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International Journal of Food Microbiology 46 (1999) 95–104

International Journal of Food Microbiology

Effect of proteolytic starter cultures of *Staphylococcus* spp. on biogenic amine formation during the ripening of dry fermented sausages

Sara Bover-Cid, María Izquierdo-Pulido, M. Carmen Vidal-Carou*

Departament de Nutrició i Bromatologia — CERTA, Facultat de Farmàcia, Universitat de Barcelona, Avinguda Joan XXIII, s/n, 08028 Barcelona, Spain

Received 29 January 1998; received in revised form 24 July 1998; accepted 27 October 1998

Abstract

The effect of proteolytic starter cultures of *Staphylococcus carnosus* and *Staphylococcus xylosus* on biogenic amine production was examined during the fermentation process of dry sausages. Microbial counts (lactic acid bacteria, *Micrococcaceae* and *Enterobactenaceae*), pH, moisture and proteolysis-related parameters were also studied. The polyamines spermine and spermidine were the main amines found in the raw material and they only showed slight fluctuations during the fermentation. The four elaborated batches presented a significant ($P < 0.001$) formation of tyramine and putrescine. The main rate of amine production was during the first three days, when a sharp pH decrease and the development of lactic acid bacteria occurred. Sausages fermented with starters had lower amounts of tyramine than naturally fermented sausages (control), but differences in the *Micrococcaceae* counts were only significant during the first week of the ripening process. A slight formation of diaminopropane, cadaverine, agmatine, tryptamine and phenylethylamine was observed. The amounts of histamine were constant and remained below 0.5 mg/kg of dry matter, while serotonin, octopamine and dopamine were not detected. The sausages with *Staphylococcus* as starter culture showed strong proteolysis that was correlated with higher pH values than those of the control sausages. However, no positive correlation was found between the proteolysis index and biogenic amine production. Since proteolysis was stronger during the second half of the ripening process, the release of free amino acids as amine precursors occurred later than the early amine production.

Keywords: Biogenic amines; Fermented sausages; Starter cultures; *Staphylococcus* spp.; Proteolysis

1. Introduction

Low concentrations of biogenic amines (BAs) are naturally present in many foods and relatively high contents of some BAs can be present in fermented foods, such as fermented sausages, cheese, wine or beer. The microorganisms involved in the fermentation process could yield much higher BA amounts than those found in the corresponding raw materials,

*Corresponding author. Tel.: +34-3-402-4513; fax: +34-3-402-1896; e-mail: mcvidal@farmacia.far.ub.es

0168-1605/99/$ – see front matter
PII: S0168-1605(98)00170-6

because some BAs are the result of amino acid decarboxylation by microbial enzymes. BAs may represent a food-poisoning hazard, which in conjunction with additional promoting factors such as monoamine oxidase inhibitor (MAOI) antidepressant drugs, alcohol, other food amines, gastrointestinal diseases and genetically deficient detoxification systems (Mariné-Font et al., 1995).

The aromatic BAs (histamine, tyramine, serotonin, β-phenylethylamine, tryptamine) have been reported as vasoactive or psychoactive amines and they have been associated with food histaminic intoxications, food-induced migraines, and severe hypertensive crisis due to MAOI drug interactions (Mariné-Font et al., 1995). Moreover, diamines such as putrescine and cadaverine could generate carcinogenic nitrosamines in the presence of nitrites (Scanlan, 1983). Interest in cadaverine, putrescine, tyramine and histamine also lies in their potential as spoilage indicators of food (Sayem-El-Daher and Simard, 1985; Vidal-Carou et al., 1990; Hernández-Jover et al., 1996b). In addition, they may have unpleasant odours (Masson et al., 1996) and also, as Flores et al. (1996) reported, agmatine, spermine, spermidine, putrescine and cadaverine could inhibit the activity of muscle aminopeptidases.

Fermentation of dry sausages offers favourable conditions for BA formation, since the main required factors are present, i.e., there is growth of microorganisms over several days, a certain degree of proteolysis takes place giving rise to the presence of free amino acids as precursors of BA and, finally, the existence of an acidic environment can favour the amino acid decarboxylase activity of microorganisms. It has been reported that bacteria could be encouraged to produce decarboxylase enzymes in such acidic environments as part of their defense mechanisms against adverse conditions (Eitenmiller et al., 1978).

More information is needed about the critical factors for BA formation during the ripening of dry sausages to avoid the presence of hazardous levels of those compounds. Since microbial flora naturally present in the raw materials seem to have a strong influence on BA formation during ripening, the choice of good quality raw materials helps to minimize the number of amine-producing bacteria (Tschabrun et al., 1990; Maijala and Eerola, 1993; Halász et al., 1994). Furthermore, BA formation may be influenced by the thawing time of raw materials (Maijala et al., 1995a) and the technological conditions used to produce the sausages (Tschabrun et al., 1990; Maijala et al., 1995b), since they affect the growth and activity of the microorganisms present. An important factor suggested for preventing amine accumulation is the addition of adequate starter cultures to complete the fermentation. Starter cultures usually consist of one or several strains of lactic acid bacteria (LAB), *Micrococcaceae*, or a combination of both (Geisen et al., 1992).

Lactic acid bacteria are being widely used as starter cultures in fermented sausages. Attention to BA formation by LAB has been paid due to their role in the fermentation process. In addition, *Micrococcaceae* play an important role in fermented dry sausages, not only because they reduce the nitrate but also because they participate in other desirable reactions, such as lipolysis and proteolysis, which have great importance in the development of flavour (Selgas et al., 1988; Geisen et al., 1992). Various authors proposed the absence of BA formation as a selection criterion for starter cultures (Buckenhüskes, 1993; Straub et al., 1995). The BA formation activity in various genera and species has been established only in some cases. The production of BAs in meat and meat products has often been related to LAB, *Enterobacteriaceae* and *Pseudomonas* (Buckenhüskes, 1993). Although LAB have been reported as being responsible for amine production in meat products (Maijala and Eerola, 1993), the use of LAB as a starter culture might also hinder the formation of BAs during the sausage fermentation (Buncic et al., 1993; Maijala et al., 1995a; Hernández-Jover et al., 1997b).

In reference to BA formation by *Micrococcaceae*, Masson et al. (1996) reported that *Staphylococcus carnosus* and *Staphylococcus xylosus* can be used as safe starter cultures according to their tyramine production in vitro. However, Straub et al. (1995) found that *S. carnosus* had a remarkable potential to form BAs, but they did not find this for *S. xylosus*. Maijala et al. (1995a) observed that *S. carnosus* did not prevent the formation of tyramine and β-phenylethylamine by the contaminant LAB during sausage ripening. In spite of these data, little is known about the formation of BAs by *Micrococcaceae* in fermented sausages and the potential relationship with their proteolytic activity. Therefore, more attention

should be paid to these microorganisms, since several species are used in sausage manufacture.

Proteolysis during the fermentation of meat products is favoured by the denaturation of proteins as a consequence of the acidity, dehydration and the action of sodium chloride (DeKetelaere et al., 1974). Non protein nitrogen (NPN) compounds are formed as a result of the enzymatic activity of microorganisms on the myofibrillar proteins of meat (Klement and Cassens, 1974). Furthermore, DeMasi et al. (1990) reported that, during meat fermentation, the activity of endogenous but also microbial proteases modifies the composition of the NPN fraction. During the ripening of fermented sausages, an increase of NPN substances usually happens, which includes the production of free amino acids. Therefore, the determination of NPN and the proteolysis index can be useful in studying the potential relationship between proteolysis and BA formation in fermented sausages.

Since the presence of BAs in dry sausages is often related to the proteolytic activity of the fermenting microbial flora (Maijala and Eerola, 1993; Halász et al., 1994; Paulsen and Bauer, 1997), the objectives of the present work were: (1) to study the changes in BA levels during the fermentation and ripening processes of *fuet*, a typical small diameter (<3 cm) dry sausage from Spain, (2) to examine the effect on BA formation of different proteolytic strains of *Staphylococcus* spp., used as starter cultures and (3) to determine if there is a correlation between proteolysis-related parameters and BA levels in the sausages. Special emphasis will be placed on comparing the formation of BAs in control sausages (naturally fermented) and in sausages fermented with proteolytic starter microorganisms.

2. Materials and methods

2.1. Sausage preparation

The meat product used was a typical and extensively consumed dry fermented sausage from Spain called *fuet*. It contains pork meat and fat, curing agents, spices (particularly pepper) and other additives. The production of *fuet* involves the processes of mincing, mixing and stuffing the raw materials into artificial or natural casings, followed by a short fermentation and ripening period (between 15 and 25 days).

Sausage mixture (20 kg per batch) was made from slightly frozen (-4°C) shoulder pork meat and lard (80:20, w/w), and the following ingredients and additives (all per kg; from Sanofi Bio-Industries, Barcelona, Spain): 25.0 g of NaCl, 0.1 g of sodium nitrite, 0.2 g of sodium nitrate, 0.5 g of sodium ascorbate, 1.5 g of sodium pyrophosphate, 7.0 g of dextrose, 10.0 g of lactose, 10.0 g of powdered skimmed milk, 10.0 g of sodium caseinate, 3.0 g of pepper and 0.015 g of natural colouring. The starter culture, curing and colouring agents were dissolved in 50 ml of water before adding them to the meat mixture.

Sausage production was carried out in a pilot manufacturing scale plant in a Spanish Meat Technology Center ('Institut de Recerca i Tecnologia–Centre de Tecnologia de la Carn', Girona, Spain; IRTA–CTC). Pork meat and fat were ground to a particle size of 6 mm at refrigeration temperature (4–5°C) in a meat cutter (Tecmaq, Spain). The minced mixture was divided into four fractions for the four different batches, i.e., control or naturally fermented without the addition of starter cultures and batches I, II and III with different starter cultures of staphylococci.

Starters were cultures from IRTA–CTC consisting of *Staphylococcus carnosus* LTH 2102 for batch I, a microorganism that is widely used as a commercial meat starter, *Staphylococcus xylosus* CTC 3037 and *Staphylococcus xylosus* CTC 3050 for batches II and III, respectively. The last two strains were isolated and selected from commercial Spanish dry fermented sausages for their high proteolytic and lipolytic activity. Starter cultures were added to the sausages to achieve a final concentration of 10^6 colony forming units (CFU)/g of sausage mixture.

Each batch of sausage mixture was homogenized with the other ingredients using a mixer (TV-VALL, Amui-80, Spain) and the final mass of each batch was stuffed into natural pork casings. Each sausage weighed approximately 300 g. The mixer was cleaned with water, dried and disinfected with ethanol (70%) between batches. Before ripening, the surfaces of the sausages were imbibed with the mould *Penicillium nalgiovense* (PNT1-NG8 Rhóne-Poulenc Texel SA, Dangé Saint Roman, France), by

immersing them in a spore suspension, to prevent the growth of undesirable yeasts or moulds. The ripening program comprised three days of fermentation, at 20 to 22°C [93 to 98% relative humidity (RH)], followed by a ripening and drying period until day 21, at 16 to 18°C (70 to 80% RH).

2.2. Sampling

Triplicate samples of the raw materials before stuffing (zero time) and three sausages from each batch were taken as samples on the first, second, third, fourteenth and twenty-first days of ripening. Microbial counts, BAs, total nitrogen (TN), NPN, the proteolysis index (PI), pH and moisture were measured in the total of 84 samples studied.

2.3. Analysis

For microbial analysis, 10 g of sausage were aseptically removed from the casings, cut into small pieces, placed in sterile Stomacher bags and homogenized using a Stomacher Lab-blender (Model 400.cooke Laboratory Products, Alexandria, VA, USA) for 2 min with 90 ml of sterile diluent containing 0.1% Bacto Peptone [catalogue number (cat. no.), 0118-01-8; Difco, Detroit, MI, USA] and 0.85% of NaCl (cat. no. 1.06404; Merck, Darmstadt, Germany) in deionized water. Serial decimal dilutions were prepared. *Enterobacteriaceae* were enumerated on Violet Red Bile Glucose agar (cat. no. 1.10275; Merck) incubated with a double layer at 30°C for 24 h, lactic acid bacteria (LAB) on Man, Rogosa and Sharpe (MRS) agar (1.10660 Merck, Darmstadt, Germany) incubated at 30°C for 48 h anaerobically and *Micrococcaceae* on Manitol Salt Agar (cat. no. 10306-17-2; Difco) incubated at 30°C for 48 h.

Amines were determined by the high-performance liquid chromatography (HPLC) method described by Hernández-Jover et al. (1996a). The method is based on the formation of ion pairs between amines previously extracted with 0.6 N perchloric acid from 5 to 10 g of sample, and octanesulphonic acid present in the mobile phase. Separation is preformed using a reversed phase column, then a postcolumn derivatization with *o*-phthalaldehyde (OPA) is followed by spectrofluorimetric detection. The method allows one to quantify, by an external standard procedure, 13 BAs, i.e., tyramine (TY), histamine (HI), tryptamine (TR), serotonin (SE), β-phenylethylamine (PHE), octopamine (OC), dopamine (DO), putrescine (PU), cadaverine (CA), diaminopropane (DP), agmatine (AG), spermidine (SD) and spermine (SM). Samples for BA determination were stored at −20°C until required. Previous work in our laboratory showed that freezing is a suitable method for keeping samples before analysis (Veciana-Nogués et al., 1995).

TN and NPN were determined by the Kjeldahl method. NPN was extracted, from 5 to 10 g of sample without casings, with 0.6 N perchloric acid (Dierick et al., 1974). The PI was calculated as described by Astiasarán et al. (1990), as the quotient between NNP and TN multiplied by 100.

The pH was measured directly from samples using a microcomputerized pH meter (Crison 507, Spain), inserting the electrode into the middle of the sausage. Moisture was determined by drying the sample at 100–105°C until a constant weight was achieved.

2.4. Statistical analysis

Statistical analyses were performed using the SPSS 6.0.1. software for Windows (SPSS, Chicago, IL, USA). The analysis of variance (ANOVA test) and its corresponding contrasts were applied to determine if there were significant differences between the control and starter culture fermentation batches. ANOVA was also used to study the statistical significance of the changes that occurred during the sausage fermentation process. To determine relationships between time of ripening, BAs, NPN, PI, microbial counts and pH, linear regression analysis and Pearson's correlation coefficient were used.

All of the values shown in the tables are the mean values obtained from the three samples analysed for each sampling point, together with their standard deviation. Due to a large decrease in relative humidity during the ripening process, all values, except for microbial counts, refer to dry matter.

3. Results and discussion

3.1. Microbiological results

Microbial changes are summarized in Fig. 1. Initial counts of *Enterobacteriaceae* (1.13±0.04 log CFU/g) decreased during ripening by more than one

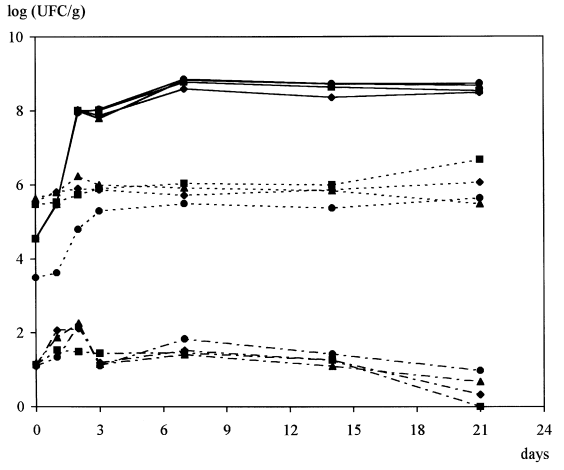

Fig. 1. Changes in counts of lactic acid bacteria (———), *Micrococcaceae* (- - - -) and *Enterobacteriaceae* (— – —) observed during the fermentation process of naturally fermented sausages (●), and sausages from batches I (■), II (▲) and III (♦).

logarithmic unit, ending up at less than 1 log CFU/g in all batches. This is a typical decrease due to the environmental conditions which make Gram-negative bacterial growth difficult (Hernández-Jover et al., 1997b). In contrast, LAB increased during the ripening process, reaching similar maximum levels at day seven in all types of sausage (8.77 ± 0.12 log CFU/g). Although in the naturally fermented batch the final levels of both *Enterobacteriaceae* and LAB were slightly higher than those found in the batches with starter culture added, the differences were not statistically significant. Therefore, the addition of *Micrococcaceae* as a starter did not prevent the development of LAB that were present naturally in the initial mixture, and LAB were able to dominate the whole ripening period in the four batches.

In the initial samples, *Micrococcaceae* profiles showed significant differences ($P < 0.001$) between the control and the inoculated batches, which reflected the starter culture growth. At the beginning of the fermentation, *Micrococcaceae* counts in batches I, II and III were more than two logarithmic units higher than those of the control batch, which contained only micrococci derived from raw materials. However, all types of sausage achieved similar counts of *Micrococcaceae* by day seven and no significant differences were found among the batches ($P > 0.05$) at the end of the ripening period.

Several authors have reported the prevalence of LAB in dry fermented sausage (Astiasarán et al., 1990; Maijala et al., 1995a; Hernández-Jover et al., 1997b), whereas other authors observed higher levels of *Micrococcaceae* than LAB at the end of the ripening process (Armengol et al., 1994).

3.2. pH, moisture and total nitrogen

The pH changed during curing of the sausages, as expected (Fig. 2). In all batches, the pH values showed an initial increase, followed by the characteristic fall and an eventual rise. However, the final rise was less marked in the control batch than in the other three batches. The first pH increase might have been due to the liberation of ammoniacal compounds as a result of endoprotease activity or the proteolytic microbial flora present in the raw meat (Armengol et al., 1994). Moreover, during the first days, the LAB population might not have been high enough to cause a pH decrease. The final rise in pH might have been due to the formation of NPN basic compounds and ammonium, together with the buffering action of the proteins (Demeyer et al., 1979; Astiasarán et al., 1990). It has been reported that, at the end of the ripening process, there is no correlation between lactic acid production and pH, and it has been suggested that pH increase is related to proteolytic activity, with the formation of peptides, amino acids and ammonia (Dierick et al., 1974; Demeyer et al., 1979). In addition, Marchesini et al. (1992) reported that the desirable growing of mould on the surface of the sausage can also contribute to the final pH rise. Here, a correlation between pH values during the second part of the ripening process (from day 7 to day 21) and PI values was found ($P = 0.008$), and

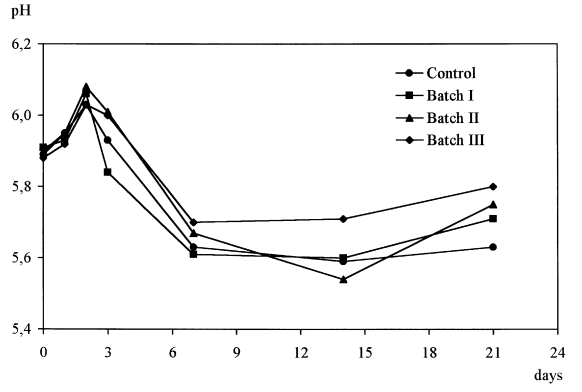

Fig. 2. Changes in pH values during the ripening of sausages from different batches.

also with NPN ($P = 0.03$). Indeed, batches with starter culture, which presented the highest PI, showed the highest pH values ($P = 0.03$) in comparison with the naturally fermented batch.

The moisture of the sausages decreased in a similar way in all sausages. The water content of the raw material was 61.5±0.6% and, at the 21st day of ripening, it was 25.4±0.4%. TN content was constant during the ripening process in all of the sausages, at about 7.2±0.2 g/100 g of dry matter. Neither of the two parameters, moisture or TN, showed significant differences ($P > 0.05$) among batches, which agrees with the fact that all of the batches were processed under the same temperature and relative humidity conditions and they were made from the same raw materials.

3.3. Proteolysis-related parameters

The initial values of NPN were similar in all of the batches [5.1±0.5 mg/g (w/w) in dry matter], while certain differences began on day seven. At the end of the ripening process, sausages from batches II and III showed the highest ($P < 0.001$) NPN contents (6.4±0.2 and 6.8±0.5 mg/g, respectively), followed by batch I (5.8±0.5 mg/g) and finally the control sausages (5.0±0.5 mg/g), which did not show any significant increase. Johansson et al. (1994) also reported an increase in NPN content during the ripening of fermented sausages with *S. xylosus* as the starter culture. In contrast, Klement et al. (1973) did not observe changes, probably due to the use of pediococci as the starter culture, which do not have any special proteolytic activity (Geisen et al., 1992).

The PI of the different sausages followed an evolution profile similar to that of the NPN. Although batches II and III presented lower staphylococci counts than batch I, these batches had higher PI values than the control and batch I (Fig. 3). Therefore, proteolytic activity in *S. xylosus* was stronger than in *S. carnosus*. These results indicated that the increase in proteolysis, with the consequent rise pH due to the production of basic NPN compounds, is related to the proteolytic activity rather than the number of proteolytic microorganisms.

3.4. Biogenic amine formation

The raw material mixture was analyzed before stuffing since it might contribute to microbial counts and BAs in sausages. Like microbial counts, the amounts of BAs were very low (Table 1). The several BA indices proposed by several authors (e.g. Sayem-El-Daher and Simard, 1985; Hernández-Jover et al., 1996b) indicated that the raw materials were of good hygienic quality.

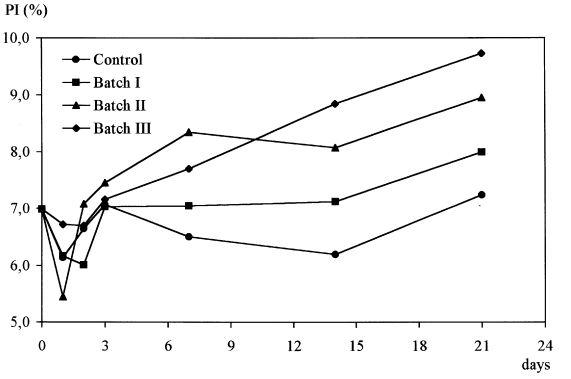

Fig. 3. Changes in the proteolysis index (PI) during the ripening of four different batches of fermented sausage.

Polyamines SM and SD were the main amines found in all of the initial samples and they did not change significantly during the ripening, nor between batches. Initial amounts of 47.9±32 1.0 mg/kg of SM and 4.0±0.2 mg/kg of SD showed a slight fluctuation during the fermentation process. Final values were 41.6±2.4 mg/kg for SM and 5.6±0.7 mg/kg for SD. Although both polyamines have been reported as microcomponents of meat (Bardócz, 1995; Halász et al., 1994; Hernández-Jover et al., 1997a) and are found in meat products (Tiecco et al., 1995; Cantoni, 1995; Hernández-Jover et al., 1997a), there are not many studies on their evolution during sausage manufacturing. Maijala et al. (1995a); Paulsen and Bauer (1997); Hernández-Jover et al. (1997b) reported slight variations of SM and SD levels. In contrast, Shalaby and Abd-El-Rahman (1995) did not find SM or SD during sausage fermentation. A decrease in SM during the ripening process could be the result of the use of this polyamine as a nitrogen source by the microorganisms (Bardócz, 1995). Our results could not demonstrate this hypothesis despite the decrease in SM levels observed (results not shown).

The four batches showed a significant ($P < 0.001$) formation of TY and PU (Figs. 4 and 5). The formation of TY was the one that showed the greatest differences between batches ($P < 0.001$).

Table 1
Changes in tryptamine, cadaverine and diaminopropane contents (mg/kg of dry matter) during the ripening of dry fermented sausages with different starter cultures

Time	Tryptamine				Cadaverine				Diaminopropane			
(days)	Control	Batch I	Batch II	Batch III	Control	Batch I	Batch II	Batch III	Control	Batch I	Batch II	Batch III
0	nd	nd	nd	nd	nd	nd	nd	nd	1.68	1.79	1.68	1.80
	–	–	–	–	–	–	–	–	(0.30)	(0.35)	(0.23)	(0.18)
1	0.39[a]	nd	nd	nd	nd	0.02	0.03	nd	1.92	1.99	2.20	1.75
	(0.15)[b]	–	–	–	–	(0.02)	(0.06)	–	(0.60)	(0.40)	(0.35)	(0.09)
2	0.35	0.23	nd	0.24	nd	0.03	0.07	nd	2.12	1.66	1.68	1.74
	(0.15)	(0.03)	–	(0.08)	–	(0.00)	(0.03)	–	(0.02)	(0.06)	(0.13)	(0.08)
3	0.93	1.09	0.40	0.56	0.57	0.56	0.10	0.04	2.44	2.33	2.29	2.36
	(1.08)	(0.08)	(0.11)	(0.28)	(0.40)	(0.28)	(0.00)	(0.00)	(0.45)	(0.15)	(0.05)	(0.17)
7	1.32	1.33	0.75	0.94	1.17	0.90	0.22	0.12	2.18	2.53	2.80	2.26
	(0.39)	(0.37)	(0.27)	(0.08)	(0.29)	(0.49)	(0.05)	(0.11)	(0.33)	(0.24)	(0.09)	(0.31)
14	1.39	1.16	0.31	0.89	1.24	1.11	0.69	0.45	2.05	1.87	2.69	2.63
	(0.72)	(0.05)	(0.08)	(0.76)	(0.39)	(1.01)	(0.11)	(0.26)	(0.12)	(0.15)	(0.13)	(0.45)
21	1.39	1.19	0.35	0.84	1.76	1.36	0.61	1.27	3.06	2.57	2.54	2.47
	(0.38)	(0.10)	(0.25)	(0.14)	(0.20)	(0.15)	(0.32)	(0.49)	(0.03)	(0.15)	(0.45)	(0.45)

[a]: Mean values. [b]: standard deviation. nd: not detected.

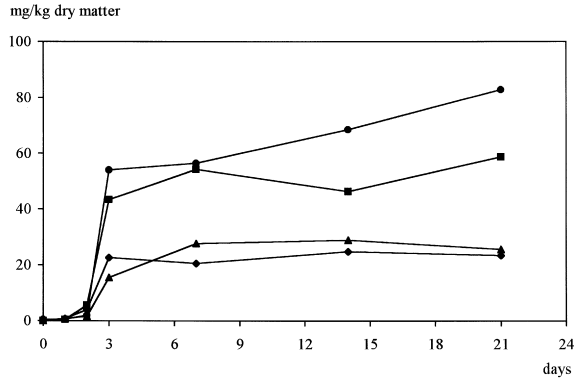

Fig. 4. Changes in tyramine levels (mg/kg of dry matter) during the ripening of naturally fermented sausages (●), and sausages from batches I (■), II (▲) and III (♦).

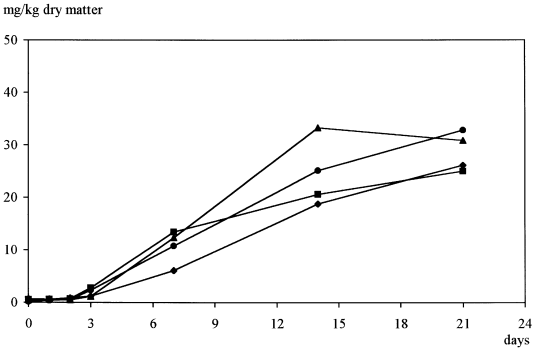

Fig. 5. Changes in putrescine levels (mg/kg of dry matter) during the ripening of naturally fermented sausages (●), and sausages from batches I (■), II (▲) and III (♦).

The highest amounts were found in naturally fermented sausages followed by batch I and finally batches II and III, both showing less than a third of the final TY content found in control sausages (Fig. 4). In all sausages, the major rate of TY formation was between the second and third day, when the pH fell and the LAB counts were 10^8 CFU/g. Also, at this stage, the highest content of *Enterobacteriaceae* was detected. Indeed, the reduction of *Enterobacteriaceae* occurred after the second week of ripening and a slight increase in TY occurred only in the control batch after this period. Other authors (Ramantanis et al., 1985; Treviño et al., 1997; Hernández-Jover et al., 1997b) also described a relationship between pH and TY formation.

The other amine formed in significant amounts was PU ($P < 0.001$), but there were no statistical differences among the four batches. The production of PU went up after the first week (Fig. 5), when the pH values dramatically dropped and LAB reached maximum levels. In contrast with TY, no formation of PU was observed during the first three days of fermentation, while a gradual increase in levels of PU occurred during the whole ripening process.

According to linear regression analysis, TY and PU contents showed a significant correlation ($P < 0.001$) with pH values and LAB counts. As mentioned earlier, BA production has been associated

with protective mechanisms of microorganisms against an acidic environment (Eitenmiller et al., 1978). This agrees with our results since the control fermentation batch showed the lowest pH and the highest total amine content, contrary to what occurred in the *S. xylosus* batches (II and III). Buncic et al. (1993) indicated the possibility that extensive proteolysis, before or during the fermentation process, could be one of the causes of the excessive formation of TY. However, no positive correlation between PI or NPN and BA production has been found here. Ramantanis et al. (1985) proposed TY as a freshness indicator of ripened meat products, due to its constant increase during storage. In our study, proteolysis increased during the second half of the process (after day 14) and, thus, the accumulation of free amino acids occurred later than the early amine production. Therefore, it is possible that those amino acids might be decarboxylated later, such as during the storage of sausage.

In the present study, the starters used yielded lower TY than the starters *Lactobacillus plantarum* and *Pediococcus pentosaceus*, both of which were used in our previous work (Hernández-Jover et al., 1997b), together with *Micrococcus carnosus*. Other sausage fermentations, with starters containing *S. carnosus*, generally yielded similar TY levels (Maijala et al., 1995a; Treviño et al., 1997). No data have been found about BA production in sausages made with *S. xylosus* as the starter.

Only slight formation of TR, CA and DP was detected after day two and usually at levels lower than 3 mg/kg. However, when staphylococci starter cultures were not used (control batch), slightly higher formation rates could be observed (Table 1). DP has been reported as an intermediate metabolite of the degradation of polyamines SD and SM (Muskiet et al., 1995) and also as a meat putrefactive indicator (Sayem-El-Daher and Simard, 1985). In our case, no evident relationship was found between DP and the polyamines. Little information is available about the presence of DP in dry sausages. Only Tiecco et al. (1995) described the presence of DP in Italian meat products. Important CA formation during the ripening of dry sausages was observed by Dierick et al. (1974); Maijala et al. (1995a). In contrast, Treviño et al. (1997) did not find CA in sausages fermented with *S. carnosus*. In our previous work (Hernández-Jover et al., 1997b), the naturally fermented batch showed more than 15 mg of CA/kg dry matter, while in the present study, much lower amounts (<2 mg/kg) were found in control and in starter fermented sausages. Therefore, it seems that not only starter cultures could reduce CA formation, but raw materials and ripening conditions could also have an effect.

Although several authors observed HI formation during the ripening of some fermented meat products (Tschabrun et al., 1990; Shalaby and Abd-El-Rahman, 1995; Cantoni, 1995; Paulsen and Bauer, 1997), the sausages studied here were nearly free of HI. HI amounts were never more than 0.5 mg/kg in any of the samples. Since remarkable HI contents were found in similar ripened meat products analyzed by us (Hernández-Jover et al., 1997a), more information is necessary about the particular factors that affect HI production.

The amines SE, OC and DO were not detected in any sample and PHE was only found at very low levels (0.5 mg/kg) in the last samples from batch I.

McCabe (1986) reported that the consumption of 6 mg of TY can produce a slight reaction, whereas 10–25 mg can cause severe headaches and hypertensive crises in patients under MAOI drug treatment. According to this, the TY amounts in the control sausages could provoke some symptoms when sensitive individuals ate more than 60 g of that sausage. In contrast, the sausages made with starters appeared to have lower TY contents, which would not produce toxicological effects. Therefore, it seems that such proteolytic microorganisms can be used as safe starter cultures to reduce BA production during the ripening of dry fermented sausages.

There is a need to study the ability of selected micrococci strains to grow and produce desired changes in the presence of the lactobacilli traditionally used as starters and to determine if they are also able to hinder BA formation during the ripening and storage of dry fermented sausages. Likewise, it is necessary to study the possibility of reducing the formation of TY during the first days of fermentation by using an adequate starter, strain and concentration, as well as technological conditions (temperature and humidity) that better favour starter development. The evolution of BAs during the storage of such products should also be studied, since proteolysis liberates free amino acids that might be decarboxylated by the remaining enzymatic activity.

Acknowledgements

The authors thank the 'Institut de Recerca i Tecnologia–Centre de Tecnologia de la Carn' (Monells, Girona, Spain) for the sausage elaboration and especially Departament of Microbiology and Biotechnology for its collaboration in the microbiological analysis. This work was financed by the 'Comisión Interministerial de Ciencia y Tecnologia' (ref. ALI95-0890-C04-01) of the 'Ministerio de Educación y Ciencia', Spain.

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