Contribution of the Microbial and Meat Endogenous Enzymes to the Free Amino Acid and Amine Contents of Dry Fermented Sausages

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The role of the starter culture and meat endogenous enzymes on the free amino acid and amine contents of dry fermented sausages was studied. Five batches of sausages were prepared. The control batch was manufactured with aseptic ingredients without microbial inoculation. The other four experimental batches were manufactured with aseptic ingredients inoculated with *Lactobacillus plantarum* 4045 or *Micrococcus*-12 or *L. plantarum* 4045 and *Micrococcus*-12 or *L. plantarum* 4045 and *Staphylococcus* sp. Their effects on pH, a_w, myofibrillar proteins, and free amino acid and amine contents were studied. Sausages inoculated only with *L. plantarum* 4045 or with this starter combined with a *Micrococcaceae* had the lowest pH as a result of carbohydrate fermentation. In all batches similar patterns were observed for myofibrillar proteins and free amino acids which could indicate that meat endogenous proteases play an important role in proteolytic phenomena. No changes were observed in the amine fraction, indicating that the strains used as starter cultures did not show amino acid decarboxylase activity.

Keywords: *Dry fermented sausages; ripening; proteolysis; starter cultures; endogenous enzymes*

INTRODUCTION

Ripening of dry fermented sausages involves several changes in the main components of the products which generate their characteristic taste and aroma. In the fermentation phase, lactic acid accumulation due to carbohydrate fermentation produces a decrease in pH and contributes to development of the texture and acid flavor. During ripening, the protein breakdown reactions yield nonproteic nitrogen compounds such as small peptides and free amino acids which participate in the taste of the sausage and also act as precursors of volatile compounds. Thus, free amino acids may be decarboxylated and deaminated to give rise amines and organic acids respectively, which may in turn lead to other changes, yielding other flavor compounds.

The reactions involved in the proteolysis of meat proteins are catalyzed by endogenous enzymes (Pezacki and Pezacka, 1986; Toldrá et al., 1992) together with the proteases produced by microorganisms. In dry fermented sausages, the proteolytic phenomena of microbial origin have been traditionally attributed to proteases of *Micrococcaceae* and lactic acid bacteria (Guo and Chen, 1991). Selgas et al. (1993) have demonstrated in vitro the existence of significant intra- and extracellular proteolytic activity of several strains of micrococci isolated from dry fermented sausages, and the latter, extracellular activity, was generally higher than the former. Other authors (Montel et al., 1992; Hammes et al., 1995) showed that some *Staphylococcus* species were hardly able to hydrolyze proteins. In relation to lactic acid bacteria, Montel et al. (1992) observed that several species of *Lactobacillus* and *Pediococcus* used as starter cultures played no role in protein hydrolysis. Nevertheless, the intracellular peptidasic activities of lactic acid bacteria could contribute to the increased levels of free amino acids (Montel et al., 1992). Despite this, the lactic acid bacteria are considered to be weakly proteolytic compared with many other groups of bacteria (*Bacillus*, *Proteus*, *Pseudomonas*, coliforms) (Kröckel, 1995) which may be present in the fresh meat and other ingredients, but there is no direct evidence that these microorganisms contribute significantly to the flavor of fermented meat and it is doubtful whether their enzymes play any role at all in meat proteolysis (Law and Kolstad, 1983).

On the other hand, several authors (Demeyer et al., 1992; Verplaetse et al., 1992; Toldrá et al., 1993) have observed an intense proteolysis due to cathepsin activities during the ham and dry sausage ripening process. Toldrá et al. (1992) in a model of cured meat systems studied the influence of the curing agents and several processing parameters on the porcine muscle cathepsins B, H, L, and D. These authors concluded that cathepsins B, L, and D were active in the mixing and fermentation stages while only cathepsin L activity remained significant in the drying stage.

In conclusion, it seems unclear to what extent microbial and/or meat endogenous enzymes participate in the proteolytic changes during the ripening of dry fermented sausages. The aim of this study was, therefore, to determine the source of these enzymes during the ripening process.

MATERIALS AND METHODS

Preparation of Sausages. Bovine M. semitendinosus, porcine M. longissimus dorsi, and lard (6−8 cm thickness) from Iberian pig were aseptically obtained as described by Ordóñez et al. (1989). The muscle exterior surface was sterilized by searing. Then, the burnt tissue was removed down to a depth

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of about 3−5 mm using sterile instruments. Likewise, the skin of Iberian pig lard was burnt and flakes of internal fat (from a depth of about 5 cm) were aseptically removed. Solutions of glucose and lactose were sterilized by filtering (0.45 μm); appropriate solutions of phosphates, NaCl, nitrates, and nitrites were autoclaved and then concentrated by lyophilization. All the procedures of sausage manufacture were performed in a laminar flow cabinet (Telstar model CE A, Tarrasa, Barcelona, Spain), and the operators used sterile surgery masks and gloves. The meat and fat were comminuted in a manual grinder (diameter of the plate holes, 5 mm) previously sterilized by autoclaving. The ingredients were aseptically mixed to give the final composition (%): pork 40; beef 40; fat 13; glucose 1.5; lactose 0.5; phosphates 0.3; NaCl 2.5; KNO_3 0.02; $NaNO_2$ 0.01. Sterilized water was used to dissolve the lyophilized ingredients.

The following batches were prepared: batch C, control (sterile ingredients and aseptic manipulation without inoculation); batch L, same as batch C but inoculated with *Lactobacillus plantarum* 4045 isolated from Spanish dry fermented sausages (Sanz et al., 1988); batch M, inoculated with an "in vitro" lipolytic and proteolytic strain of *Micrococcus*-12, isolated from Spanish dry fermented sausages (Selgas et al., 1993); batch L+M, inoculated with *L. plantarum* 4045 and *Micrococcus*-12; and batch L+S, inoculated with *L. plantarum* 4045 and *Staphylococcus* sp., the latter was kindly given by Dr. M. L. García from the Faculty of Veterinary Science (León, Spain).

Brain heart infusion (BHI) and de Man, Rogosa, Sharpe (MRS) broths (Oxoid, Unipath Ltd., Basingstoke, Hampshire) were used, respectively, for *Micrococcaceae* and lactobacilli growth. One milliliter of a bacterial suspension (absorbance at 600 nm = 1.0, $\sim 10^7$ cfu/mL), washed twice and resuspended in sterile physiological saline solution, was inoculated into the corresponding batch. The final mixture was aseptically stuffed into artificial casings soaked (48 h) in sterile 15% (w/v) saline solution and washed just before use in abundant sterile distilled water. After this treatment the bacterial load of the casings was lower than 10^2 cfu/cm^2. The casings were, likewise, aseptically stuffed with a manual grinder in which the cut plate was replaced by an appropriate sterile funnel. The weight of each batch was about 2.5 kg which was made into 10 sausages of 250 g each. They were ripened in a laboratory ripening cabinet (Kowell model CC3AFY) programmed for 48 h at 22 °C and a relative humidity (RH) of 90%, followed by 48 days at 12 °C and 85% RH. Samples were taken at different times of ripening.

Microbial Analyses. Total viable counts (TVC) were determined on Plate Count Agar (PCA) (Oxoid) and *Micrococcaceae* on mannitol salt agar (MSA) (Oxoid), both incubated at 32 °C for 2 days. Lactobacilli were enumerated on double-layer MRS agar (Oxoid) at pH 5.6 and incubated for 4 days at 32 °C.

Physical and Chemical Analyses. Measurement of the pH was done with a Crison 2001 pH meter (Crison Instruments S.A., Barcelona, Spain) in a homogenate prepared with an aliquot of sausage (1.5 g) and distilled water (10 mL). Dry matter was determined by drying at 110 °C to constant weight. A Decagon CX1 dew point hygrometer (Decagon Devices Inc., Pullman, WA) was used to measure the water activity (a_w) at 25 °C.

Myofibrillar proteins were extracted according to Toldrá et al. (1993) and after extraction they were exhaustively dialyzed against distilled water and then lyophilized. Proteins were analyzed by PAGE using a "Phast-system" electrophoresis equipment (Pharmacia LKB, Uppsala, Sweden). Sodium dodecyl sulfate (SDS)−PAGE was performed on 20% homogeneous gels in accordance with the manufacturer's instructions. The electrophoretograms were run with standards (Sigma Chemical CO, St. Louis, MO) of known molecular weight [α-lactalbumin (14.2 kDa), trypsin-inhibitor (20.1 kDa), trypsinogen (24 kDa), carbonic anhydrase (29 kDa), ovalbumin (45 kDa), phosphorylase B (97.4 kDa), β-galactosidase (116 kDa), and myosin (205 kDa)]. Gels were stained with Coomassie Blue and the intensity of the bands was measured at 610 nm in a Shimadzu CS-9000 densitometer (Shimadzu Corporation, Kyoto, Japan).

For free amino acid (FAA) determination, samples of sausage (10 g) were homogenized with 50 mL of distilled water. As internal standard, an L-norleucine (Sigma) solution (10 mg/mL) in 0.2 M HCl was added. The homogenate was centrifuged at 6500*g* at 4 °C for 6 min, and the supernatant was filtered through a Whatman no. 2 paper. An aliquot (10 mL) of the supernatant was mixed with the same volume of 10% sulfosalicylic acid solution. The mixture was left at 4 °C for 17 h. Insoluble materials were removed by filtration through Whatman paper no. 2. Phenylthiocarbamil derivatives of the different amino acids were obtained according to Yang and Sepúlveda (1985) and analyzed by HPLC in a Beckman Model 332 apparatus equipped with Model 110 A pumps, a Beckman Model 160 UV detector operating at 254 nm and a column (25 cm × 4.6 mm) filled with ODS-2 on Spherisorb. Chromatography was carried out at 35 °C. Peak resolutions were accomplished using a gradient elution, with the mobile phase in pump A consisting of 0.05% triethylamine in pH 6.8, 0.03 M sodium acetate buffer, and that in pump B of acetonitrile/water (9/1) (v/v). The mobile phase began at a flow rate of 1.0 mL/min at 4% B and increased to 38.5% B over 32 min, and then increased to 99% B and a flow rate of 1.5 mL over 5 min, and was finally held at this ratio for 15 min. At the end of the isocratic period B was decreased to 4%. Free amino acid identification was made by comparing the retention times with those of authentic standards (Sigma) and quantified by peak area measurement against the normalized internal standard.

Nonvolatile amines were extracted according to the methodology of Spinelli et al. (1974) and analyzed by HPLC in the same chromatograph and column equipped with a Spectra/flo fluorometer. Chromatography was carried out according to the method of Ordóñez et al. (1991).

Due to the complexity of aseptic sausage elaboration, results are only the mean of two replications performed with different ingredients but the same formulation and starters. Microbial and physical−chemical analysis were done in duplicate.

RESULTS AND DISCUSSION

Microbial Flora. The microbial changes throughout ripening have been reported in a preceding paper (Hierro et al., 1997). At day 0 the control batch (aseptically manufactured) presented total flora, lactobacilli, and *Micrococcaceae* counts lower than 10^3 cfu/g. After ripening, counts did not reach values higher than 10^5 cfu/g. This means that in the case of lactobacilli (the dominant microbiota), the counts were about 5000-fold lower than those reached in conventional dry sausages (Sanz et al., 1988; Selgas et al., 1988).

All lactobacilli inoculated dry fermented sausages (L, L+M, and L+S) showed similar MRS counts. Lactobacilli was, as usual, the dominant microbiota with values close to 10^7 cfu/g at day 0 and 10^8−10^9 cfu/g after 3−5 days of ripening, leveling off toward the end of the process. Total viable counts showed a similar trend to the lactobacilli.

Sausages inoculated with *Micrococcaceae* (M, L+M, and L+S) also showed a similar pattern in the MSA counts. Counts started at around 10^6 cfu/g at day 0 reaching 10^7 cfu/g after 4−5 days. Afterward, counts showed a slightly decreasing trend throughout ripening similar to that recorded in conventional sausages (Selgas et al., 1988; Samelis et al., 1993).

Batch M showed a maximum lactobacilli count of around 10^3 cfu/g and batch L reached a maximum MSA count of 10^4 cfu/g, both counts were always much lower (>10000-fold for lactobacilli and about 1000-fold for micrococci) than those described in conventional sausages (Lücke, 1987; Sanz et al., 1988).

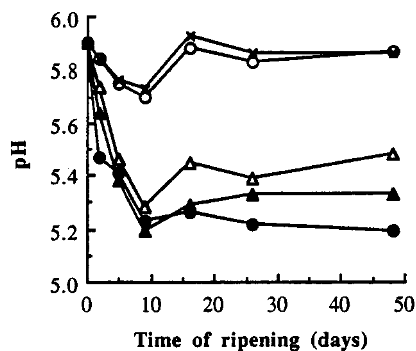

Figure 1. Changes in pH during the ripening of experimental dry fermented sausages: (×) control, aseptic batch without inoculation; (△) aseptic batch inoculated with *L. plantarum* 4045; (○) aseptic batch inoculated with *Micrococcus*-12; (▲) aseptic batch inoculated with *L. plantarum* 4045 and *Micrococcus*-12; and (●) aseptic batch inoculated with *L. plantarum* 4045 and *Staphylococcus* sp.

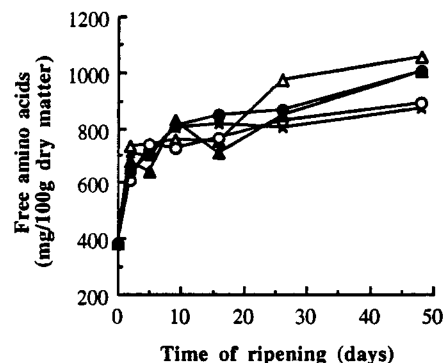

Figure 2. Changes in the free amino acid content during the ripening of experimental dry fermented sausages: (×) control, aseptic batch without inoculation; (△) aseptic batch inoculated with *L. plantarum* 4045; (○) aseptic batch inoculated with *Micrococcus*-12; (▲) aseptic batch inoculated with *L. plantarum* 4045 and *Micrococcus*-12; and (●) aseptic batch inoculated with *L. plantarum* 4045 and *Staphylococcus* sp.

Due to microbial activities the experimental sausages developed two different colors. Those related to *Micrococcaceae* (M, L+M, and L+S) showed a typical deep pink color reflecting the reduction of nitrates to nitrites and the formation of nitrosylmyoglobin. The other two batches (C and L), without inoculation of *Micrococcaceae*, showed a brown color, indicating no nitrate or nitrite reduction. The absence of the pink color in batches C and L means that the contaminating microbial population was not large enough to affect sausage ripening and that its effects were probably negligible.

pH. Changes in pH in the dry fermented sausages during ripening are shown in Figure 1. The pH of the initial mixture prepared for sausage manufacture was similar in all cases (around 5.9). After casing, two clearly different trends were observed depending on the microorganisms inoculated. The pH of batches inoculated with lactobacilli (L, L+M, and L+S) are within the interval of values reported for this type of product (Ferrer and Arboix, 1986; Genigeorgis et al., 1986; Sanz et al., 1988; Nagy et al., 1989). Batches C and M showed higher pH values, which are obviously due to the absence of sugar fermentation.

Moisture and Water Activity (a_w). Both parameters have been reported in a previous paper (Hierro et al., 1997). No differences between batches were observed. All of them showed a similar trend with moisture ranging from 63 to 25−30% and a_w from 0.97 to 0.85.

Myofibrillar Proteins. All batches showed the same electrophoretic pattern when they were compared at the same ripening time (data not shown). None of the bands detected at the beginning of the ripening disappeared in all batches during the ripening, and some new bands of low molecular weight (<18 kDa) were observed after 5 days of the process. Similar results have been described by Díaz et al. (1996, 1997), but the data of the present experiments indicate that endogenous meat enzymes are mainly responsible for myofibrillar proteolysis.

Free Amino Acids (FAA). Figure 2 shows the changes in the total FAA content during ripening. In all batches an increase of these substances was observed throughout the ripening process. During the first 10−20 days of ripening the levels of FAA showed an important increase (from 380 to about 800 mg/100 g dry matter). After this time, the amount of amino acids released was considerably reduced and this decrease was progressive until the end of ripening. Individual free amino acid concentrations of experimental sausages are given in Table 1. From Figure 2 and Table 1, it is evident that in the first stage of ripening (first 5 days) all batches followed a similar pattern. This indicates that proteolysis was mainly due to meat tissue proteases and not to microbial enzymic activities. After about the first 10−20 days there was a slight divergence in the graphs corresponding to three batches inoculated with lactobacilli (independent of whether one of the two *Micrococcacae* were also inoculated), the control batch, and the batch exclusively inoculated with *Micrococcacae*. This difference was not very pronounced since the rise produced at the end of ripening compared to day 0 in the level of free amino acids was 2.6× in batches L, L+M, and L+S and 2.3× in batches C and M. At the end of ripening the ratio of the values of the free amino acids with the highest (L) and lowest (C) concentrations was approximately 1.2×. This difference was not sufficiently large to be able to attribute it to the effect of the microorganisms inoculated. It is noteworthy that in batch C, produced aseptically, the maximum total load of microorganisms was around 10^5 cfu/g and in batch L was the same as in the other batches inoculated with lactobacillus (L+M and L+S), exceeding the level of 10^8 cfu/g (1000-fold higher than the level of bacth C). In light of this difference if there had been a clear proteolysis of microbial origin this would have undoubtedly been reflected in a rise in the level of free amino acids in these batches. One can, therefore, conclude that the proteolysis which takes place during sausage ripening is almost completely due to endogenous proteases from the muscle tissue.

These results coincide with those of other authors (Verplaetse et al., 1992; Johansson et al., 1996) who also studied proteolytic activity in dry fermented sausages by manufacturing products to which microbial growth inhibitors (antibiotics) or protease inhibitors (pepstatin) had been added. They described that the latter substances inhibited degradation of actin and myosin to a greater extent than when microbial growth was inhibited by the addition of antibiotics. Johansson et al. (1996) demonstrated that the addition of antibiotics only inhibited peptide and amino acid release to a small extent and this inhibition was significantly greater when pepstatin was added. These authors concluded that most peptides and amino acids were released as a

Table 1. Changes in the Free Amino Acid Contents during the Ripening of Experimental Dry Fermented Sausages

	batch[a]															
	C				L			M			L+M			L+S		
	day				day			day			day			day		
	0	5	26	48	5	26	48	5	26	48	5	26	48	5	26	48
Asp	27	80	54	81	59	120	118	80	73	94	79	116	137	71	92	131
Glu	34	44	93	80	50	88	138	49	77	81	31	97	135	68	97	149
Asn+Ser	11	21	30	51	23	62	57	26	33	36	24	48	51	22	30	48
Gly+Gln	58	75	76	56	83	81	71	86	73	61	71	54	66	75	52	67
Tau+His	57	78	61	77	83	78	69	65	68	80	53	63	68	51	71	68
Thr	6	26	42	21	45	59	38	22	22	30	16	27	28	19	29	33
Ala	57	72	93	116	71	90	111	71	105	116	63	88	99	81	89	93
Arg	21	49	44	61	51	69	72	49	68	61	48	53	60	45	68	66
Tyr	11	26	26	26	26	24	24	27	30	31	23	24	29	25	23	25
Val	14	25	43	51	32	49	48	34	36	51	37	43	49	37	41	49
Met	6	11	16	32	21	28	28	26	16	30	17	28	33	21	32	29
Ile	12	37	52	38	35	52	58	25	52	40	34	46	57	39	53	57
Leu	29	61	68	76	68	73	85	59	74	81	57	65	78	65	71	75
Phe	20	31	39	33	37	27	40	57	32	33	28	26	33	26	27	30
Trp	10	9	11	11	15	13	15	17	14	13	10	12	13	15	19	15
Lys	10	50	56	63	40	60	82	50	59	56	48	57	67	43	71	69
total	383	695	804	873	739	973	1054	743	832	894	639	847	1003	703	865	1004

[a] Values are expressed as mg/100 g dry matter. Abbreviations: C, control, aseptic batch without inoculation; L, aseptic batch inoculated with *L. plantarum* 4045; M, aseptic batch inoculated with Micrococcus-12; L+M, aseptic batch inoculated with *L. plantarum* 4045 and *Micrococcus*-12; L+S, aseptic batch inoculated with *L. plantarum* 4045 and *Staphylococcus* sp.

consequence of the action of endogenous enzymes, principally acid and cystein proteinases.

In the analysis of the individual free amino acids (Table 1), Ala and Glu were the predominant amino acids in the initial mass (day 0), as Langner (1969) and Cantoni et al. (1974) also reported both in “Campagnolo” and “Varzi” salamis, Dierick et al. (1974) in Belgian sausages, and Díaz et al. (1993, 1997) in “salchichón”. It therefore appears that the presence of these amino acids as the majority components in the initial stage of sausage ripening is an usual phenomenon. The presence of Ala is not unexpected since this is one of the most abundant amino acids in meat (Nishimura et al., 1988). It is also noteworthy that the quantitatively most important amino acids at the start of the ripening process were Tau and His which eluted together. The presence of Tau as one of the majority amino acids in both the meat (Nishimura et al., 1988) and in the initial mixture (Cantoni et al., 1974; Domínguez et al., 1989) suggests that in the sum of Tau+His, Tau is predominant. Several authors (Domínguez et al., 1989; DeMasi et al., 1990) have reported Gln to be one of the predominant amino acids in the original mixture. In this study, Gln and Gly eluted together amounted to 58 mg/100 g DM of the initial mixture; this was the highest value but it was not possible to determine the amount corresponding to Gln and Gly, respectively.

Moreover, it is noteworthy that the amino acid Lys underwent the greatest rise (×5.6−8.2) in all the batches and Gly+Gln, Tau+His, and Trp increased the least (×1.04−1.40) in all the batches. Finally, after Lys the amino acids which underwent the greatest increase in each batch were Met (×4.7−5.5), Asn+Ser (×3.3−5.2), Val (×3.4−3.6), Thr (×3.5−6.3), Ile (×3.2−4.8), and Asp (×3.0−5.1). Some of these amino acids (Lys, Val, and Asp) have been described as the most released ones during the ripening of dry-cured ham (Toldrá and Flores, 1998). These results confirm once again the conclusion reached in the previous section from analysis of the total values of free amino acids. Given the fact that batch C was not inoculated with any microorganism and that the other batches had a different starter culture mixture, one would expect different increases in each amino acid. However, this was not the case since the increases observed were reasonably similar. It is possible, therefore, to draw the same conclusion once again that muscle proteases are the main agents responsible for proteolysis in dry fermented sausages, at least in those with a short or medium ripening period (less than 2 months). It is worth bearing in mind that many dry fermented sausages, especially those of smaller calibre, are available on the market before the ripening period is complete.

Nonvolatile Amines. The concentrations of amines in the experimental dry fermented sausages are presented in Table 2. Only putrescine, spermidine, and spermine were regularly detected and, occasionally, 2-phenylethylamine. Triptamine, cadaverine, histamine, and tiramine were not found. No important changes were observed in the levels of amines during ripening. Undoubtedly, there was no amino acid decarboxylation during the ripening process. These results are very different to those recorded by other authors (Dierick et al., 1974; Rice et al., 1975; Vandekerckhove, 1977; Eitenmiller et al., 1978; Koehler and Eitenmiller, 1978; Santos-Buelga et al., 1986) who detected a greater variety of amines and in higher concentrations in different kinds of dry fermented sausages. For this reason, these results have to be carefully analyzed.

Although the presence of putrescine and cadaverine in dry fermented sausages has been reported (Dierick et al., 1974; Vandekerckhove, 1977), lactic acid bacteria are not producers of these diamines (Dainty et al., 1986; Edwards et al., 1987). The lack of cadaverine and low levels of putrescine were expected in experimental sausages because these diamines are formed during raw meat storage by the metabolic activities of *Pseudomonas* and *Enterobacteriaceae* (Slemr, 1981), and the sausages in this work were manufactured using ingredients obtained aseptically, with total flora counts before inoculation less than 10^3 cfu/g. Lactic acid bacteria are also unable to produce spermidine and spermine (Edwards et al., 1987), whereas eukaryote cells can produce them (Davis, 1996). Therefore, both amines can be

Table 2. Changes in the Amine Contents during the Ripening of Experimental Dry Fermented Sausages[a]

		amine			
batch	day	2-phenyl-ethylamine	putrescine	spermidine	spermine
C	0	ND	0.2	1.0	9.8
	5	ND	0.3	0.7	7.5
	9	1.2	0.5	0.8	6.3
	26	ND	0.4	0.8	7.2
	48	ND	0.6	0.7	6.0
L	0	ND	0.2	1.0	9.8
	5	ND	0.1	0.6	8.3
	9	ND	ND	ND	7.0
	26	ND	1.4	0.9	7.0
	48	ND	0.4	0.4	9.5
M	0	ND	0.2	1.0	9.8
	5	ND	0.3	0.9	8.9
	9	0.2	0.4	0.6	6.3
	26	ND	0.6	0.3	8.0
	48	ND	0.4	0.4	8.4
L+M	0	ND	0.2	1.0	9.8
	5	ND	0.3	ND	8.6
	9	0.3	0.6	0.2	6.8
	26	ND	1.2	0.2	7.5
	48	ND	1.1	2.2	8.2
L+S	0	ND	0.2	1.0	9.8
	5	ND	0.2	0.4	7.7
	9	ND	0.1	0.8	8.5
	26	ND	0.2	0.1	8.2
	48	ND	0.1	0.1	7.0

[a] Values are expressed as mg/100 g dry matter. Triptamine, cadaverine, histamine and tyramine were not detected. Abbreviations: C, control, aseptic batch without inoculation; L, aseptic batch inoculated with *L. plantarum* 4045; M, aseptic batch inoculated with *Micrococcus*-12; L+M, aseptic batch inoculated with *L. plantarum 4045* and *Micrococcus*-12; L+S, aseptic batch inoculated with *L. plantarum 4045* and *Staphylococcus* sp.; ND, not detected.

present in the initial mixture. For the same reason, changes were not observed throughout ripening. This behavior has also been reported in dry fermented sausages (Díaz et al., 1996, 1997) and in refrigerated meat (Nakamura et al., 1979), vacuum-packaged meat (Edwards et al., 1987), and meat stored in modified atmospheres (Ordóñez et al., 1991). However, the presence of phenylethylamine in the experimental batches was expected, because some lactobacilli strains and *Micrococcaceae* species are producers of this amine (Masson et al., 1996). Lactic acid bacteria may also play an important role in histamine and tyramine formation, as has been reported by some authors (Edwards et al., 1987; Chander et al., 1989). Nevertheless, both amines were not detected in our experimental sausages probably because the strain used as the starter culture was not endowed with His or Tyr decarboxylase activity. Wide variations in the tyramine concentration from 4 to 30 mg/100 g have been reported in dry fermented sausages (Santos et al., 1985). Santos-Buelga et al. (1986) and Chander et al. (1989) found that pH had an influence on tyramine formation with the highest tyramine level coinciding with the lowest pH. Aciduric lactobacilli (cluster II of Shaw and Harding, 1984) have tyrosine decarboxylase activity. These lactobacilli, as their name indicates, give rise to an important decrease in pH (<5.0) which explains the results obtained by the latter authors. In the present work tyramine was not even detected in the batches inoculated with lactobacilli. Although the final pH values of these batches were the lowest, they never went below 5.0. Therefore, the lactobacilli strain used (*L. plantarum* 4045) may be considered as nonaciduric and, therefore, not able to form tyramine.

CONCLUSION

The proteolytic activity occurring during at least the first 50 days of the ripening of dry fermented sausages is due to endogenous proteases. The present bacteria play a minor role, if any, in this phenomenon.

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