	Z	5.8 ± 0.6	0.96 ± 0.01	55.4 ± 6.0	1.3 ± 0.7	9.4 ± 11.2
		(4.3–6.4)	(0.95–0.97)	(48.5–66.7)	(0.6–2.9)	(0.5–39.8)
	M	5.6 ±0.6	0.95 ± 0.02	49.2 ± 10.3	1.8 ± 0.6	7.1 ± 5.9
		(4.6–6.3)	(0.93–0.97)	(35.6–65.5)	(1.3–2.8)	(0.5–16.6)
	F	5.4 ± 0.8	0.91 ± 0.07	41.8 ± 15.6	1.8 ± 0.6	11.9 ± 18.8
		(4.4–6.6)	(0.77–0.97)	(19.1–65.6)	(1.2–2.9)	(1.1–63.3)
Portugal (*n* = 11)	Z	5.9 ± 0.4	0.98 ± 0.01	59.5 ± 5.6	0.9 ± 0.3	8.3 ± 3.3
		(5.4–6.6)	(0.97–0.98)	(48.9–66.4)	(0.4–1.6)	(3.8–12.5)
	M	5.5 ± 0.4	0.95 ± 0.02	45.4 ± 9.0	1.9 ± 1.0	10.7 ± 3.4
		(5.0–6.1)	(0.93–0.98)	(27.5–61.0)	(0.6–3.7)	(4.7–15.7)
	F	5.5 ± 0.3	0.90 ± 0.03	30.5 ± 8.9	2.0 ± 0.8	10.4 ± 4.3
		(5.0–6.1)	(0.85–0.93)	(17.7–48.3)	(0.9–3.7)	(3.9–18.6)
Slovakia (*n* = 3)	Z	6.0 ± 0.1	0.96 ± 0.00	49.8 ± 3.3	1.0 ± 0.1	8.0 ± 0.4
		(5.9–6.1)	(0.96–0.97)	(47.6–53.6)	(0.9–1.1)	(7.6–8.4)
	M	5.4 ± 0.5	0.96 ± 0.01	47.9 ± 6.6	1.8 ± 0.3	10.9 ± 4.4
		(5.0–6.0)	(0.95–0.97)	(43.3–55.5)	(1.5–2.2)	(6.9–15.5)
	F	5.2 ± 0.56	0.93 ± 0.03	39.5 ± 8.6	1.9 ± 0.4	14.7 ± 10.2
		(5.0–6.0)	(0.90–0.95)	(32.9–49.2)	(1.7–2.3)	(7.8–26.5)

Proteolytic parameters: α-amino nitrogen (AAN) and Proteolytic index (PI). Z: products just before stuffing, M: products after fermentation, F: products after ripening (ready for consumption).

[a] Mean ± standard deviation of all processing units (PUs) of each country.

[b] Range (minimum–maximum) of all processing units (PUs) of each country.

Five groups, A–E, were distinguished (Table 2). Group A included sausages showing very low or low amine contents (from not detected to 150 mg/kg of total of biogenic amines) and accounted for one third of the sausages examined. In this group, tyramine was practically the only biogenic amine, except in few cases in which putrescine reached similar values to those of tyramine. Cadaverine, histamine and other minor amines were absent or the levels were extremely low. This group would be the most desirable option from the hygienic point of view. Up to 28% of the products were included in group B and presented moderate total biogenic amine content, with a range from 150 to 350 mg/kg, with tyramine as the major amine. Putrescine was the second amine, followed by cadaverine, whereas histamine was practically absent. It is well known that tyramine is the major amine in fermented meat products, and it is generally associated with the tyrosine-decarboxylase activity of some lactobacilli and other microbial populations that usually participate in the fermentation and ripening of sausages (i.e. some gram-positive catalase-positive cocci and most enterococci) (Suzzi & Gardini, 2003). Therefore, moderate levels of tyramine, as in the sausages of group B, would be acceptable. Nevertheless, since some samples of group A showed low or even extremely low levels of tyramine, it seems that it could be feasible to elaborate nearly tyramine-free and biogenic amine-free sausages. Group C included 18% of the products and these also showed moderate total biogenic amine content, but cadaverine was the main biogenic amine followed by tyramine, while putrescine content remained low. Group D included 11% of the products and was characterized by high total biogenic amine content (from 350 to 550 mg/

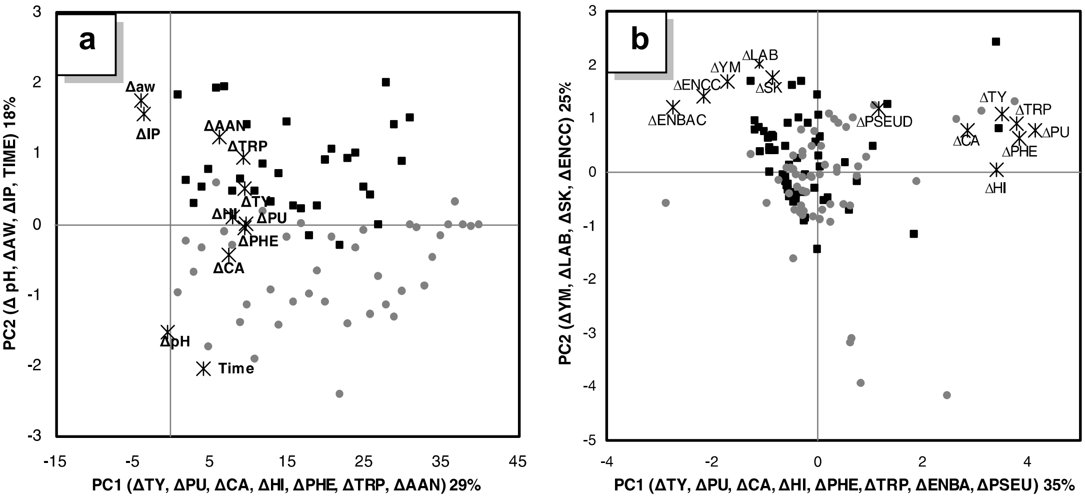

Fig. 2. Principal component analysis (PC1 and PC2) of the biogenic amine increase with technological conditions and physico-chemical changes (graph a), or with microbial changes (graph b) during the fermentation (■) and maturation (○) of European traditional fermented sausages. Asterisks (∗) show the relative position of the variables. △: Change between two consecutive sampling points (M–Z, for fermentation and F–M for ripening). TY: tyramine, PU: putrescine, CA: cadaverine, HI: histamine, PHE: phenylethylamine, TRP: tryptamine, AAN: α-amino nitrogen, Aw: water activity, IP: proteolytic index, ENBA: enterobacteria, PSEU: pseudomanads, YM: yeast and moulds, LAB: lactic acid bacteria, SK: staphylococci, ENCC: enterococci.

kg). The qualitative amine profile of Group D was similar to that of Group C, cadaverine being the most abundant amine, followed by tyramine. Finally, the products clustered into group E showed very high levels of biogenic amines (total content higher than 550 mg/kg), especially cadaverine, putrescine, tyramine and histamine. This group contained also the 11% of the products examined. Cadaverine and also histamine are usually related to the activity of decarboxylase-positive contaminant microbiota, such as enterobacteria (Durlu-Özkaya, Ayhan, & Vural, 2001), which may not be totally inhibited during fermentation. On the basis of the amount and type of amines, sausages included in groups C, D and E could be considered less desirable than the others. Indeed, the fermented sausages in which biogenic amine accumulation was already detected in Z samples were included in one of these three groups. Therefore, a relationship between the hygienic quality of raw materials and the final contents of biogenic amines could be suspected. In order to evaluate the influence of hygienic conditions of raw materials on the accumulation of biogenic amines, additional batches from some selected PUs of each country were analyzed. In most of the additional batches the amine profiles differed to those found in the first manufacture, both from quantitative and qualitative points of view (Table 4). These results indicate that the batch, mainly because of differences in hygienic quality of raw materials, would be a critical factor in explaining the differences observed between batches from the same PU.

3.4. Biogenic amines in final products: toxicological interest

The other objective of this study was to assess whether the consumption of the traditional fermented sausages could imply a health risk for their biogenic amine contents. Several genetic, pharmacological and dietary factors are responsible for the wide inter- and

Table 4
Biogenic amine content (mg/kg dry matter) of the fermented sausages (end-product) of two different batches (a and b) manufactured within the processing units (PUs) of each country

Country	PUs	TY	PU	CA	HI	PHE	TRY
France	2a	5.25	0.43	0.55	0.39	0.01	0.01
	2b	8.68	6.87	0.01	0.01	0.01	0.01
	8a	133.21	42.20	259.79	10.47	4.30	19.13
	8b	102.01	14.50	103.31	25.55	0.01	0.01
Spain	2a	86.82	43.94	0.01	0.01	0.01	0.01
	2b	14.46	3.08	1.42	0.01	1.89	0.01
	3a	174.72	17.56	610.96	0.01	3.69	0.01
	3b	276.94	57.25	456.29	0.01	11.56	0.01
Italy	2a	229.19	209.87	29.28	4.88	10.21	9.56
	2b	188.96	95.08	318.75	23.20	2.61	5.41
	7a	70.30	15.39	195.47	0.01	0.01	3.76
	7b	99.01	4.43	143.93	19.74	0.01	0.01
Greece	1a	14.29	0.01	0.01	0.01	0.01	0.01
	1b	148.26	126.39	1.18	91.64	6.02	0.01
	8a	135.70	96.60	199.68	0.01	0.01	10.51
	8b	14.96	0.01	0.01	1.74	0.01	0.01
Portugal	2a	9.71	7.28	4.91	6.22	0.01	0.01
	2b	72.77	84.90	30.65	0.73	0.09	0.32
	11a	82.03	83.69	121.35	4.13	0.01	0.68
	11b	93.03	111.30	52.80	8.98	0.01	13.21
Slovakia	1a	117.94	49.29	2.51	2.19	4.52	1.89
	1b	106.87	238.11	15.87	0.72	0.01	8.04
	2a	110.98	61.71	1.40	15.29	1.05	0.90
	2b	242.87	109.51	1.74	41.41	77.55	2.38
	3a	0.01	0.01	0.01	0.01	0.01	0.01
	3b	109.37	11.26	2.11	0.21	0.01	0.01

TY: tyramine, PU: putrescine, CA: cadaverine, HI: histamine, PHE: phenylethylamine, TRP: tryptamine.

intra-individual variability of sensitivity to biogenic amines, which makes their toxic threshold difficult to establish (Mariné-Font et al., 1995). For the healthy population, intestinal and hepatic barriers (mainly mono- and di-amine oxidases) are highly effective, lowering the risk of health troubles. In susceptible individuals, some food migraines have been reported to be produced by the ingestion of 100 mg of tyramine (Hannington, 1980). Moreover, individuals receiving treatment with MAOI drugs are usually recommended to avoid food sources of tyramine and other biogenic amines. A dose of 6 mg of tyramine can cause hypertensive symptoms during classical MAOI treatments (McCabe, 1986), whereas up to 50–100 mg of tyramine can be tolerated by individuals treated with third generation MAOI drugs (Dingemanse et al., 1998).

In the light of these data, the lowest hazardous dose of tyramine (i.e. the 6 mg in classical MAOI treatments) could be easily reached by consuming 80 g of some traditional fermented sausages included in Groups A and B. To reach the 50–100 mg of tyramine, it would be necessary to eat more than 700–1300 g of sausage, which far exceeds the approximately 50 g of a usual serving of sausage. In the worse case, for instance, taking into account the average tyramine content of sausages of Group E (196 mg/kg), a serving of 50 g would result in a consumption of 98 mg of tyramine, practically the limit dose for triggering migraine or causing interaction with third generation MAOI drugs.

Histamine is the only biogenic amine subjected to legal regulations in some fish species, with an upper limit of 100 mg/kg in Europe (EC, 2005). However, there is no regulation about limits of biogenic amines in fermented sausages. Some authors have suggested 100 mg/kg of histamine as a limit to establish potential risk for healthy individuals (Brink et al., 1990).

Di-amine oxidase (DAO) is the main histamine catabolising enzyme in the intestinal tract. The hypersensitivity to histamine, ingested with food, could appear when DAO is affected by genetic deficiencies or by the use of common drugs which are DAO inhibitors (DAOI), such as acetylcysteine, clavulanic acid, verapamil and metoclopramide. These drugs are taken by approximately a 20% of the population (Sattler, Hafner, Klotter, Lorenz, & Wagner, 1988). Although histamine in traditional fermented sausages was found in amounts below the proposed limit for healthy population, the consumption of these products could cause adverse effects in persons treated with DAOI.

Adverse effects of biogenic amines as a result of consumption of European traditional fermented sausages would be unlikely, except for individuals on classical MAOI and DAOI drugs. Therefore, sensitive individuals following a biogenic amine-restricted diet should be recommended to avoid dry fermented sausages, like other foods such as cheese, wine, fish products that are also potential sources of biogenic amines.

4. Conclusions

The findings of the present study indicate that biogenic amines in European traditional fermented sausages show variable levels of accumulation, tyramine being the main biogenic amine followed by putrescine and cadaverine.

On the basis of the quantitative and qualitative profile of biogenic amines in the final products, it was possible to distinguish five groups. Thus, very low amounts of biogenic amines or absence of these compounds could be regarded as a quality attribute while in contrast, the considerable occurrence of certain biogenic amines (especially cadaverine) could be considered indicators of poor hygienic quality. In this regard, more than a half of the products included in the groups classified as ‘‘undesirable” from a hygienic point of view (C, D and E), showed relatively high amounts (>30 mg/kg) of one or more biogenic amines in the raw materials. The hygienic quality of raw materials is a crucial factor that could affect the biogenic amine content of final products. High levels of biogenic amines in final products are usually related with the high occurrence of microflora possessing amino acid decarboxylase activity. However, not always raw meat of poor hygienic quality has high levels of biogenic amines.

The potential risk of harmful effects of biogenic amines stress the importance of ensuring proper hygienic practices during the manufacturing process to avoid the risks associated to these compounds.

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