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Original article

Comparison of the effects of a commercial and an autochthonous *Pediococcus acidilactici* and *Staphylococcus vitulus* starter culture on the sensory and safety properties of a traditional Iberian dry-fermented sausage ''salchichón''

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Summary The effect of the addition of an autochthonous starter culture and commercial starter culture on the physico-chemical and sensory characteristics of the dry-fermented sausage 'salchichon' was investigated. Sausages were prepared with a mixture of *Pediococcus acidilactici* MC184 and *Staphylococcus vitulus* RS34 as the autochthonous starter culture (P184S34), ripened for 86 days, and compared with a commercial starter batch (CS1). Strains of acid lactic bacteria and *Staphylococcus* were identified in the samples along the ripening process which demonstrated that the inoculation of sausages assures their implantation during the ripening process. However, P184S34 starter culture showed a higher inhibitory effect on enterobacteria and coliform flora than the CS1 batches, guaranteeing a better quality hygienic sausages. Dry-fermented sausages ripened with P184S34 showed higher amounts of NPN and volatile compounds derived from amino acid catabolism than the CS1 batches. Especially important was the result found in biogenic amines, since the P184S34 reduced their accumulation compared to the commercial starter batch. The inoculation of a decarboxylase-negative autochthonous starter culture P184S34 reduced the biogenic amine accumulation guaranteeing safety and homogeneity of the products without producing a negative effect on the sensory characteristics of the traditional fermented sausages.

Keywords Autochthonous starter cultures, biogenic amines, dry-fermented sausages, *Pediococcus acidilactici*, *Staphylococcus vitulus*.

Introduction

Iberian dry-fermented sausages are high-value products manufactured with traditional technologies without adding starter cultures. The quality of the final product is closely related to the ripening that takes place during drying. Proteolysis and lipolysis are the most important biochemical changes occurring during the ripening of dry fermented sausages.

Protein hydrolysis during ripening yields peptides and free amino acids, and hydrolysis of triglycerides involves the liberation of fatty acids, which undergo later enzymatic processes to yield, as final products, small molecular weight compounds which alter the taste and flavour development (Díaz *et al*., 1993; Naes *et al*., 1995; Ordóñez *et al*., 1999; Fernández *et al*., 2000). These reactions are often catalysed by enzymes produced by microorganisms involved in the ripening process. The role of microbial proteases and lipases in the generation of volatile compounds in semi-dry and dry-fermented sausages is well documented (Berdagué *et al*., 1993; Stahnke, 1995; Montel *et al*., 1998). Ethyl esters, methyl aldehydes, methyl ketones, and other volatile compounds in these products have been attributed to lactic acid bacteria and *Micrococcaceae* (Guo & Chen, 1991; Stahnke, 1995; Montel *et al*., 1996). However, biogenic amines can also be produced in high amounts by microorganisms through the activity of amino acid decarboxylases. Excessive consumption of amines can be a health concern because their unbalanced uptake by the human organism can generate diseases with differing degrees of severity due to their action on the nervous and gastro-intestinal systems, and on blood pressure. Biogenic amines have been studied as hygiene indicators in meat and meat products and for

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their potential risk for human health. The amines of particular interest to this study are mainly histamine, tyramine, cadaverine and putrescine as they are more relevant from a food safety perspective. Clearly bacteria intended for starter culture development should be selected on the basis of their lack of biogenic amine production (Bover-Cid *et al*., 2000, 2001).

It is considered that non-autochthonous starter cultures could have a negative impact on the sensory properties of the product, and indeed their use often results in a decrease in their desirable sensory characteristics (Samelis *et al*., 1998). Hence, autochthonous starter cultures are necessary to achieve the desired fermentation parameters specific for the product type (Erkkilä *et al*., 2001).

LAB and staphylococci strains have been selected from indigenous populations to obtain starter cultures better adapted to Iberian dry-cured sausages production and to help preserve the typical characteristics of these products. Indeed autochthonous LAB and staphylococci have been shown to make good starter cultures (Benito *et al*., 2007; Martín *et al*., 2007). Especially, the strains *P.acidilactici* MS184 and *S. vitulus* RS34 have shown desirable technological properties of proteolytic and lipolytic activities, and low or null production of biogenic amines. So, the use of the mixed starter culture P184S34, which combines these two strains, could be of great interest to improve the sensory quality and to minimize the risks associated with biogenic amines formation of dry-fermented sausages.

The aim of the present work was to investigate the effect of the selected autochthonous starter culture P184S34 on the development of sensory characteristics in the dry-fermented sausage 'salchichón' in comparison with a commercial starter. In addition, how the selected autochthonous starter culture could favour health safety by preventing biogenic amine formation in an effort to improve the global quality of these products.

Materials and methods

Autochthonous starter culture preparation

The strains *Pediococcus acidilactici* MC184 and *Staphylococcus vitulus* RS34 were chosen to form starter culture P184S34. These strains were selected for having the best aptitudes for use as starter cultures in the production of Iberian fermented sausages (Benito *et al*., 2007, 2008a,b; Martín *et al*., 2007). The *P. acidilactici* MC184 was cultured in Man Rogosa Sharpe broth (MRS) (Oxoid, Basingstoke, UK) at 37 °C for 24 h in anaerobic conditions, and *S. vitulus* RS34 in Brain Heart Infusion broth (BHI) (Oxoid) at 37 °C for 24 h individually. The cultures were then inoculated under the same conditions into flasks with nutrient broth plus 5% NaCl and 10% mannitol (Scharlau Chemie S.A., Barcelona, Spain) which have proven protective during drying. The flasks with the same strain were mixed until the cell concentration reached 10^{12} CFU mL^{-1}. Then, cells were harvested under aseptic conditions by centrifugation (6000 ***g*** for 10 min) at room temperature. The cell pastes obtained were washed once with 2.5% sucrose, re-centrifuged, and concentrated to 10^{12} CFU mL^{-1} in 2.5% sucrose. The cells were quick-frozen by immersion in a dry ice/isopropanol bath prior to lyophilization, and then lyophilized at low temperatures (-46/-52 °C) and pressures (0.17/0.22 mbar) in a Cryodos-50 freeze-drier (Telstar, Barcelona, Spain). The dried starter culture was stored in vacuum-sealed bags at −80 °C until use (Carvalho *et al*., 2004). Before use, lyophilized starter was analysed for total viable cell counts (TVC). The TVC was performed by the standard dilution method on MRS and BHI agar (Oxoid) after incubation at 37 °C for 48 h. Powders with a TVC of more than 10^{11} CFU g^{-1} were used in the production of the dry fermented sausages.

Preparation of dry-fermented sausages

Iberian dry-fermented sausages (diameter 60 mm, weight 1600 g) were prepared using the following formulation: Iberian pork, 2.4% NaCl, 1 g kg^{-1} white pepper, 1.5 g kg^{-1} black pepper, and a commercial mixture of spices and additives (Anvisa, Arganda del Rey, Madrid) specially prepared for this type of meat product and which contains dextrin, dextrose, lactose, sodium nitrate, and sodium ascorbate.

Lyophilized starter P184S34 (0.1 g kg^{-1} of meat mixture) was added to 50 kg of meat mixture. An initial population of 5×10^7 CFU g^{-1} was obtained for each strain. Commercial starter culture from Rhodia (Boulogne-Billancourt, France) was added as starter culture according with the instructions of the manufacturers. In the fermentation trials, two different batches were prepared: (i) commercial batch with commercial starter culture usually added in that industry (CS1); (ii) added starter culture P184S34.

The sausages were ripened for 86 days at the temperature and relative humidity (RH) values listed in Table 1. Samples were taken from each batch at the following times of incubation: 0 h, 3, 7, 25, 63, and 86 days. Each analysis was made in triplicate.

Table 1 Sampling of dry-fermented sausages 'salchichón'

Processing conditions	Temperature (°C) and relative humidity (%)
0–3 days	4 °C; 70%
3–27 days	7 °C; 65–70%
27–62 days	10–12 °C; 70%
62–86 days	10–12 °C; 70%
Total days	86

Microbiological analysis

The *Staphylococcus* count was determined in Mannitol Sal Agar (MSA) at 30 °C, and lactic acid bacteria were grown in MRS (Oxoid) at 37 °C for 24 h in anaerobic conditions at pH 5.6.

To investigate the presence of strains inoculated at high levels in the sausages, the samples at the different days of incubation listed above were used. To get the DNA template, five colonies obtained from these plates which contained counts from the highest dilutions were isolated at random in MRS or MSA broth. Then the DNA was obtained and 16S rRNA gene sequence analysis was performed (Benito *et al*., 2008a,b). The same method was used for the commercial batches to identify the microorganism added.

Total enterobacteria (Gram-negative and cytochrome oxidase-negative) were inoculated on Violet Red Bile Glucose agar (VRBG; Oxoid), the plates were covered with a layer of the same medium before incubation at 37 °C for 24 h, and colonies that were rose-coloured and surrounded by a halo of purple precipitate were counted. Violet Red Bile Agar (VRBA) was used for coliform counts and the inoculated plates of this medium were also covered with a layer of the same medium before incubation at 30 °C for 48 h. Typical dark red colonies (> 0.5 mm in diameter) surrounded by a zone of precipitated bile acids were considered as coliforms for the counts.

Moisture, water activity, and pH determination

The moisture content of the dry-fermented sausages was determined by dehydration at 100 °C to constant weight by the ISO recommended methods (ISO, 1973). Water activity (a_w) was determined using an FA-St/1 apparatus from GBX (France Scientific Instrument). The pH was measured using a Crison mod. 2002 pH meter (Crison Instruments, Barcelona, Spain).

Parameters related to protein fraction

Analysis of sarcoplasmic and myofibrillar proteins

To obtain the sarcoplasmic and myofibrillar proteins, 1 g of sample was homogenized as in Benito *et al*. (2005). The protein concentration was determined following the Bradford method and the results were expressed as mg protein g^{-1} dry matter of protein.

Non-protein nitrogen and amino acid nitrogen analysis

Non-protein nitrogen (NPN) was determined by the Nessler method using 4 g of sample after protein precipitation with 0.6 M perchloric acid. Amino acid nitrogen (AN) was determined from the 0.6 M perchloric acid protein precipitation fraction after peptide precipitation with 10% sulfosalycilic acid (Benito *et al*., 2005).

Biogenic amine production (BA)

The samples were prepared following Křížek & Peliková (1998). A 10 g sample was homogenized and then shaken in a closed Erlenmeyer flask with 75 mL of 0.6 M $HClO_4$ for 1 h. The mixture was filtered through filter Whatman-3MM paper, washed with $HClO_4$, and the volume adjusted to 100 mL. In accordance with the expected amine content, 5 mL of acidic extract was transferred to a test tube, the volume adjusted to 5 mL with $HClO_4$, and then spiked with 125 μL of internal standard solution (1,7-heptanediamine, 400 mg L^{-1}). The mixture was then made alkaline by adding 1 mL of 9.8 M NaOH solution. After brief vortexing, 100 μL of pure benzoyl chloride was added. The test tube was shaken for 2.5 min. Then it was allowed to stand in the water bath of the laboratory ultrasound cleaner for 15–20 min. Subsequently, 2.5 g of NaCl were shaken with the solution for 1 min. This was followed by a two-step extraction with 3 mL of diethyl ether. Combined extracts (1 mL in each step) were dried under a stream of hot air. The dry residue was dissolved in 400 μL of methanol–water (1:1, v/v). Finally, the content of the biogenic amines was determined by micellar electrokinetic capillary chromatography (MECC) (Benito *et al*., 2007; Martín *et al*., 2007) using an automated PACE 5500 (Beckman Instrument Inc., Palo Alto, CA). Tryptamine hydrochloride, histamine dihydrochloride, putrescine dihydrochloride, cadaverine dihydrochloride, and tyramine hydrochloride standards (Scharlab) were used as standards.

Parameters related to lipid fraction

The total lipid fraction was extracted from the final product according to the method of Bligh & Dyer (1959). The acidity value was determined using the ISO 660:1996 method. The extent of lipid oxidation of the final product was estimated as thiobarbituric acid-reactive substances (TBARs) by the extraction method described in Jørgensen & Sørensen (1996). Absorbance at 532 nm (A532) was measured in three replicates of each sample on a Shimadzu UV-1201 spectrophotometer (UNICAM, Mod. Helios). Correction for sample turbidity was made by subtracting the absorbance at 600 nm (A600) from the absorbance at 532 nm (A532). TBARs were expressed as mg of malonaldehyde (MDA) kg^{-1} of sample using tetraethoxypropane (TEP) as a standard.

The fatty acid composition of lipids was determined by gas chromatography after acidic-trans-esterification in the presence of sulfuric acid (5% sulfuric acid in methanol; Cava *et al*., 1997). The gas chromatograph, model Hewlett-Packard 4890 Series II, was equipped with a split/splitless injector and a flame ionization detector. Fatty acid methyl esters (FAMEs) were separated on a nitroterephthalic acid modified polyethylene

glycol (HP-FFAP) fused silica semicapillary column (30 m long, 0.250 mm i.d., 0.25 μm film thickness) maintained at 220 °C. Injector and detector temperatures were 300 °C. Nitrogen was used as carrier gas at 2 mL min^{-1}. The individual FAMEs were identified by comparison of their retention times with those of reference standard mixtures (Sigma Chemical Co., St. Louis, MO). Results were expressed as a percentage of the total fatty acid methyl esters present.

Volatile compound analysis

Volatile compounds were determined from portions of dry-fermented sausages vacuum packaged and stored at −80 °C until analysis (Ruiz *et al.*, 1998). Volatile compounds were extracted by the solid phase micro-extraction (SPME) technique with a 10 mm long, 100 μm thick fibre coated with poly-dimethylsiloxane (Supelco Co., Bellefonte, PA). The SPME fibre was inserted into the vial headspace through the septum and exposed to the headspace for 30 min at 40 °C in a water bath. Gas chromatography/mass spectrometry analyses were performed using a Hewlett-Packard 216 5890 S II gas chromatograph coupled to a Hewlett-Packard 5971A ion-trap mass spectrometer. A 5% phenyl–95% dimethyl polysiloxane column (50 m × 0.32 mm i.d., 1.05 m film thickness; Hewlett-Packard) was used for the separation of volatile compounds. The carrier gas was helium. The injection port was in a splitless mode. The SPME fibre was maintained at 220 °C in the injection port throughout the chromatographic run. The temperature program was isothermal for 15 min at 35 °C, increased to 150 °C at 4 °C min^{-1}, and then to 250 °C at 20 °C min^{-1}. The NIST/EPA/NIH mass spectrum library and Kovats indices were used to identify the volatile compounds.

Texture analysis

Texture profile analysis (TPA) of the samples was performed at room temperature, using a $TA.XTA_{2i}$ texture analyzer (Stable Micro Systems, Godalming, UK) equipped with a cylindrical probe of 50 mm in diameter. This procedure involved cutting slices approximately 1.5 cm thick that were compressed twice to 25% of their original height. Force–time curves were recorded at a cross-head speed of 2 mm s^{-1}. Hardness (N), springiness (cm), cohesiveness, gumminess (N), chewiness (N cm), and adhesiveness (N s) were evaluated at the end of the ripening process.

Instrumental determination of colour

L^*, a^* and b^* values were measured using a Minolta Colorimeter CR-300 (Minolta Camera Co., Japan). The hue angle ($H^\circ = \arctan(b^*/a^*)$) and chroma ($C^* = ((a^*)^2 + (b^*)^2)^{0.5}$) parameters were calculated. The instrument was calibrated before each measurement with an identical wrap to that used for the samples placed over a white tile standard. Lightness, L^*, and chromaticity coordinates, a^* (redness/greenness) and b^* (yellowness/blueness), were measured in quintuplicate for all samples at the end of the ripening process.

Sensory analyses

The sensory analysis was performed by triangle test between the batches at the end of ripening according to the International Standards Organization (ISO, 1981) by asking the tasters to choose the sample they thought was different and their reason for choosing it. The sensory panel consisted of workers from the School of Agricultural Engineering, Badajoz (17 people). Samples were also examined by panellists to judge the overall quality according to a hedonic scale from 1 (very bad) to 10 (very good).

Statistical analysis

Statistical analysis of the data was carried out using one-way analysis of variance, and the means were separated by Tukey's honest significant difference test, using SPSS for Windows, 15.0. (SPSS Inc., Chicago, IL).

Results and discussion

Microbial counts during processing

The microbiological analysis revealed no significant differences between the commercial starter (CS1) and the autochthonous starter culture P184S34 batches in MRS and MSA medium during the ripening process (Fig. 1). Counts in MRS agar were around 10^7 CFU g^{-1} for both batches at the beginning of the process, reached maximum levels of 10^8 CFU g^{-1} after 25 days. These levels remained to the end of ripening. After 16S rRNA gene sequence analysis, the isolates obtained from samples inoculated with the autochthonous starter culture P184S34 were identified as *P. acidilactici* (100% identity with *P. acidilactici* MC184, HQ315857 accession number, Benito *et al.*, 2008a). This shows the strain *P. acidilactici* MC184 to be well adapted to the conditions of processing. The identifications obtained from CS1 batches were *Lactobacillus sakei* (99% identity with *L. sakei* AB494726.1 accession number).

Counts in MSA agar were around 10^7 CFU g^{-1} 0 h of ripening (Fig. 1). These counts decreased in both batches during the first 7 days which may indicate that *Staphylococcus* spp. adapted poorly to the cooling conditions during these early days. Both batches reached maximum levels of 10^9 CFU g^{-1} and decreased to 10^4–10^5 CFU g^{-1} by the end of the process. The

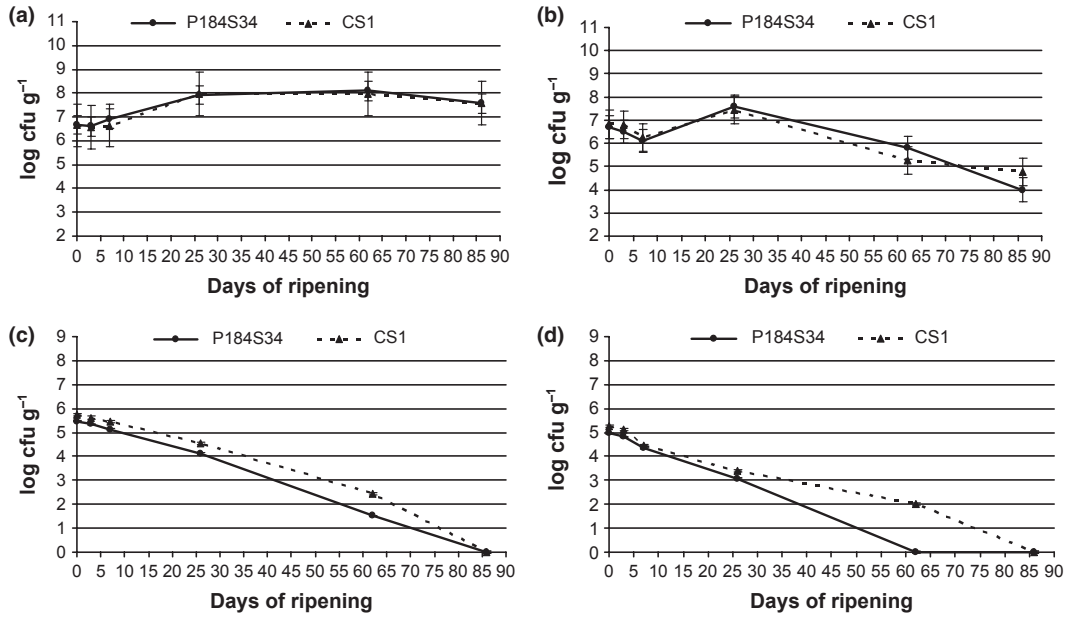

Figure 1 Evolution of lactic acid bacteria (log CFU g^{-1}) in MRS medium (a), *Staphylococcus* sp. (log CFU g^{-1}) in MSA medium (b), enterobacteria (log CFU g^{-1}) in VRBG medium (c) and coliform (log CFU g^{-1}) in VRBA medium (d) during ripening of salchichón.

isolates obtained from samples inoculated with the P184S34 starter culture were identified as *S. vitulus* (100% identity with *S. vitulus* RS34, HQ315860 accession number, Benito *et al*., 2008b). The levels of *Staphylococcus* sp. in the CV1 batches increased during the process. These were mainly identified as *Staphylococcus carnosus* (95% identity with *S.carnosus* AB009934.1 accession number). This specie is commonly used as a starter culture in fermented meat products.

The enterobacteria and coliform counts in VRBG and VRBA plates are shown in Fig. 1. In general, counts for both batches (P184S34 and CS1) were decreasing during the ripening process. However, the inhibitory effect exerted by the starter culture P184S34 on the pathogenic flora was therefore evident, especially from the middle of the process onwards. The enterobacteria and coliform counts at the beginning of the process depend on the hygienic quality of the raw materials and the handling conditions during processing. Although the initial levels found indicate that contamination was high, these values are similar to those found by other authors (González & Díez, 2002; González-Fernández *et al*., 2006; Benito *et al*., 2007; Lebert *et al*., 2007) and the evolution of their populations was similar to that obtained in the present study, being lower than 10 CFU g^{-1} at the end of the maturation process. One of the factors causing the reduction of enterobacteria and coliform is the inhibitory effect of the starter culture, which is crucial to obtain high quality hygienic sausages (Coppola *et al*., 1995; Papa *et al*., 1995; Benito *et al*., 2007; Martín *et al*., 2007). In this sense, the autochthonous starter culture, P184S34, showed an inhibitory effect on this pathogenic flora. The antimicrobial activity of these strains (*P. acidilactici* MC184 and *S. vitulus* RS34) has been shown in '*in vitro*' assays by Benito *et al*. (2007) and Martin *et al*. (2007); thus, P184S34 starter culture could be applied to meat products as a protective culture.

Moisture, water activity, and pH changes

Moisture content, water activity (a_w), and pH values did not differ significantly between batches throughout the ripening period (Fig. 2). In general, the moisture content decreased during ripening from initial values of around 63% to 29%. The water activity ranged from initial values of 0.98 to 0.79 at the end of ripening in all batches. Finally, the pH decreased from about 6.3 to 4.9 after 63 days of ripening, and then rose to 5.7 at the end of process in all batches.

The moisture, a_w, and pH values of the dry-fermented sausages were similar to those reported elsewhere during the ripening process for this kind of traditional product (Benito *el al*., 2007; Casaburi *et al*., 2007; Martín *et al*., 2007).

Changes in the nitrogen fraction during processing

The changes in proteins content was monitored throughout the ripening process. The results given in Table 2 are only for the end of the ripening. The

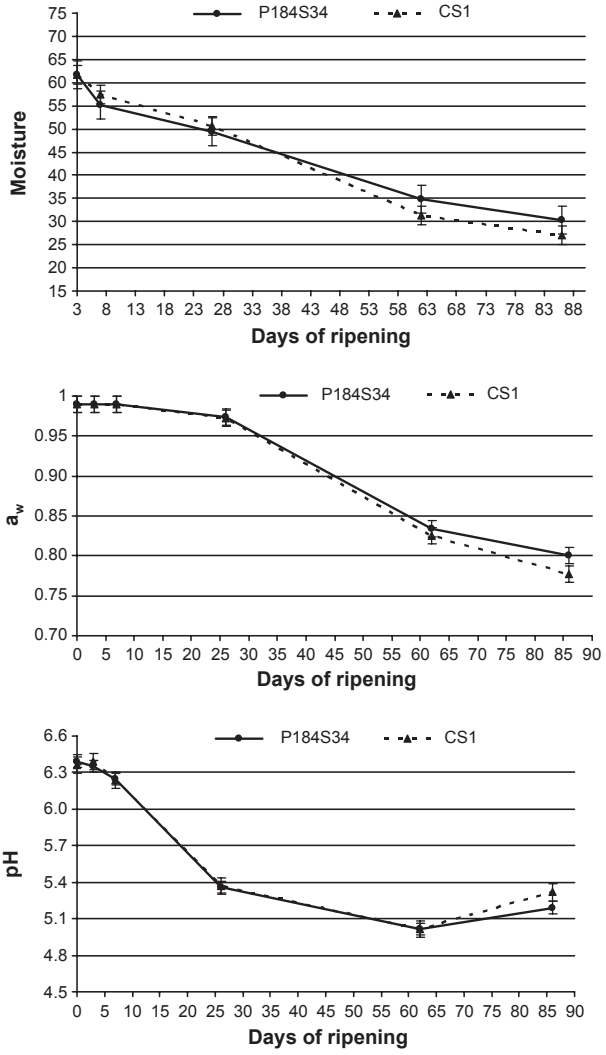

Figure 2 Moisture content, water activity (a_w), and pH values during the ripening process of salchichón.

sarcoplasmic protein concentration of the raw material was 240 mg of protein g^{-1} of dry matter. The myofibrillar protein concentration was 260 mg g^{-1} of dry matter. Sarcoplasmic (SAR) and myofibrillar (MYO) proteins showed significant ($P < 0.01$) decreases during ripening. At the end of processing, there was a reduction that ranged between 82% and 92% in both protein fractions compared to the initial values, with no differences between batches (Table 2). These changes have also been reported in sausages treated with proteases and starter cultures during ripening (Benito *et al*., 2005; Casquete *et al*., 2011).

The decrease of concentration in the sarcoplasmic and myofibrillar proteins in all batches during the ripening process is mainly due to proteolysis at the beginning of the process and protein denaturation at the end. This

Table 2 Compounds related to the nitrogen fraction in the dry-fermented sausages studied at the end of the ripening process

	P184S34	SVC1	
	Mean ± SD	**Mean ± SD**	***P*[1]**
Nitrogen compounds			
mg protein g^{-1} dry matter (SAR)	43.3 ± 3.92	41.3 ± 1.78	
mg protein g^{-1} dry matter (MYO)	16.1 ± 3.47	20.2 ± 4.36	
mg NNP g^{-1} dry matter	4.7 ± 0.30	2.5 ± 0.33	***
mg NA g^{-1} dry matter	0.3 ± 0.01	0.3 ± 0.01	
Biogenic amines (mg kg^{-1})			
Putrescine	7.5 ± 1.75	9.0 ± 1.93	
Cadaverine	35.6 ± 2.48	106.6 ± 9.66	***
Tryptamine	51.4 ± 2.46	49.1 ± 1.26	
Histamine	24.08 ± 12.53	39.9 ± 17.27	
Tyramine	67.4 ± 9.80	69.5 ± 5.61	
Total amines	**185.9 ± 30.05**	**274.1 ± 39.61**	**
Volatile compounds derived from Amino acids catabolism (arbitrary area units and %)			
3-Methylbutanal	6.25 ± 0.56	5.12 ± 1.72	
Benzaldehyde	3.37 ± 0.92	4.53 ± 1.06	
Benzeneacetaldehyde	26.51 ± 4.78	6.07 ± 1.66	***
Di-2-propenyl disulfide	0.71 ± 0.12	1.41 ± 0.30	*
Benzenemethanol	0.43 ± 0.07	0.38 ± 0.09	
Benzeneethanol	0.78 ± 0.15	1.04 ± 0.19	
Total	**31.8 ± 6.04**	**18.5 ± 3.30**	***

[1]*P*: values for inoculation factor.
*$P < 0.1$.
**$P < 0.05$.
***$P < 0.01$.

proteolytic effect is of great interest as it increases the peptides and free amino acids, and their catabolism results in the generation of volatile compounds associated with the flavour of traditional dry fermented meat products (Ordóñez *et al*., 1999; Fernández *et al*., 2000).

The highest values of non-protein nitrogen (NPN) were detected in P184S34 batches (Table 2), although similar free amino acid nitrogen values (AN) were detected in both batches. It should be considered that the main component of AN (free amino acids) is continually used by the microbial population as an energy source and in the process generating volatile compounds or amines, thus it is very difficult to detect differences in AN between batches. The high NPN values of the P184S34 could be due to the microbial proteases from the autochthonous *P. acidilactici* and *S. vitulus* strains (Benito *et al*., 2007; Martín *et al*., 2007). The release of free amino acids plays an important role in the development of flavour (Nishimura *et al*., 1988), and the presence of these compounds has been associated with such taste descriptors as spicy, beefy, sweet, bitter, and astringent (Ansorena *et al*., 1998), and their catabolism results in the generation of volatile compounds associated with the flavour of traditional dry fermented meat products.

Furthermore, the strains *P. acidilctici* MC184 and *S. vitulus* RS34 were selected for their non-production of biogenic amines (BA) because of the low activity of their amino acid decarboxylases (Benito *et al.*, 2007; Martín *et al.*, 2007). Indeed, there were significant differences between batches, with the P184S34 batch having a lower level of BA than the CS1 batch (Table 2). The level of cadaverine was the highest and putrescine was the lowest in both batches. Several authors (Leuschner & Hammes, 1998; Leuschner *et al.*, 1998; Bover-Cid *et al.*, 2001) tested in vitro potential amine degradation by many bacteria isolated from foods and, in particular, in strains belonging to the genera *Lactobacillus*, *Pediococcus*, and *Staphylococcus*, and found that this enzymatic activity can be present at very different levels. In addition, amounts detected in our study were low compared with those reported by other authors at the end of shorter ripening processes (Roseiro *et al.*, 2010). The batches with the autochthonous starter culture, P184S34, were inoculated with strains with low amine-producing capacity, which confirms that the inclusion as starter culture of strains without amino acid descarboxylase activity prevents BA accumulation in Iberian sausages (Leuschner *et al.*, 1998; Bover-Cid *et al.*, 2000; Casquete *et al.*, 2011). Thus, addition of selected starter culture P184S34 to sausages could avoid risk for the consumers due to formation of high biogenic amines concentration.

Volatile compounds attributed to catabolism of amino acids are shown in Table 2. Branched carbonyls and alcohols were present at higher levels in sausages of the P184S34 batches. Moreover, the inoculated P184S34 batches exerted a greater impact than CV1 batches on benzeneacetaldehyde compound ($P < 0.01$). The ability of LAB and *Staphylococcus* strains for producing these products of phenylalanine metabolism have previously been described in several studies (Nierop Groot & De Bont, 1999; Tjener *et al.*, 2004). These compounds have been described as being produced during the ripening of these kinds of fermented sausages (Sunesen *et al.*, 2001). Thus, the starter culture P184S34 could have a positive impact on these important flavour compounds.

Changes in the lipid fraction

The amount of fat in the batches was 47% (Table 3). Lipids are the source of many aromatic substances generated by lipolytic and oxidative phenomena that take place during the ripening process (Navarro *et al.*, 1997). The intensity of these phenomena in the batches studied was determined by parameters related to fat stability such as fatty acid index, MDA production, total fatty acid, and volatile compounds generated from lipid oxidation (Table 3). The fatty acid index of the fat and the amount of MDA ranged from 2.8 to 3.3 mg NaOH g^{-1} of fat and 5.3–5.4 mg kg^{-1}, respectively, and there were no significant differences between batches. These values were similar to those

Table 3 Compounds and parameters related to the lipid fraction in the Iberian dry-fermented sausages at the end of the ripening process

	P184S34	SVC1	
	Mean ± SD	Mean ± SD	P^1
Lipid			
Total fat (%)	47.3 ± 4.54	47.2 ± 2.53	
Fatty acid index (mg NaOH g^{-1})	2.8 ± 0.46	3.3 ± 0.88	
MDA (mg kg^{-1})	5.4 ± 0.39	5.3 ± 1.53	
Total fatty acids (%)			
SFA[3]	39.1 ± 1.59	39.9 ± 0.54	
MUFA[4]	51.0 ± 0.85	49.1 ± 0.47	*
PUFA[5]	9.9 ± 2.47	11.0 ± 0.269	
Volatile compounds derived from lipid autoxidation (arbitrary area units and %)			
Propanal	6.3 ± 0.89	7.7 ± 1.65	
1-Propanol	6.2 ± 0.77	4.4 ± 0.81	**
Pentanal	4.9 ± 0.56	7.4 ± 1.44	***
1-Pentanol	2.1 ± 0.61	0.6 ± 0.17	**
Hexanal	1.5 ± 0.28	1.9 ± 0.43	
1-Hexanol	26.7 ± 5.64	42.6 ± 8.02	***
Hexanoic acid	5.1 ± 0.71	1.9 ± 0.36	***
Heptanal	0.3 ± 0.05	7.1 ± 1.68	***
1-Heptanol	2.4 ± 0.43	7.7 ± 1.85	**
Octanal	0.6 ± 0.11	1.4 ± 0.31	***
2-Octenal	2.9 ± 0.55	4.7 ± 1.04	
1-Octanol	1.1 ± 0.22	3.4 ± 0.76	***
Nonanal	0.4 ± 0.07	0.7 ± 0.14	
Octanoic acid	3.1 ± 0.53	6.7 ± 1.52	**
Decanal	0.3 ± 0.06	0.8 ± 0.18	
2.4-Nonadienal	0.8 ± 0.13	0.7 ± 0.19	
2-Decenal	0.3 ± 0.05	n.d.[2] ± 0.00	*
Nonanoic acid	n.d. ± 0.00	0.7 ± 0.16	*
2.4-Decadienal	0.5 ± 0.10	1.0 ± 0.24	
Decanoic acid	n.d. ± 0.00	0.5 ± 0.12	*
Tetradecanoic acid	0.3 ± 0.05	0.8 ± 0.17	
Total	**65.8 ± 11.81**	**102.7 ± 21.24**	***
Volatile compounds derived from microbial β-oxidation activity (arbitrary area units and %)			
1-Penten-3-ol	1.2 ± 0.22	0.9 ± 0.21	
2-Heptanone	2.2 ± 0.34	0.9 ± 0.19	**
2.3-Octanedione	0.8 ± 0.17	4.2 ± 1.16	***
1-Octen-3-ol	2.8 ± 0.41	2.4 ± 0.46	
2-Octanone	0.4 ± 0.10	1.4 ± 0.32	*
2-Nonanone	3.4 ± 0.45	0.8 ± 0.11	**
2-Undecanone	n.d. ± 0.00	0.8 ± 0.16	*
Total	**10.8 ± 1.69**	**11.4 ± 2.45**	

[1]*P*: values for inoculation factor.
[2]Not detected.
[3]Saturated fatty acid.
[4]Monounsaturated fatty acid.
[5]Polyinsaturated fatty acid.
*$P < 0.1$.
**$P < 0.05$.
***$P < 0.01$.

Table 4 Analysis of texture, colour, and sensory characteristics at the end of ripening

	P184S34	CS1	
	Mean ± SD	Mean ± SD	P^1
Texture analysis			
Hardness (N)	286.9 ± 23.79	190.4 ± 43.83	***
Springiness (cm)	0.8 ± 0.03	0.8 ± 0.04	
Cohesiveness	0.9 ± 0.02	0.8 ± 0.04	
Gumminess (N)	247.6 ± 23.44	154.4 ± 37.26	***
Chewiness (N cm)	210.0 ± 13.11	121.5 ± 28.13	***
CIE color values			
a	16.4 ± 4.23	13.9 ± 0.35	
*C**	17.9 ± 4.84	15.4 ± 0.26	
*H**	23.6 ± 2.87	25.8 ± 0.97	
b	7.3 ± 2.48	6.7 ± 0.13	
L	40.4 ± 3.26	41.5 ± 6.77	
Overall acceptability	7.1 ± 0.67	6.1 ± 0.68	

[1]*P*: values for inoculation factor.
***$P < 0.01$.

found by other authors in similar meat products (Marco *et al*., 2006; Olivares *et al*., 2010). With respect to the total fatty acids, the greatest percentages corresponded to C18:1n-9, C16:0, and C18:0, with 45%, 25%, and 12% of the total fatty acids. There were no differences between batches (Table 3). These results agree with the ones obtained by Rubio *et al*. (2008) for these kinds of products.

The volatile compounds formed by lipid autoxidation and microbial β-oxidation are listed in Table 3. Compounds such as 1-propanol, propanal, and 1-hexanol were present in the greatest amounts in the samples. The highest values of total volatile compounds were detected in sausages of the CS1 batches. An additional increase in fatty acid precursors in these sausages due to the lipase activity of this commercial starter culture could explain this result.

Texture, colour, and sensory analyses

The results of the texture profile analysis (TPA) at the end of ripening are shown in Table 4. In general, hardness, gumminess, and chewiness were lower in CS1 than in the P184S34 samples. The differences could be due to the different enzymatic activities of the starter cultures used. However, these values are commonly described in these kinds of products (Casquete *et al*., 2011). With respect to the colour measurements, there were no differences in the hue (*H**), chroma (*C**), *L**, *a**, or *b** components (Table 4). These results are consistent with those found by other authors in these kinds of products (Chasco *et al*., 1996).

Finally, the triangle test made comparing P184S34 with commercial starter batches were well distinguished ($P < 0.001$). However, the overall quality revealed no significant differences between batches although the P184S34 batches were ranked better (Table 4).

In conclusion, the autochthonous starter culture P184S34 was able to compete well and control the process. This autochthonous starter culture showed a higher inhibitory effect on enterobacteria and coliform flora than the commercial starter culture (CS1), guaranteeing a better quality hygienic sausages. Especially important was the result found for biogenic amines, since the P184S34 reduced their accumulation compared to the commercial starter batch. The inoculation of a decarboxylase-negative autochthonous starter culture P184S34 reduced the biogenic amine accumulation guaranteeing healthiness and homogeneity of the products without producing a negative effect on the sensory characteristics of the traditional fermented sausages.

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