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Improvement of the sensory properties of dry-fermented sausages by the addition of free amino acids

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Abstract

The addition of free amino acids in the manufacture of dry-fermented sausages, as a method to enhance their flavour, was evaluated. For this purpose, three batches of dry-fermented sausages were manufactured: a control batch (C), and the same formula treated with 0.159% (g/100 g sausage) of a mixture of valine, isoleucine and leucine (58/35/66) (w/w) (batch L1) or treated with 1.01% of a "pool" of free amino acids (batch L2) (gly/asn/his/arg/thr/ala/pro/tyr/val/met/ile/leu/phe/trp/lys/asp/glu/ser /gln/cys) (19/19/105/41/55/ 69/90/10/58/27/35/66/35/62/110/56/106/18/19/10) (w/w). The pH values, dry matters and water activities of experimental sausages did not show significant differences ($p < 0.05$) among different batches. However, an increase in the microbial counts (lactic acid bacteria and micrococci) and in the level of free amino acids and ammonia was observed in batches L1 and L2. A higher amount of volatile compounds was also detected in both experimental batches (L1 and L2), particularly those compounds derived from amino acid breakdown, such as branched aldehydes and their corresponding alcohols. In the sensory analysis, bach L1 showed a better overall quality than the control and batch L2.

Keywords: Fermented sausages; Flavour; Free amino acids

1. Introduction

The flavour of dry-fermented sausages is the result of a complex combination of several volatile (aldehydes, esters, alcohols, ketones) and non volatile (amines, amino acids, small peptides) compounds. Most of these substances are formed by enzymatic reactions (glycolysis, proteolysis, oxidative deamination, transamination, decarboxylation) or chemical processes (lipid autooxidation, Strecker degradation, Maillard reaction) taking place during the ripening of sausages (Montel, Masson, & Talon, 1998; Ordóñez, Hierro, Bruna, & Hoz, 1999).

Numerous methods have been used to accelerate the ripening and/or enhance the flavour of ripened foods: the use of elevated ripening temperatures (El-Soda & Pandian, 1991), the addition of slurries (Kristoffersen, Mkoljcik, & Gould, 1967) and exogenous enzymes (reviewed by Fernández, Ordóñez, Bruna, Herranz, & de la Hoz, 2000), the use of genetically modified starters (Christensen, Johnson, & Steele, 1995; McGarry et al., 1994; Rijnen, Bonneau, & Yvon, 1999; Yvon, Berthelot, & Gripon, 1998) and the addition of intracellular cell-free extracts (Bruna, Fernández, Hierro, de la Hoz, & Ordóñez, 1999, 2000a, 2000b, 2001a, 2001b; El-Deeb, 1989; Engels & Visser, 1996). The addition of exogenous

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enzymes (proteases and lipases) has been one of the most studied methods because of its relatively low cost and their specific action on the substrate. When added to dry-fermented sausages, these enzymes are able to accelerate the proteolytic and lipolytic phenomena, but only in some cases will the free amino acids and fatty acids formed cause a slight flavour enhancement (Fernández, Hoz, Díaz, Cambero, & Ordóñez, 1995; Zapelena, Zalacain, Paz de Peña, Astiasarán, & Bello, 1997a Zapelena, Zalacain, Paz de Peña, Astiasaran, & Bello, 1997b, 1999). Moreover, when proteases are used, the overripening of the product is possible if the doses are not well adjusted or the technological conditions of the sausage manufacture are modified (Bruna, 2000).

Wallace and Fox (1997) added a *Lactococcus lactis* subsp *cremoris* 223 starter and different amounts (1.4–8.5 g/kg cheese curd) of a mixture of free amino acids to Cheddar cheese in an attempt to improve the flavour. The addition of intermediate levels of free amino acids (2.8 or 5.7 g/kg) seemed to enhance the secondary proteolytic phenomena, i.e., the breakdown of small peptides to amino acids, giving cheeses with a better flavour and texture than the controls. These authors concluded that the addition of 2.8 or 5.7 g of amino acids/kg of cheese curd during manufacture, seemed to have a beneficial effect on the development of cheese flavour.

In accordance with these previous experiences, it was reasonable to assume that the addition of free amino acids as ingredients in the manufacture of dry-fermented sausages (as precursors of sapid and aromatic compounds) could have a beneficial effect on the flavour of the final product. The typical microbiota of sausages (lactic acid bacteria and micrococci) would have a high amount of substrate for deamination, decarboxylation and transamination reactions, and the amino acids could be transformed into volatile compounds, resulting on an enhancement of the flavour of the product.

The purpose of the present work was to assay the use of free amino acids as ingredients in the manufacture of sausages. Leucine, valine and isoleucine are precursors of the compounds mainly associated with the "ripened" aroma of dry-fermented sausages, namely, branched aldehydes and their respective alcohols (Careri et al., 1993; Hinrichsen & Pedersen, 1995; Montel et al., 1998). Yvon et al. (1998, 2000) have shown that transaminases, which catalyze the first step in the catabolism of amino acids, have a high affinity for these amino acids. A "pool" of different amino acids was also tested in the present work. The composition of the mixture of amino acids used in the present work was previously described by Díaz, Fernández, García de Fernando, Hoz, and Ordóñez (1993) and corresponds to an experiment in which 600 units of Pronase E/kg sausage were added to dry-fermented sausages.

2. Materials and methods

2.1. Preparation of sausages

In the present work, "salchichón" type dry-fermented sausages were manufactured according to the following formula (% w/w): pork (55), beef (13.49), pork fat (25), NaCl (2.5), dextrin (1.8), lactose (1.0), glucose (0.8), monosodium glutamate (0.25), sodium ascorbate (0.046), $NaNO_3$ (0.0095), $NaNO_2$ (0.0065) and equal amounts of whole grain and ground black pepper (0.1). The ingredients were processed at 2 °C in a mincer, equipped with an adjustable plate set at a hole diameter of 5 mm, and then inoculated with 1% (v/w) of a starter culture mixture of *Lactobacillus plantarum* 4045, *Staphylococcus carnosus* and *Staphylococcus xylosus*. The mixture was divided into three parts, which were used to manufacture three different batches of fermented sausages. The first, batch C (control), consisted of the initial mixture alone. Batch L1 was like batch C, but treated with 0.159% (g/100 g sausage) of the branched amino acids valine, isoleucine and leucine in the proportions (58/35/66) (w/w). These amino acid proportions were calculated according with the study by Díaz et al. (1993). The third batch (L2) was treated with 1.01% (g/100 g sausage) of the following amino acids (gly/asn/his/arg/thr/ala/pro/tyr/val/met/ile/leu/phe/trp/lys/asp/glu/ser/gln/cys) (19/19/105/41/55/69/90/10/58/27/35/66/35/62/110/56/106/18/19/10) (w/w). The proportions were also established according to the experiments performed by Díaz et al. (1993).

2.2. Microbial analysis

Total viable microorganisms were counted in plate count agar (PCA) (Condalab, Madrid, Spain) and *Micrococcaceae* in manitol salt agar (MSA) (Condalab, Madrid, Spain), both incubated at 32 °C for 2 days. Lactic acid bacteria were grown in MRS agar (Condalab, Madrid, Spain) at pH 5.6 in a double-layer at 32 °C for 2 days.

2.3. Chemical analysis

Dry matter (D.M.) was determined by drying the sample at 110 °C to constant weight. Water activity (a_w) was determined using a Decagon CX1 hygrometer (Decagon Devices, Pullman, USA) at 25 °C. The pH was measured in a homogenate of the sample with distilled water (1:10) (w/v), using a Crison Digit-501 pH meter (Crison Instruments, Barcelona, Spain).

Free amino acids were extracted as described by Yang and Sepúlveda (1985) and analysed by HPLC, as described by Bruna et al. (2001a). After extraction, amino acids were derivatised with phenylisothiocyanate (PITC). Amines were extracted according to Spinelli,

Lakritz, and Wasserman (1974) and analysed after derivatisation with dansyl chloride (Ordóñez, de Pablo, Pérez de Castro, Asensio, & Sanz, 1991). The amino acids and amine derivatives were analysed in a Beckman System Gold *Nouveau* chromatograph (Beckman, Fullerton, USA) equipped with a column Spherisorb S5 ODS2 (25 cm × 4.6 mm, 5 μm particle size, Waters Spherisorb, Milford, USA) maintained at 35 °C in a column oven (Jones Chromatography, Hengoed, UK). Detection was performed at 254 nm in both cases. The different amino acids and amines were identified by comparing the samples with standard solutions (Sigma, Madrid, Spain) analysed under the same conditions.

Ammonia levels were determined using an enzymatic test (Boehringer Mannheim, Mannheim, Germany) following the manufacturer instructions for meat products.

2.4. Analysis of volatile compounds

A Purge & Trap concentrator Tekmar 3000 (Tekmar, Cincinnati, OH, USA), connected to a Hewlett-Packard 5890 Series II gas chromatograph and coupled to a HP5972 mass spectrometer (Hewlett-Packard, Palo Alto, CA, USA), was used for the volatile compound analyses. Seven grammes of sample were minced and thoroughly mixed with 10 g of Na_2SO_4. Eight grammes of the mixture were transferred into a 25 ml fritless sparger and were purged under the following conditions: 30 ml/min flow of ultrapure helium was used as a purge gas, purge was 15 min at 30 °C controlled by a thermal sleeve. The compounds concentrated in a Tenax trap were thermally desorbed at 220 °C for 3 min. The transfer line and the valves were maintained at 180 °C. A CP-Sil 8 CB low bleed/MS fused silica capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness, Chrompack, Middelburg, The Netherlands) was used with helium as the carrier gas at a flow rate of 1 ml/min. Immediately before the desorption of the trap, 1 μl of an internal standard (131 ng/μl 1,2-dichlorobenzene in methanol) was injected into the gas chromatograph. During the desorption period of 3 min, volatile compounds were cryofocussed by immersing 15 cm of column adjacent to the heater in a solid CO_2 bath while the oven was held at 40 °C. The bath was then removed, and chromatography achieved by holding at 40 °C for 2 min, followed by a programmed rise to 280 C at 4 °C/min and held for 5 min. A series of *n*-alkanes (C_6–C_{22}) was analysed under the same conditions to obtain linear retention index (LRI) values for the aroma components.

The mass spectrometer was operated in electron impact mode with an electron energy of 70 eV and an emission current of 50 μA. Compounds were identified by first comparing their mass spectra with those contained in the HP Wiley 138 Mass Spectral Database and then comparing the LRI values with either those of authentic standards or with published values. Approximate quantities of the volatiles were estimated by comparing their peak areas with those of the 1,2-dichlorobenzene internal standard, obtained from the total ion chromatograms using a response factor of 1.

2.5. Texture analysis

Texture profile analysis (TPA) (Bourne, 1978) was used to evaluate the texture of the experimental sausages. A Stable Micro Systems TA-XT2i texture analyzer (Stable Micro Systems, Surrey, UK), equipped with a cylindrical probe P/25 was used. This procedure involved cutting sausage samples approximately 1.5 cm high and 2.5 cm wide, after discarding the external layer (2 cm). Samples were allowed to reach room temperature and were then compressed twice to 50% of their original height. The following parameters were determined from the curves obtained from the analysis: hardness (*H*), maximum force required to compress the sample; springiness (*S*), ability of the sample to recover its original shape after the deforming force was removed; adhesiveness (*A*), area under the abscissa after the first compression; cohesiveness (*C*), extent to which the sample could be deformed prior to rupture; gumminess (*G*), force to disintegrate a semisolid meat sample for swallowing ($H \times C$); chewiness (*Ch*), work to masticate the sample before swallowing ($S \times G$). To determine the maximum cutting force and the cutting work (Bourne, 1978), a reversible probe calibrated with 5 kg was used.

Five slices of sausage per batch were used in the texture analysis.

2.6. Sensory analysis

Triangle and acceptance tests were carried out on the last day of ripening (day 22) by a panel of 20 tasters in a tasting room designed according to I.S.O./DP 66.58 (I.S.O., 1981a). Tasters were members of Departamento de Nutrición, Bromatología y Tecnología de los Alimentos and they had been previously trained in the sensory assessment of meat products. A triangle test (I.S.O., 1981b) was performed by the forced-choice option, in which the tasters must choose the sample that, in their opinion, is different. The acceptance test was performed by the hedonic rating option, presenting one sample at a time and asking the panellists to rate the colour, texture, odour and flavour by using a non-structured hedonic scale in which samples were given scores of 1 (very poor) to 10 (excellent). The overall quality was calculated from the expression: Overall quality = (Colour × 0.1) + (Texture × 0.25) + (Odour × 0.15) + (Flavour × 0.5). This equation was calculated from a study on commercial fermented sausages, in which the tasters were asked to assess the relative importance of the different sensory characteristics (Bruna et al., 2000a).

2.7. Statistical analysis

ANOVA was used to search for significant differences between mean values of the different results. Comparison between batches was performed by the Student Newman–Keul's test ($p < 0.05$) using SigmaStat 3.0 (Jandel Corporation, San Rafael, USA).

3. Results and discussion

3.1. Changes in microbiota

Total microbiota and lactic acid bacteria counts are shown in Fig. 1.The Figure shows the usual microbial behaviour observed in this kind of product (Bruna et al., 1999, Bruna, Fernández, Hierro, de la Hoz, & Ordóñez, 2000a Bruna, Fernández, Hierro, de la Hoz, & Ordóñez, 2000b, 2001a Bruna, Fernández, Ordóñez, & de la Hoz, 2002).

It is important to note that batch L2 showed a higher final micrococci count (day 22) than batches C and L1. This fact might be attributed to a more intense metabolic activity of the starter due to a larger amount of available free amino acids (Bruna et al., 2000a, 2002).

3.2. Changes in pH, dry matter and water activity (a_w)

The addition of free amino acids did not affect these general parameters, and all batches showed the same pattern. The mean values for pH, dry matter content and a_w at the end of ripening were 4.7, 71% and 0.807, respectively (data not shown). The values and changes observed during ripening were similar to those described by other authors for different fermented sausages (Beriain, Lizaso, & Chasco, 2000b; Bruna et al., 1999, 2000a, 2000b, 2001a, 2002; Díaz et al., 1993, Dıaz, Fernández, García de Fernando, Hoz, & Ordóñez, 1996, 1997; Fernández et al., 1995; Hierro, Hoz, & Ordóñez, 1997; Samelis, Aggelis, & Metaxopoulos, 1993).

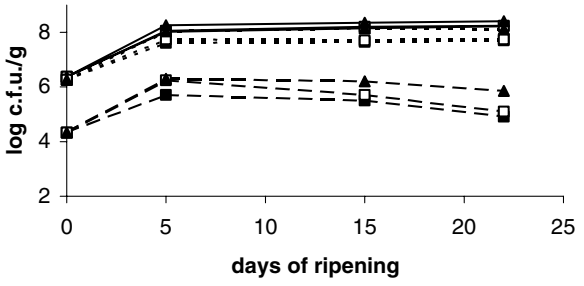

Fig. 1. Changes observed in the microbiota during the ripening of the experimental sausages. (■) control batch, (□) batch L1 (control treated with 0.159% (w/w) of (val/ile/leu) (58/35/66)), (▲) batch L2 (control batch treated with 1.01% (w/w) of (gly/asn/his/arg/thr/ala/pro/tyr/val/met/ile/leu/phe/trp/lys/asp/glu/ser /gln/cys) (19/19/105/41/55/ 69/90/10/58/27/35/66/35/62/110/56/106/18/19/10)). Plain lines: counts of total bacteria. Short-dotted lines: counts of lactic acid bacteria. Long-dotted lines: counts of micrococci.

3.3. Changes in free amino acids, ammonia and amines

The total free amino acid content of the experimental sausages is shown in Table 1. All batches showed an increase during the ripening. The initial results of the control batch (C) are within the range reported by many authors (Beriain, Lizaso, & Chasco, 2000a, 2000b; Bruna et al., 2000b, 2001a, Bruna, Ordóñez, Fernández, Herranz, & de la Hoz, 2001b; Hierro, Hoz, & Ordóñez, 1999), while the values of batches L1 and L2 are, obviously, higher, since a significant amount of amino acids was added to these sausages. As a consequence, at the end of ripening, batches L1 and L2 showed significantly higher free amino acid contents ($p < 0.05$) than the control sausages (Table 1). The final free amino acid contents in batches L1 and L2 were 1.6 and 4.4 times higher than batch C, respectively.

The final content of total amino acids in the control sausages increased 1.8-fold from day 0 while, in batches L1 and L2, the amino acid contents rose 1.6- and 1.3-fold, respectively, compared to the initial values. The lower ratios of increase observed in batches L1 and L2 could be attributed to an enhancement of the amino acid breakdown due to the addition of free amino acids, as previously reported by Wallace and Fox (1997) in cheese. On the other hand, the higher initial contents of free amino acids in batches L1 and L2 might have exerted an inhibitory effect on the activity of both, endogenous and microbial proteases.

Most of the individual free amino acid showed significant differences ($p < 0.05$) among batches at the end of ripening (Table 1). When batches L1 and L2 are compared, the amino acids Val, Ile, Leu did not show significant differences between them, but they did when compared to the control sausages, due to the addition of free amino acids to the formula. However, the ratios of release of those amino acids (obtained as the quotient between the final and the initial contents for each batch) were lower for Val and Ile in batches L1 and L2 than in the control, namely, approximately 1.2 for Val and 1.5 for Ile in both batches vs 1.4 and 2.4 in the control, respectively. The lower ratios obtained for Val and Ile in the sausages treated with amino acids might be explained again by the above-mentioned reasons, namely, the enhancement of the amino acid breakdown and the inhibition of proteolysis. Finally, the ratio of release of Leu was not affected by the addition of this amino acid to sausages, showing a value of 1.4-fold in all batches.

Table 1 also shows the changes observed in the ammonia content during the ripening of the experimental sausages. At the end of ripening, batch L2 showed the highest amount of ammonia ($p < 0.05$), followed by batches L1 and C. The final ammonia content of

Table 1
Free amino acids and total ammonia (mg/100 g D.M.) of the experimental sausages at days 0 and 22 of the ripening

Amino acid	Day 0			Day 22		
	C	L1	L2	C	L1	L2
Asp	7.45^{b}	7.30^{b}	232^{a}	26.3^{b}	24.4^{b}	288^{a}
Glu	13.2^{b}	13.0^{b}	437^{a}	57.7^{b}	65.9^{b}	514^{a}
Hpx	1.82	1.78	1.94	14.2^{b}	15.3^{b}	21.0^{a}
Ser	14.3^{b}	14.2^{b}	86.3^{a}	21.8^{b}	26.6^{b}	99.8^{a}
Asn	19.4^{b}	19.8^{b}	95.4^{a}	58.4^{b}	50.5^{b}	142^{a}
Gly	19.9^{b}	20.2^{b}	95.9^{a}	34.8^{b}	32.4^{b}	106^{a}
Gln	126^{b}	126^{b}	202^{a}	218^{b}	223^{b}	413^{a}
His	59.8^{b}	59.6^{b}	480^{a}	87.7^{b}	82.3^{b}	545^{a}
Tau + GABA	64.2	64.6	65.0	78.3^{b}	82.0^{b}	114^{a}
Thr	91.5^{b}	90.0^{b}	312^{a}	126 ^{b}	124^{b}	313^{a}
Ala + Arg	54.7^{b}	54.2^{b}	495^{a}	96.0^{b}	98.9^{b}	586^{a}
Pro	39.8^{b}	40.0^{b}	400^{a}	62.1^{b}	63.4^{b}	404^{a}
Tyr	5.40^{b}	6.33^{b}	45.4^{a}	19.5^{b}	19.9^{b}	59.3^{a}
Val	45.0^{b}	277^{a}	277^{a}	63.6^{b}	337^{a}	343^{a}
Met	17.2^{b}	17.4^{b}	125^{a}	41.9^{b}	42.4^{b}	232^{a}
Cys	22.7^{b}	23.2^{b}	62.7^{a}	71.8^{b}	75.0^{b}	273^{a}
IIe	20.1^{b}	160^{a}	160^{a}	49.0^{b}	240^{a}	248^{a}
Leu	51^{b}	315^{a}	315^{a}	69.4^{b}	445^{a}	454^{a}
Phe	22.8^{b}	22.7^{b}	163^{a}	48.3^{b}	49.6^{b}	265^{a}
Trp	32.8^{b}	33.2^{b}	281^{a}	70.9^{b}	78.2^{b}	321^{a}
Lys	44.3^{b}	48.1^{b}	484^{a}	72.2^{b}	74.2^{b}	522^{a}
Cis	2.29	2.67	2.87	22.8	24.8	25.1
Total	775^{c}	1416^{b}	4817^{a}	1410^{c}	2275^{b}	6286^{a}
NH_3	15.9	16.4	17.0	34.2^{c}	45.3^{b}	63.7^{a}

C, control batch; L1, control batch treated with 0.159% of (val/ile/leu) (58/35/66); L2, control batch treated with 1.01% of (gly/asn/his/arg/thr/ala/pro/tyr/val/met/ile/leu/phe/trp/lys/asp/glu/ser /gln/cys) (19/19/105/41/55/69/90/10/58/27/35/66/35/62/110/56/106/18/19/10) (w/w).
a–c: Values in a row with different letters are significantly different ($p < 0.05$).

batch L2 was approximately 1.9-fold higher than batch C, and batch L1 had approximately 1.3-fold higher levels than batch C. Wallace and Fox (1997) also found higher ammonia contents at the end of ripening in cheeses treated with free amino acids. Therefore, it seems that the addition of free amino acids promoted the deaminative activity of microbiota.

At the end of ripening, the amine levels (Table 2) were similar to those reported by other authors (Bover-Cid, Schoppen, Izquierdo-Pulido, & Vidal-Carou, 1999; Bruna et al., 1999, 2000a, 2000b, 2001a, 2001b; Díaz et al., 1993, Díaz, Fernández, Garcıa de Fernando, Hoz, & Ordóñez, 1997) although some significant ($p < 0.05$) differences were found between batches. Batch L2 showed a final total content approximately 1.4-fold higher than batch C and the final content of batch L1 was approximately 1.2-fold higher than C (Table 2). This fact would indicate an enhancement of the

Table 2
Amines (mg/100 g D.M.) of the experimental sausages at days 0 and 22 of the ripening

Amine	Day 0	Day 22		
	C, L1 and L2	C	L1	L2
Tryptamine	3.03	9.50^{b}	9.75^{b}	13.0^{a}
Phenylethylamine	2.49	10.6^{b}	14.3^{a}	15.8^{a}
Putrescine	1.67	7.37^{b}	8.04^{b}	9.28^{a}
Histamine + Cadaverine	nd	6.14	6.40	6.48
Tyramine	2.38	9.33^{b}	11.3^{b}	15.7^{a}
Spermidine	0.47	0.51	0.52	0.53
Spermine	3.67	4.72	5.01	5.12
Total	13.7	48.0^{b}	55.3^{a,b}	65.9^{a}

C, control batch; L1, control batch treated with 0.159% of (val/ile/leu) (58/35/66); L2, control batch treated with 1.01% of (gly/asn/his/arg/thr/ala/pro/tyr/val/met/ile/leu/phe/trp/lys/asp/glu/ser /gln/cys) (19/19/105/41/55/69/90/10/58/27/35/66/35/62/110/56/106/18/19/10) (w/w).
a,b: Values in a row with different letters are significantly different ($p < 0.05$).
nd: Not detected.

decarboxylative activity developed in the amino acid-treated sausages. Some individual differences were found for tryptamine, phenylethylamine, putrescine and tyramine, and batch L2 showed the highest values.

3.4. Volatile compounds

A total of 40 volatile compounds were identified and quantified across the three different batches. They included 12 terpenes, 9 alcohols, 8 aldehydes, 3 sulfur compounds, 3 hydrocarbons, 2 esters, 2 ketones and one furan. Table 3 shows the values after 22 days of ripening. Compounds are grouped according to their chemical class. Terpenes were not included as they come from black pepper. Neither were hydrocarbons, as they have relatively high odour threshold values (Drum & Spanier, 1991) and are, therefore, very unlikely to contribute to sausage flavour.

Table 3
Volatile compounds (ng/100 g) found in the experimental sausages after 22 days of ripening

LRI[A]	Compound	C	L1	L2	Method of identification[B]
	Alcohols				
503	Ethanol	1362^{c}	2166^{b}	2813^{a}	ms + lri
524	2-Propanol	213^{b}	326^{b}	586^{a}	ms + lri
560	1-Propanol	103^{b}	367^{a}	307^{a}	MS + LRI
629	2-Methylpropanol	149^{c}	448^{a}	296^{b}	ms + lri
653	1-Butanol	358	364	327	MS + LRI
672	1-Penten-3-ol	90	80	78	ms + lri
740	3-Methylbutanol	298^{b}	1419^{a}	1200^{a}	MS + LRI
744	2-Methylbutanol	58^{b}	230^{a}	268^{a}	MS + LRI
	4-Hexen-1-ol	174^{b}	292^{a}	294^{a}	ms
		2805^{b}	**5692**^{a}	**6169**^{a}	
	Aldehydes				
551	2-Methylpropanal	171^{c}	736^{b}	1071^{a}	MS + LRI
654	3-Methylbutanal	219^{c}	1437^{b}	2065^{a}	MS + LRI
662	2-Methylbutanal	12^{b}	50^{a}	60^{a}	MS + LRI
705	Pentanal	442^{c}	1309^{a}	1096^{a}	MS + LRI
802	Hexanal	2248	2957	2752	MS + LRI
902	Heptanal	121^{b}	146^{b}	181^{a}	MS + LRI
954	2-Heptenal (*E*)	n.d	58	59	MS + LRI
1105	Nonanal	196	174	215	MS + LRI
		3409^{b}	**6867**^{a}	**7499**^{a}	
	Ketones				
683	2-Pentanone	460	440	371	MS + LRI
898	2-Heptanone	186^{b}	277^{a}	205^{b}	MS + LRI
		646	**717**	**576**	
	Esters				
615	Ethyl acetate	656^{b}	1328^{a}	1207^{a}	MS + LRI
849	3-Methylethylbutanoate	49^{b}	488^{a}	579^{a}	MS + LRI
		705^{b}	**1816**^{a}	**1786**^{a}	
	Furans				
604	2-Methylfuran	169	155	100	MS + LRI
	Sulfur compounds				
538	Carbon disulfide	121^{b}	113^{b}	191^{a}	MS + LRI
748	Dimethyl disulfide	65^{b}	80^{b}	114^{a}	MS + LRI
784	3-Methylthiophene	86^{b}	94^{b}	191^{a}	MS + LRI
		272^{b}	**287**^{b}	**496**^{a}	
	Total volatiles	**8006**^{b}	**15,534**^{a}	**16,626**^{a}	

C, control batch; L1, control batch treated with 0.159% of (val/ile/leu) (58/35/66); L2, control batch added with 1.01% of (gly/asn/his/arg/thr/ala/pro/tyr/val/met/ile/leu/phe/trp/lys/asp/ glu/ser/gln/cys) (19/19/105/41/55/69/90/10/58/27/35/66/35/62/110/56/106/18/19/10) (w/w).
a–c: Values in a row with different letters are significantly different ($p < 0.05$).
[A] Linear retention index on a CP-Sil 8 CB low bleed/MS column.
[B] MS + LRI, mass spectrum and LRI agree with those of authentic compounds; ms + lri, mass spectrum and LRI in agreement with the literature; ms, mass spectrum agrees with spectrum in the HP Wiley 138 Mass Spectral Database.

The main quantitative differences in the volatile compounds were detected in the substances derived from amino acid catabolism and microbial activity.

Three sulfur compounds were identified in all samples. These compounds could derive from methionine which is converted to methanethiol through a transamination reaction, followed by a decarboxylation and, probably, a subsequent enzymatic conversion, or through a deamination and demethiolation (Smit et al., 2000). Methanethiol is a very potent flavour compound and a precursor of other sulfur compounds. However, high concentrations of these compounds contribute to an undesirable odour (Dainty & Mackey, 1992). Batch L2 showed the highest concentrations of sulfur compounds. This fact could be attributed to the methionine enrichment in batch L2 and the consequent high content of this amino acid in those sausages.

On the other hand, it can be observed in Table 3 that the concentrations of compounds derived from branched amino acids (branched aldehydes and alcohols) were much higher in batches L1 and L2. They reached 4 to 6-fold (2- and 3-methylbutanol, 2-methylpropanal or 2-methylbutanal) and 7-9-fold (3-methylbutanal) higher values than those recorded in batch C. Therefore, the metabolic activity of the starter might have been enhanced by the addition of amino acids; the starter could have used Val, Leu and Ile to generate 2-methylpropanal, 3-methylbutanal and 2-methylbutanal, respectively. These compounds have been described by several authors as responsible for the ripened aroma in cured meat products (Careri et al., 1993; Ruiz et al., 1999; Søndergaard & Stahnke, 2002).

Many authors have studied the role of the starters in the production of branched aldehydes. Species of the genus *Staphylococcus* can be more important than *Lactobacillus sakei*, *Pediococcus acidilactici* or *Pediococcus pentosaceus* in relation to the production of these volatile compounds (Berdagué, Monteil, Montel, & Talon, 1993). In previous experiments both, dry sausage models and fermented sausages inoculated with *S. carnosus* or *S. xylosus* had high concentrations of 3-methylbutanal and 2-methylbutanal and their corresponding acids, 3-methylbutanoic and 2-methylbutanoic acids (Berdagué et al., 1993; Montel et al., 1996; Stahnke, 1999). Larrouture, Masson, Talon, and Montel (1998) inoculated the same strains, *S. carnosus* and *S. xylosus*, in culture media and other strains of staphylococci (*S. warneri* and *S. saprophyticus*) and they observed higher amounts of Leu metabolites than when different strains of lactic acid bacteria, such as *L. sakei*, *L. plantarum* and *P. pentosaceus*, were used. Later, Stahnke (1999) confirmed (in sausage models) that as much *S. xylosus* as *S. carnosus* produced a high amount of branched aldehydes and their corresponding acids and alcohols from Val, Leu and Ile. More recently, Larrouture, Ardaillon, Pépin, and Montel (2000) analysed the ability of lactic acid bacteria and staphylococci, both of meat origin, to produce volatile compounds from Leu; they observed that lactic acid bacteria could only generate these volatiles via transamination, as mentioned above. In contrast, staphylococci can use two pathways: oxidative deamination and transamination.

Although the starter used in the present work was the same in batches L1 and L2 (a mixture of *L. plantarum* 4045, *Staphylococcus xylosus* and *Staphylococcus carnosus*) and the same amounts of Val, Ile and Leu (2.32, 2.64 and 1.40 g/kg sausage, respectively) were added to both batches, the concentration of branched aldehydes was higher in batch L2. This phenomenon could be attributed to an increase of the oxidative deamination and transamination reactions in batch L2, due to the higher count of micrococci (Larrouture et al., 2000).

Esters are another group of compounds which showed the greatest differences between the control batch and the sausages treated with free amino acids. They arise from microbial esterification of acids (derived from sugar fermentation or lipolytic activity) with alcohols. Ethanol, the most abundant alcohol, derives mainly from pyruvate (Kandler, 1983). Although the type of microorganism responsible for ester formation, especially for ethyl esters, is not clear, some authors relate their formation to the *Micrococcaceae* family. In this sense, Montel, Reitz, Talon, Berdagué, and Rousset-Akrim (1996) studied 19 strains of *Micrococcaceae* in fermented sausages, finding that *S. xylosus* and *S. carnosus* were the main producers of ethyl esters, giving rise to sausage models with better flavour. Stahnke (1995), in sausages manufactured without spices and inoculated with *S. xylosus*, reported high amounts of ethyl esters, although it could not be established whether commensural microbiota also participated in their formation. Oleson and Stahnke (2000) also reported this phenomenon in fermented sausages when a *Candida utilis* strain was used.

In the present work, sausages treated with free amino acids (batches L1 and L2) showed higher levels of 3-methylethylbutanoate and ethyl acetate than control batch (Table 3). This fact could be attributed to an enhancement of the growth of *Staphylococcus* species, which can use free amino acids throughout ripening or, in the case of 3-methylethylbutanoate, it could be explained by the higher availability of Leu for transformation by micrococci or lactobacilli. The greater amounts of ethyl esters in batches L1 and L2 can be considered as favourable, because they are essential for the overall flavour of fermented sausages, providing fruity notes (Barbieri et al., 1992; Meynier, Novelli, Chizzolini, Zanardi, & Gandemer, 1999; Montel et al., 1996) or masking rancid odour (Stahnke, 1994).

Table 4
Texture analysis (means ± SD) of the experimental sausages after 22 days of ripening

	C	L1	L2
Hardness (N)	140.2 ± 7.7	153.0 ± 12.2	131.8 ± 7.8
Adhesiveness (N s)	−1.35 ± 0.27	−1.32 ± 0.34	−1.33 ± 0.37
Cohesiveness	0.38 ± 0.06	0.43 ± 0.06	0.49 ± 0.08
Springiness × 10^{-2} (m)	0.54 ± 0.07[b]	0.68 ± 0.06[a]	0.50 ± 0.03[b]
Gumminess (N)	53.6 ± 5.3[b]	75.0 ± 10.8[a]	82.1 ± 8.3[a]
Chewiness (J)	0.29 ± 0.04[b]	0.51 ± 0.01[a]	0.41 ± 0.01[a]
Cutting force (N)	180.4 ± 24	173.6 ± 11	160.8 ± 25
Cutting work (J)	2.28 ± 0.05	2.12 ± 0.03	2.01 ± 0.02

C, control batch; L1, control batch treated with 0.159% of (val/ile/leu) (58/35/66); L2, control batch treated with 1.01% of (gly/asn/his/arg/thr/ala/pro/tyr/val/met/ile/leu/phe/trp/lys/asp/glu /ser/gln/cys) (19/19/105/41/55/69/90/10/58/27/35/66/35/62/110/56/106/18/19/10) (w/w).
a, b: Values in a row with different letters are significantly different ($p < 0.05$).

3.5. Texture analysis and sensory analysis

Table 4 shows the texture profile of the experimental sausages after 22 days of ripening. Significant differences ($p < 0.05$) were observed between batches L1 and L2 for springiness and between batch C and batches L1 and L2 for gumminess and chewiness. These differences could be attributed to the activity of the microbiota (either starter or commensural) as a consequence of the addition of amino acids.

In the sensory analysis, the triangle test showed statistically significant differences ($p < 0.05$) among all batches (data not shown). Maximum differences ($p < 0.001$) were found when comparing batches L1 and L2. In the acceptance test (Fig. 2) batch L1 was the best scored, followed by batches L2 and C. These results were based mainly on odour and, flavour contribution and, for this reason, the overall quality was also significantly better ($p < 0.05$). The flavour scores of batches L1 and L2 were also better ($p < 0.05$) than for batch C, although the texture score of L2 was the worst. A similar finding was described by Wallace and Fox (1997), who observed that when high levels of free amino acids (8.5 g/kg) were added to cheese, the panellists described the texture as "weak" and "pasty", although these authors did not give an explanation for these phenomena.

The better flavour score of batches L1 and L2 could be linked to a higher concentration of volatile compounds derived from free amino acids, mainly Val, Leu and Ile. Wallace and Fox (1997) also observed a better odour and flavour in their experimental cheeses treated with intermediate levels of free amino acids (2.8 or 5.7 g/kg). When higher concentrations of free amino acids were added (8.5 g/kg), Wallace and Fox (1997) observed a loss of odour and flavour and, therefore, a lower overall quality. These results were attributed by the authors to a possible inhibition of the cheese microbiota during the last stage of the ripening, due to an excess of free amino acids.

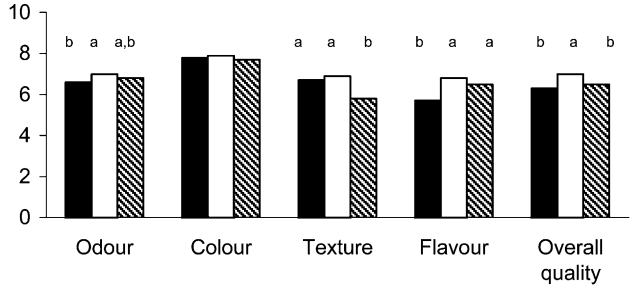

Fig. 2. Sensory analysis of the experimental sausages at the end of the ripening (scale 1–10). (■) control batch, (□) batch L1 (control batch treated with 0.159% (w/w) of (val/ile/leu) (58/35/66)), (▨) batch L2 (control batch treated with 1.01% (w/w) of (gly/asn/his/arg/thr/ ala/pro/tyr/val/met/ile/leu/phe/trp/lys/asp/glu/ser/gln/cys) (19/19/105/41/55/69/90/10/58/27/35/66/35/62/110/56/106/18/19/10)).

4. Conclusions

From the results obtained in the present work it can be concluded that the addition of free amino acids to dry-fermented sausages can result in an improvement of the overall quality of the product due to an increase in the generation of typical taste and aroma compounds.

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