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Evolution of microbial populations and biogenic amine production in dry sausages produced in Southern Italy

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Aims: To evaluate the occurrence and evolution of biogenic amines during ripening of fermented sausages and their relationship with physico-chemical and microbiological properties of the product.

Methods and Results: Salsiccia and Soppressata were obtained from artisanal and industrial plants in Basilicata and pH, a_W, microbial counts and biogenic amine content were measured. A high variability in amine content was observed. 2-Phenylethylamine and histamine were rarely found, while the tyramine, putrescine and cadaverine content increased during ripening. No correlation was found between individual biogenic amine content, microbial counts or physico-chemical parameters.

Conclusions: Starter cultures did not necessarily prevent the production of biogenic amines whose total contents were usually higher in Soppressata, a product with a larger diameter and a_W compared with Salsiccia.

Significance and Impact of the Study: Literature findings on biogenic amine content and the evolution of microbial populations were confirmed. Normal ranges for amine content in Salsiccia and Soppressata are reported.

INTRODUCTION

High levels of biogenic amines in foods are of public health significance because of their potential toxic effects. Toxic levels of biogenic amines cause reddening of the skin, stomach trouble and migraine (Brink *et al*. 1990). Histamine, tyramine and 2-phenylethylamine have vasoactive properties and, in some cases, they can reach concentrations in foods which are dangerous for the most sensitive consumers (Rice *et al*. 1975). In fact, the presence of these biogenic amines in food, especially in conjunction with other factors such as the consumption of monoamine oxidase-inhibiting drugs, alcohol and other food amines (e.g. spermine, spermidine, putrescine, cadaverine), may cause food poisoning (Maijala and Eerola 1993). A histamine intake of 8–40 mg, 40–100 mg and higher than 100 mg may cause slight, intermediate and intensive poisoning, respectively, while tyramine intake exceeding 100 mg may cause migraine (Maijala and Eerola 1993). Usually, biogenic amines are produced by microbial decarboxylation of amino acids. This requires the availability of amino acids, the presence of micro-organisms capable of decarboxylating amino acids, and favourable conditions for their growth and for the development of their decarboxylase activity (Ordóñez *et al*. 1999). In general, fermented foods can be regarded as matrices potentially contaminated by biogenic amines. For example, the manufacture of fermented sausages provides both the micro-organisms and free amino acids required for amine formation, together with environmental factors favouring bacterial growth and decarboxylase activity (Silla Santos 1996). Tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine have been detected in dry fermented sausages and other meat products (Rice *et al*. 1975; Vidal-Carou *et al*. 1990). Production of histamine and tyramine has been related to

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the presence of histidine- or tyrosine decarboxylase-containing lactic acid bacteria (Maijala 1993). However, other microbial groups, such as enterobacteria (Roig-Sagués *et al*. 1996; Marino *et al*. 2000), pseudomonads (Tiecco *et al*. 1986), aerobic spore-forming micro-organisms (Rodriguez-Jerez *et al*. 1994a), streptococci (Edwards and Sandine 1981) and micrococci (Rodriguez-Jerez *et al*. 1994b) have histidine and tyrosine decarboxylase activites. In dry fermented sausages, the quality of the raw material appears to be one of the main factors affecting biogenic amine formation (Kranner *et al*. 1991) whose levels increase in conjunction with microbial spoilage (Sayem-El-Daher *et al*. 1984; Maijala *et al*. 1995a, b).

Salsiccia and Soppressata are two of the most widely consumed ripened dry sausages in Southern Italy. Their composition, size, type of casing and technology depend on the customs of the geographical region in which they are produced. The composition of the two kinds of sausage includes pork, pork fat, curing salts, pepper and spices. Their production involves the preparation of sausage mix, stuffing into natural casings, fermentation and drying. The main differences between these products are the proportion of fat and meat, the type of spices used, the diameter of the sausage, and/or the length of the ripening process (from 15 to 30 days).

The main objective of this work was to study the occurrence of amines, their relative abundance and evolution during the ripening processes in Salsiccia and Soppressata sausages produced in the Basilicata region (Southern Italy) by different manufacturers with artisanal and industrial technology. In addition, the relationship between amine levels and some microbiological and chemico-physical parameters was investigated.

MATERIALS AND METHODS

Samples and sampling

Dry sausages (Salsiccia and Soppressata) were produced at different plants located in Basilicata (Southern Italy). Ten batches of Salsiccia (with a total of 30 samples obtained at different ripening times) and nine of Soppressata (27 samples) were analysed. The samples were produced in three industrial (B, E and H) and seven artisanal (A, C, D, F, G, I and L) plants. None of the artisanal plants used starter cultures, while two of the industrial plants used commercial starter cultures (*Pediococcus pentosaceus* and *Staphylococcus xylosus* in plant B, and *Lactobacillus sakei* and *Staph. xylosus* in plant E). The formulation of both sausage types and the ripening conditions differed among plants. Salsiccia is produced by stuffing a mixture of pork meat (usually shoulder and a low proportion of bacon), NaCl (2–4% w/w) and spices (dill seed or red pepper flakes) into 20–25 mm natural casings, followed by drying (24°C at 80% RH for 24 h, 18–22°C at 75–80% RH for 5 days) and ripening (15–20°C at 80–85% RH for 20–25 days). Soppressata is produced by stuffing a mixture of lean pork meat (usually ham), lard (2–3% w/w), NaCl (2–4% w/w) and spices (black pepper grains) into 45–60 mm natural casings, followed by drying and ripening (thermohygrometric conditions are similar to Salsiccia ripening) for up to 40 days. In industrial plants, sugars (sucrose and/or lactose 0·2–0·6% w/v), potassium nitrate and nitrite (50–150 mg kg^{-1}), and ascorbic acid (0·08% w/w), were used and ripening was carried out in rooms with controlled temperature, air speed and RH conditions. In artisanal plants, ripening was carried out in cellars with poor control of temperature and RH.

The whole sausage was taken as the sample each time and transported to the laboratory under refrigerated conditions (4°C). Samples were taken immediately after stuffing (T0), after 7–10 days of ripening (T1) and at the end of ripening process, i.e. 17–20 days for Salsiccia and 20–40 days for Soppressata (T2).

Microbiological analyses

A 10 g aliquot of each sample was taken aseptically and homogenized with 90 ml sterile Ringer's solution for 3 min in a Stomacher Lab-Blender 400 (Seward Medical, London, UK); serial dilutions of the homogenate were then made in sterile 0·1% (w/v) peptone water and plated in duplicate using the media and the incubation conditions described below.

Lactic acid bacteria were enumerated by pour-plating on MRS Agar with cycloheximide (100 mg l^{-1}) and bromocresol purple (0·16 g l^{-1}), followed by incubation at 30°C for 48 h. Since this medium lacked selectivity, Rogosa Agar, pH 5·4, was also used, with incubation at 30°C for 5 days. Microstaphylococci were enumerated on Baird Parker Medium supplemented with Egg Yolk Tellurite, followed by incubation at 37°C for 36 h. All colonies were enumerated, while colonies with the typical *Staphylococcus aureus* morphology were tested on Baird Parker Medium with rabbit plasma and bovine fibrinogen (RPF) supplement, followed by incubation at 37°C for 24 h to test for the production of coagulase. Enterobacteriaceae were enumerated by pour-plating on Violet Red Bile Glucose Agar, incubated at 37°C for 24 h. Yeasts and moulds were enumerated by spread-plating on Rose Bengal Chloramphenicol Agar, with incubation at 25°C for 5 days.

pH, a_w and biogenic amine determinations

The pH was measured by inserting a spear-tip electrode of 3 mm diameter (Orion Research, Beverly, MA, USA)

connected to a pH meter (Hanna Instruments, Vila do Conde, Portugal, model 8417) into two different points of the mass of each sausage. The result is expressed as the mean of the determinations.

Water activity was measured on two replicate samples at 25°C using a Rotronic Hygroskop BT (International PBI, Milan, Italy).

Biogenic amines were determined in the homogenized samples by HPLC, using the method proposed by Moret and Conte (1996), after amine derivatization performed according to Eerola *et al.* (1993).

Media and reagents

Unless otherwise stated, all microbiological media and ingredients were obtained from Oxoid, while chemicals were obtained from Sigma.

Statistical methods

All the statistical tests were performed using Systat 7·0 for Windows (SPSS Inc., Chicago, IL, USA). Results are given as the mean obtained in the two trials.

RESULTS

Evolution of microbial populations

The samples of Soppressata and Salsiccia analysed after production and during ripening had different origins. Some of them were produced with industrial technology (samples from B, E and H), whereas others were traditionally manufactured in artisanal plants (samples from A, C, D, F, G, I and L). This implies different technological procedures concerning the meat mixture, the use of starter cultures, the addition of sugars, and the control of the process parameters such as temperature and RH. Therefore, the microbial counts of the initial samples showed significant differences in relation to their origins. Differences were also found between Soppressata and Salsiccia, reflecting the different characteristics of the two products. The counts of Enterobacteriaceae, lactic acid bacteria and microstaphylococci during fermentation and ripening of the sausages were higher in Soppressata than in Salsiccia. Generally, the initial counts of Enterobacteriaceae decreased during ripening (Fig. 1). The sausages produced in A (both Salsiccia and Soppressata) had initial high counts of this microbial group. During ripening, only in Salsiccia did their concentration decrease below 10^3 cfu g^{-1}. One sample of Salsiccia produced in plant D showed an Enterobacteriaceae concentration higher than 10^4 cfu g^{-1} after ripening. As expected, in Salsiccia and

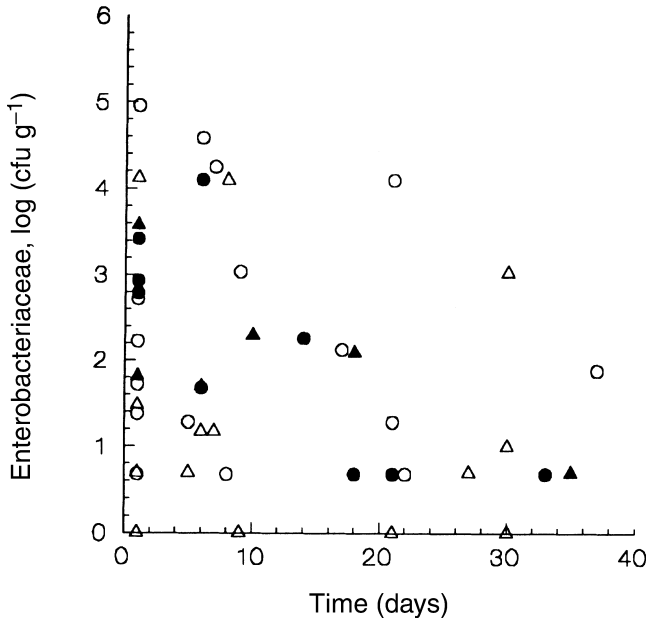

Fig. 1 Enterobacteriaceae counts detected in Salsiccia with (●) and without (○) starter cultures, and Soppressata with (▲) and without (△) starter cultures, during ripening

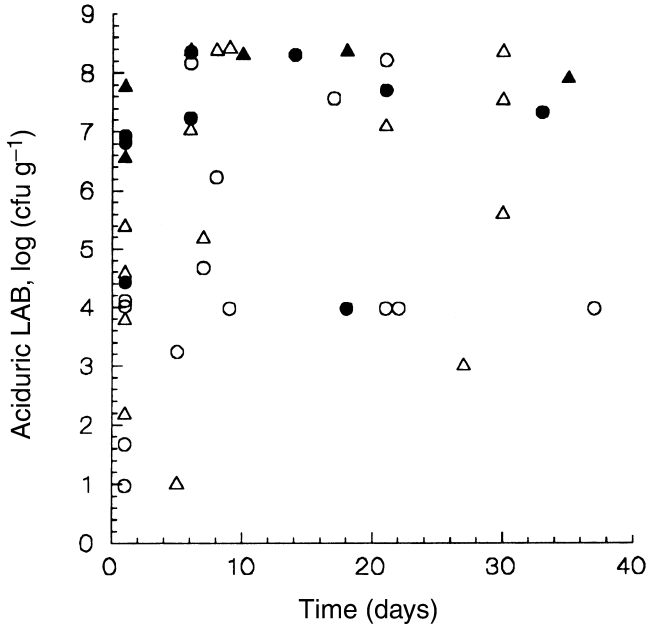

Fig. 2 Lactic acid bacteria counts detected in Salsiccia with (●) and without (○) starter cultures, and Soppressata with (▲) and without (△) starter cultures, during ripening

Soppressata manufactured with the addition of starter cultures (plants B and E), the decrease in the number of Enterobacteriaceae was more evident. However, Entero-

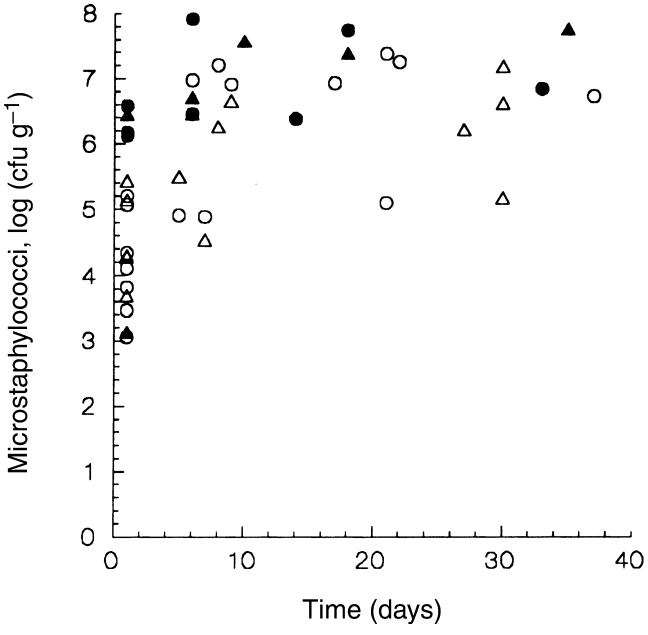

Fig. 3 Microstaphylococci counts detected in Salsiccia with (●) and without (○) starter cultures, and Soppressata with (▲) and without (△) starter cultures, during ripening

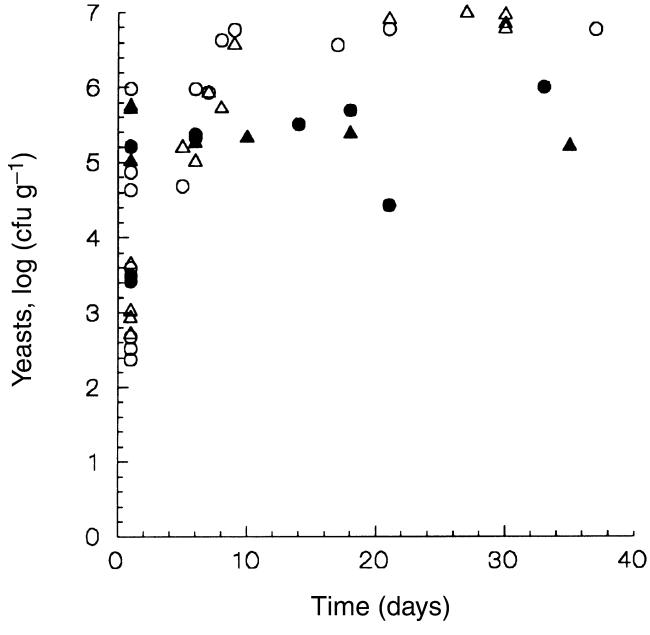

Fig. 4 Yeasts counts detected in Salsiccia with (●) and without (○) starter cultures, and Soppressata with (▲) and without (△) starter cultures, during ripening

bacteriaceae were still detectable in these samples at the end of ripening (about 15 days for Salsiccia and 25 days for Soppressata), even though their numbers were relatively low (less than 10^3 cfu g^{-1}).

The counts of lactic acid bacteria (LAB) are shown in Fig. 2. When starter cultures (pediococci or *Lactobacillus sakei*) were used, the numbers of LAB rapidly increased from about 10^7 to more than 10^8 cfu g^{-1}, and then slightly decreased. The initial numbers of LAB in sausages produced without starter cultures were usually lower (often less than 10^5 cfu g^{-1}) but, with a few exceptions, increased to 10^7–10^8 cfu g^{-1} during the first week of ripening. In a few samples, the numbers of LAB remained lower than 10^6 cfu g^{-1} (Soppressata produced in L and Salsiccia produced in C) throughout ripening.

In both Salsiccia and Soppressata, the initial numbers of microstaphylococci (Fig. 3) were always higher than 10^3 cfu g^{-1} and their evolution followed a pattern similar to that observed for LAB. As expected, the initial number was higher (10^6 cfu g^{-1}) when starter cultures were used (sausages produced in plants B and E). Generally, the initial population increased during ripening, and numbers of microstaphylococci as high as 10^8 cfu g^{-1} were obtained in some samples (Salsiccia from B and Soppressata from D). Even in sausages produced without the addition of starter cultures, the numbers of microstaphylococci were usually higher than 5×10^6 cfu g^{-1}, confirming the important role of this wild microflora in the fermentation and ripening processes.

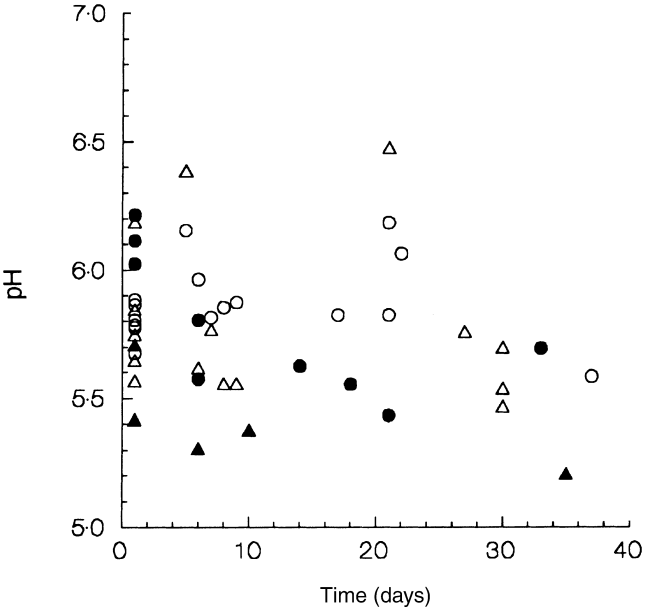

Fig. 5 pH values detected in Salsiccia with (●) and without (○) starter cultures, and Soppressata with (▲) and without (△) starter cultures, during ripening

Yeasts were always present in high numbers in both Salsiccia and Soppressata. Their counts increased during ripening and were often higher than 10^6 cfu g^{-1} (Fig. 4) in artisanal plants; lower numbers were usually observed in

Table 1 Biogenic amine content in Soppressata during ripening

Origin and time of ripening*		Biogenic amine content (mg kg^{-1} sausage)†						
		PHE	PUT	CAD	HIS	TYR	SPD	SPE
A	T0	n.d.‡	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	T1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	T2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B	T0	n.d.	n.d.	n.d.	n.d.	n.d.	0	20·49
	T1	n.d.	59·22	n.d.	20·27	124·62	24·85	32·42
	T2	n.d.	128·24	6·84	71·45	206·92	26·11	n.d.
C	T0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	T1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	T2	n.d.	n.d.	n.d.	n.d.	133·25	17·96	n.d.
D	T0	n.d.	20·15	218·42	n.d.	n.d.	34·12	64·03
	T1	n.d.	4·92	n.d.	n.d.	97·32	47·71	77·45
	T2	n.d.	n.d.	n.d.	n.d.	193·77	64·08	97·86
E	T0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	T1	n.d.	211·37	163·82	8·32	427·52	79·18	n.d.
	T2	10·56	416·14	269·29	24·39	511·44	90·57	47·61
F	T0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	T1	n.d.	n.d.	n.d.	n.d.	n.d.	39·96	41·14
	T2	n.d.	n.d.	n.d.	n.d.	n.d.	79·95	62·96
G	T0	n.d.	n.d.	n.d.	n.d.	8·12	n.d.	n.d.
	T1	17·83	n.d.	n.d.	61·86	n.d.	50·32	64·73
	T2	19·90	344·83	271·36	100·88	556·88	80·99	68·32
H	T0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	T1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	T2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
L	T0	n.d.	n.d.	n.d.	n.d.	41·73	78·93	43·52
	T1	n.d.	n.d.	n.d.	n.d.	20·33	91·25	51·20
	T2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	42·93

*Samples were analysed immediately after production (T0), after a week (T1) and at the end (T2) of ripening.

†The biogenic amines tested were 2-phenylethylamine (PHE), putrescine (PUT), cadaverine (CAD), histamine (HIS), tyramine (TYR), spermidine (SPD) and spermine (SPM).

‡Not detectable under the analytical condition adopted.

sausages produced in industrial plants. Mould counts were usually lower in Soppressata in which initial counts of 10^3 cfu g^{-1} were only occasionally found. In Salsiccia, higher mould counts were observed (sometimes more than 10^5 cfu g^{-1}). Nevertheless, the presence of these micro-organisms was characterized by a great variability (data not shown).

During ripening, the initial counts of *Staph. aureus* (which reached 10^3 cfu g^{-1}) decreased in Soppressata to final levels lower than 300 cfu g^{-1}. However, in two samples of Salsiccia (from D and F), dangerous levels of *Staph. aureus* (higher than 10^4 cfu g^{-1}) were found (data not shown).

A high variability was observed in the chemico-physical parameters between products at any given ripening. The initial pH ranged between 5·4 and 6·2, depending on the raw material used (Fig. 5), indicating that meats with different qualitative characteristics were used for sausage manufacturing. Also, the changes in pH during ripening depended on the type of sausage and its origin. In fact, in many artisanal products, the pH did not change significantly during ripening, while in products of industrial origin in which starter cultures and sugars were used, significant drops of pH were observed during the first part of ripening.

The moisture content and a_w values were rather low immediately after casing, and decreased constantly during

Table 2 Biogenic amine content in Salsiccia during ripening

Origin and time of ripening*		Biogenic amine content (mg kg^{-1} sausage)†						
		PHE	PUT	CAD	HIS	TYR	SPD	SPE
A	T0	n.d.‡	n.d.	n.d.	n.d.	n.d.	63·78	n.d.
	T1	6·99	n.d.	24·51	n.d.	n.d.	22·65	n.d.
	T2	n.d.	n.d.	25·88	n.d.	n.d.	n.d.	n.d.
B	T0	n.d.	n.d.	n.d.	n.d.	17·03	n.d.	n.d.
	T1	n.d.	n.d.	n.d.	n.d.	87·98	n.d.	n.d.
	T2	n.d.	77·74	2·54	n.d.	100·51	n.d.	n.d.
C	T0	n.d.	n.d.	n.d.	n.d.	n.d.	26·61	n.d.
	T1	n.d.	n.d.	n.d.	n.d.	n.d.	23·72	n.d.
	T2	n.d.	n.d.	n.d.	n.d.	27·58	41·03	n.d.
D	T0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	T1	n.d.	15·13	n.d.	n.d.	108·84	n.d.	n.d.
	T2	n.d.	60·20	n.d.	n.d.	177·85	n.d.	n.d.
E	T0	n.d.	n.d.	n.d.	n.d.	129·27	n.d.	n.d.
	T1	n.d.	17·93	38·64	n.d.	297·31	59·12	n.d.
	T2	n.d.	34·35	38·98	n.d.	338·85	57·17	n.d.
F	T0	n.d.	n.d.	n.d.	n.d.	264·56	85·54	n.d.
	T1	n.d.	n.d.	n.d.	n.d.	15·79	46·37	n.d.
	T2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
G	T0	n.d.	n.d.	n.d.	n.d.	10·33	n.d.	n.d.
	T1	n.d.	n.d.	n.d.	n.d.	41·31	n.d.	n.d.
	T2	n.d.	n.d.	n.d.	n.d.	25·29	26·62	n.d.
H	T0	n.d.	n.d.	n.d.	n.d.	30·01	n.d.	n.d.
	T1	n.d.	n.d.	n.d.	n.d.	18·43	15·08	n.d.
	T2	n.d.	24·97	n.d.	n.d.	78·30	38·93	28·08
I	T0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	T1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	T2	n.d.	n.d.	n.d.	n.d.	18·43	n.d.	n.d.
L	T0	n.d.	n.d.	n.d.	n.d.	56·94	23·48	n.d.
	T1	n.d.	n.d.	n.d.	n.d.	33·47	35·90	18·43
	T2	n.d.	n.d.	n.d.	n.d.	n.d.	23·93	n.d.

* Samples were analysed immediately after production (T0), after a week (T1) and at the end (T2) of ripening.
† The biogenic amines tested were 2-phenylethylamine (PHE), putrescine (PUT), cadaverine (CAD), histamine (HIS), tyramine (TYR), spermidine (SPD) and spermine (SPM).
‡ Not detectable under the analytical condition adopted.

ripening. Almost all the samples at the end of ripening were characterized by final a_w values lower than 0·90 (data not shown).

Biogenic amine production

The biogenic amine (2-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine) content of Salsiccia and Soppressata was evaluated immediately after casing and during ripening. The results are shown in Tables 1 and 2.

2-Phenylethylamine and histamine were found in only a few samples of Soppressata and their amounts were always rather low. Nevertheless, in two samples of Soppressata (B and G), histamine was present at the end of ripening at a level higher than 70 mg kg^{-1}.

Some Salsiccia (samples from A, E, F and L) and Soppressata (B, D and L) batches were characterized by a high biogenic amine content immediately after casing. In these samples, the most significant amines were spermine, spermidine and cadaverine in Soppressata, and tyramine and spermidine in Salsiccia. During ripening, tyramine was

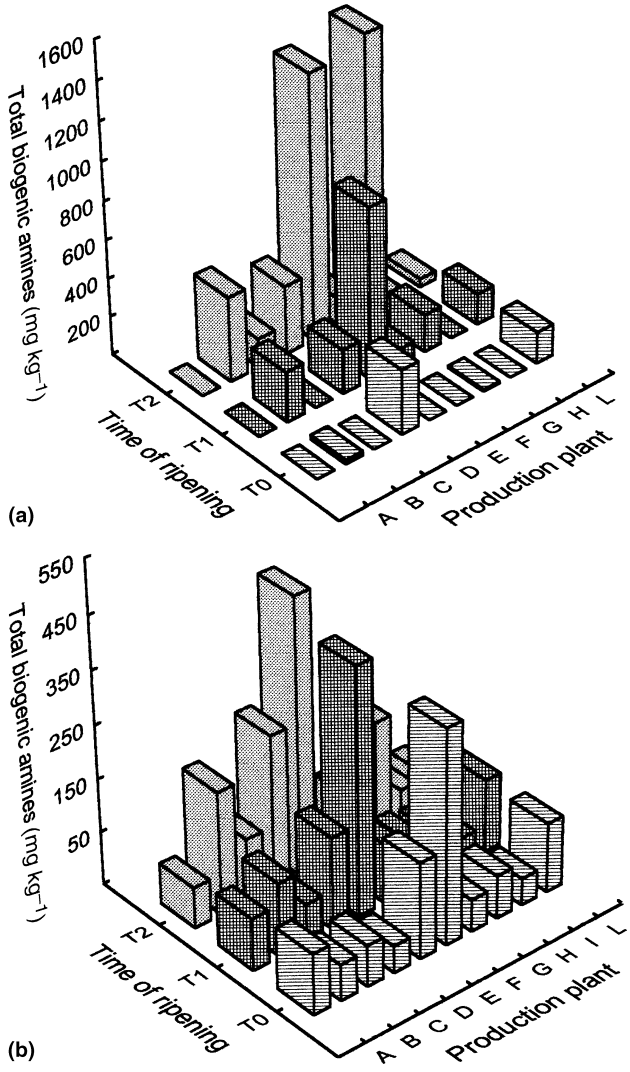

Fig. 6 Total biogenic amine amounts detected in Soppressata (a) and Salsiccia (b) during ripening

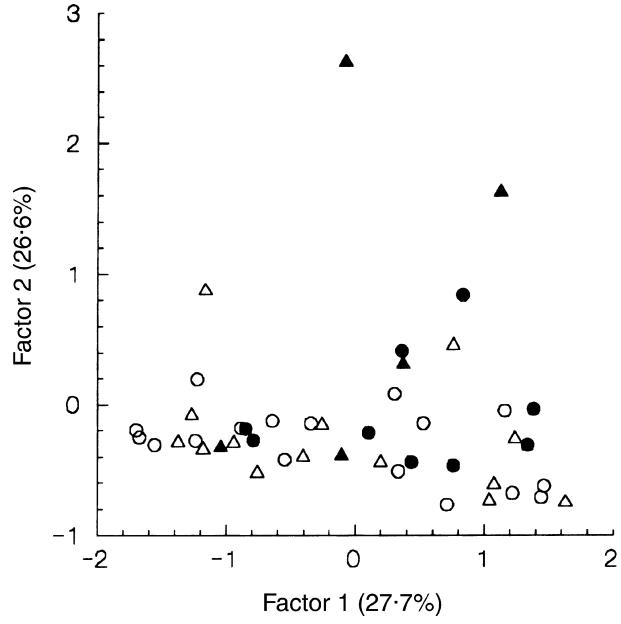

Fig. 7 Score plot obtained by Principal Component Analysis of physico-chemical analyses, microbial counts, histamine, tyramine, putrescine and cadaverine amine content and ripening time of samples of Salsiccia with (●) and without (○) starter cultures, and Soppressata with (▲) and without (△) starter cultures, during ripening. The first two principal components, explaining 54·2% of the total variability, are shown

usually present in a higher quantity, accounting for more than half of the total amines in most samples. Putrescine and, to a lesser extent, cadaverine, also increased during ripening. Spermidine and spermine did not increase significantly during ripening and a decrease in their concentrations was observed in many cases.

Figure 6 shows the evolution of the total biogenic amine content during ripening in Salsiccia and Soppressata. Soppressata had a higher mean biogenic amine content than Salsiccia. It is also interesting to note that the Soppressata and Salsiccia produced in industrial plant E, with the addition of starter cultures, had the highest amounts of biogenic amines. Other sausages produced at industrial scale (samples from B) showed a high amount of amines after ripening. By contrast, in the sausages manufactured artisanally in A, C, I and L, biogenic amines reached a lower final content. Finally, it is worth noting that in some products (Salsiccia produced in A, F and L, and Soppressata manufactured in L), biogenic amines decreased during ripening, suggesting the presence of micro-organisms with monoaminooxidase activities.

Principal component analysis

Principal component analysis with Varimax rotation was carried out on the ripening time, microbial counts, pH, a_W, level of putrescine, cadaverine, tyramine and histamine. These amines can be considered indicators of hygienic conditions and manufacturing practices (Eerola *et al.* 1998). The first four rotated components explained 75% of the total variance (respectively, 27·6, 26·6, 11·4 and 9·4 for components 1, 2, 3 and 4). A score plot for the first two components is shown in Fig. 7. The second component was associated with increasing amine levels (which were significantly correlated). The first component was positively associated with ripening time, counts of microstaphylococci, LAB and yeasts, and negatively

associated with a_w. No correlation was found between individual amine content, ripening time, pH or counts, nor with the sausage type or with the use of starter cultures.

DISCUSSION

The presence of biogenic amines in fermented food is generally considered as inevitable. During dry sausage manufacturing, endogenous and exogenous proteolysis takes place, and free amino acids are available to the amino acid decarboxylases of bacterial origin. Some studies (Vidal-Carou *et al.* 1990; Maijala *et al.* 1995a, b) have suggested that the amount of biogenic amines cannot be used directly as a qualitative parameter for the evaluation of the raw materials for dry sausages since the same raw materials can lead to very different amine levels in the final products. In the dry sausages analysed in this study, the presence of 2-phenylethylamine, and values higher than 20 mg kg^{-1} for putrescine, 40 mg kg^{-1} for cadaverine, 50 mg kg^{-1} for spermine and spermidine and 100 mg kg^{-1} for tyramine, were detected in less than 10% of the samples. Lower levels of these amines are considered usual in Salsiccia and Soppressata.

The great variability observed in the amine levels characterizing the different types of products and manufacture can be related to the hygienic quality of the raw material, the presence of micro-organisms with amino acid decarboxylase activity, the different chemico-physical conditions during ripening and processing, and the presence of micro-organisms with monoamine oxidase activity (Martuscelli *et al.* 2000). Several authors have also reported a great variability in amine contents (Bauer *et al.* 1989; Cantoni 1995; Hernandez-Jover *et al.* 1997b; Bover-Cid *et al.* 1999). In addition, many studies have reported a significant correlation between pH and amine contents, the lowest pH generally being characterized by highest amine levels (Eitenmiller *et al.* 1978; Halász *et al.* 1994; Bover-Cid *et al.* 1999) according to the hypothesis that biogenic amine production could be a protective mechanism for micro-organisms against acidic environmental conditions. The lack of increase in spermidine and spermine concentration during ripening is in agreement with the hypothesis that these polyamines are naturally present in meat and do not derive from the microbial decarboxylation of amino acids (Bardócz 1993; Hernandez-Jover *et al.* 1997a).

Sausage diameter affects the fermentation process, providing more anaerobic conditions for the meat mixture; salt concentration is usually lower and a_W is higher in sausages with a larger diameter (Bover-Cid *et al.* 1999). Vidal-Carou *et al.* (1990) observed that more anaerobic conditions favoured histamine production in sausages. This could explain the higher amount of biogenic amine found in Soppressata, which is characterized by a larger diameter than Salsiccia.

In this study, the commercial starter cultures did not inhibit the production of biogenic amines in the samples analysed. Similar results were reported by Rice and Koehler (1976) and Bauer *et al.* (1994), whereas other studies reported beneficial effects of starter cultures (Eitenmiller *et al.* 1978; Taylor *et al.* 1978). In Salsiccia and Soppressata manufactured in Basilicata according to industrial practices, high levels of tyramine, putrescine and cadaverine were detected; their total contents were similar to, or often higher than in artisanal products. Similar results were obtained in studies carried out on French industrial and artisanal sausages (Montel *et al.* 1999). Starter cultures are used extensively for meat fermentation to reduce the pH rapidly and ensure the safety of the product. Generally, they prevail on spoilage micro-organisms in the first stage of fermentation. However, the levels of biogenic amines in the industrial Salsiccia and Soppressata showed that starter cultures were unable to control the decarboxylase-positive strains, which are able to grow at the late phase of ripening. Biogenic amine formation in fermented sausages has often been related to amine-positive non-starter lactic acid bacteria (Maijala and Eerola 1993; Bauer *et al.* 1994; Paulsen and Bauer 1997). This could explain the increase in biogenic amine contents of sausages at the end of ripening. Microbial counts of artisanal dry sausages were quite similar to those in the industrial ones, suggesting that in spontaneous fermentation, the natural microflora was more efficient than starter cultures in inhibiting amino acid-decarboxylating micro-organisms. Micro-organisms capable of oxidizing amines to aldehydes can be present in the natural microflora of salami (Leuchsner *et al.* 1998), and *Staphylococcus xylosus* strains capable of oxidizing histamine and tyramine have been isolated from artisanal sausages (Martuscelli *et al.* 2000). The use of starter cultures able to compete with amino acid-decarboxylating micro-organisms and/or to reduce biogenic amine contents in association with good manufacturing practices, could be a way of obtaining products with the typical sensorial properties but with reduced risks for the health of consumers.

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