



Effect of commercial starter cultures on free amino acid, biogenic amine and free fatty acid contents in dry-cured foal sausage



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ABSTRACT

This study assessed the effects of commercial starter cultures on microbial counts, proteolytic and lipolytic changes of dry-cured foal sausage. Four different batches of foal sausage were manufactured: **CO** batch: control without starter culture; **CX** batch was fermented with *Staphylococcus carnosus* + *Staphylococcus xylosum* + *Pediococcus pentosaceus*; **FL** batch with *Debaryomyces hansenii* + *S. xylosum* and **TH** with *P. pentosaceus* + *S. xylosum*. The results revealed that the use of starter cultures increase the number of lactic acid bacteria and the total viable counts, while completely reduce the *Enterobacteriaceae* count compared with **CO** batch (spontaneous fermentation). The use of starter cultures increases free amino acids release. The **CO** batch showed the lowest content of free amino acid (952 vs. about 1200 mg/100 g dry matter). Total level of biogenic amines ranged from 61 to 143 mg/kg. Sausages inoculated with TH starter culture displayed the highest accumulation of total biogenic amines. Lipolytic activity was lower in starter-inoculated sausages than in **CO** batch (8726–9503 vs. 12858 mg of total free fatty acid/100 g of fat). In conclusion, the use of starter cultures contributes to improve the hygienic quality, however, additional studies should be undertaken to understand the effect of lipolysis and proteolysis on the sensory properties of this product.

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1. Introduction

Salchichón is a traditional Spanish dry-ripened sausage whose basic ingredients are lean pork, pork backfat, salt and spices (Fonseca, Gómez, Domínguez, & Lorenzo, 2015). In general, pork is the meat used to elaborate this Spanish traditional sausage, but, another type of meat is susceptible of being used.

It is well known that the typical characteristics of fermented sausages are generated by chemical, biochemical, physical and microbiological changes that occur during fermentation, ageing and drying (Aro, Nyam-Osor, Tsuji, Shimada, Fukushima, & Sekikawa, 2010; Lorenzo, Purriños, Bermúdez, Temperán, & Franco, 2011). Two of these changes including degradation of lipids and proteins play a very important role in determining final organoleptic characteristics of product (Lorenzo, Temperán, Bermúdez, Cobas, & Purriños, 2012). During ripening of meat products, the proteins undergo degradation processes; large peptides are first

generated and then degraded into oligopeptides, and these are in turn degraded to free amino acids. The free amino acids are then catabolized, giving rise to different compounds such as ammonia, α -ketoacids, methylketones, and amines (Bermúdez, Lorenzo, Fonseca, Franco, & Carballo, 2012). An excessive intake of these amines might represent toxicological effects on human health (Lorenzo et al., 2010).

Independently of flavour, it is fundamental to keep the safety in this type of fermented products. The addition of starter cultures has become common in the manufacture of several types of fermented products in order to ensure safety by restraining the development of wild microbiota, thus reducing the risk of pathogenic and spoilage bacteria, as well as to contribute to colour and flavour development and extend shelf-life, maintaining the typical characteristics obtained in artisanal productions (Casaburi et al., 2008; Ciuciu Simion, Vizireanu, Alexe, Franco, & Carballo, 2014; Essid & Hassouna, 2013; Lorenzo, Gómez, Purriños, & Fonseca, 2016). Thus, the aim of this work was to study the effect of the starter cultures on free amino acids, biogenic amines and free fatty acids produced during the manufacturing process of foal sausage.

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2. Material and methods

2.1. Sausage production and sampling procedures

Four different batches of foal sausage were manufactured according to traditional techniques, one of them without starter culture and the other three batches with addition of different commercial starter cultures (Cargill and Sacco) in a proportion defined by the manufacturer in each case. The batches were named as follows: (i) **CO** batch, control without starter culture, (ii) **CX** batch, with CXP (Cargill, Barcelona, Spain) (*Staphylococcus carnosus* + *Staphylococcus xyloso* + *Pediococcus pentosaceus*), (iii) **FL** batch, with Flavor Start P406 (Cargill, Barcelona, Spain) (*Debaromyces hansenii* + *S. xyloso*) (iv) **TH** batch, with Lyocarni THM-17 (Sacco, Cadorago, Italy) (*P. pentosaceus* + *S. xyloso*). Sausage manufacture was done two different times. The four batches mentioned before were manufactured with the same ingredients, formulation and technology in May and June 2014.

Foal sausage formulation includes lean foal meat (85%), pork back fat (15%), NaCl (25 g/kg), lactose (10.7 g/kg), dextrin (9.3 g/kg), sodium caseinate (20 g/kg), glucose (7 g/kg), black pepper (1.5 g/kg), white pepper (1 g/kg), sodium ascorbate (0.5 g/kg), sodium nitrite (0.15 g/kg) and potassium nitrate (0.15 g/kg). The lean foal meat and the pork back fat were ground through a 12 and 8 mm diameter mincing plate, respectively, and vacuum mixed together with the other ingredients and the starter cultures (depending on the batch) during 3 min. The mix was maintained at 4 °C for 24 h and then stuffed into natural casings with a diameter of 60 mm and a length of 40 cm. The sausages were fermented for 2 days at 20 °C and 80–85% of relative humidity and then transferred into a drying-ripening chamber where they were kept for 51 more days at 12 °C and 75–80% relative humidity. Samples were taken at the end of the ripening for subsequent analysis.

2.2. Microbial analysis

For microbiological analysis, 10 g of sausage was aseptically weighted in a sterile plastic bag, previously removing and discarding the outer plastic. Subsequently samples were homogenized with 90 mL of a sterile solution of 0.1% (w/v) peptone water (Oxoid, Unipath, Basingstoke, UK), containing 0.85% NaCl and 1% Tween 80 as emulsifier, for 2 min at 20–25 °C in a Masticator blender (IUL Instruments, Barcelona, Spain), thus making a 1/10 dilution. Serial 10-fold dilutions were prepared by mixing 1 mL of the previous dilution with 9 mL of 0.1% (w/v) sterile peptone water. Total viable counts were enumerated in Plate Count Agar (PCA; Oxoid, Unipath Ltd., Basingstoke, UK) and incubated at 30 °C for 48 h; lactic acid bacteria (LAB) were determined on the Man Rogosa Sharpe medium Agar (Oxoid, Unipath Ltd., Basingstoke, UK) (pH 5.6) after an incubation at 30 °C for 5 days; *Enterobacteriaceae* was determined on Violet Red Bile Glucose Agar (Merck, Darmstadt, Germany) after an incubation at 37 °C for 24 h and yeasts were enumerated using OGYE Agar Base (Merck, Darmstadt, Germany) with OGYE Selective Supplement (Merck, Darmstadt, Germany), previously ready and incubated at 25 °C for 4–5 days. After incubation, plates with 30–300 colonies were counted. The microbiological data were transformed into logarithms of the number of colony forming units (CFU/g).

2.3. Chemical composition and pH values

Moisture, fat and protein were determined according to the ISO recommended standards (ISO 1442:1997, ISO 1443:1973 and ISO 937:1978, respectively). The pH of samples was measured using a digital pH meter (model 710 A+, Thermo Orion, Cambridgeshire,

UK) equipped with a penetration probe.

2.4. Free amino acid analysis

Amino acids were extracted following the procedure described by Lorenzo, Cittadini, Bermúdez, Munekata, and Domínguez (2015a). Amino acids were derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Waters AccQ-Fluor reagent kit) and analyzed by RP-HPLC using a Waters 2695 Separations Module with a Waters 2475 Multi Fluorescence Detector, equipped with a Waters AccQ-Tag amino acids analysis column. The free amino acids results were expressed in mg/100 g of dry matter.

2.5. Biogenic amine analysis

The extraction of biogenic amines from samples was performed according to the method developed by Zhai et al. (2012) while the derivatization was performed with dansyl chloride using the method reported by Lu et al. (2010). Biogenic amines were analyzed by RP-HPLC using a Waters 2695 Separations Module with 996 photodiode array detector (Waters Milford, USA). The analysis was performed using a reverse-phase column (SunFire™ C18, 4.6 mm ID × 150 mm, 5 µm particle size, Waters, Milford, MA, USA), with UV–Vis photodiode array detection (254 nm). The temperature of the column oven was adjusted at 40 °C. Data regarding biogenic amines composition were expressed in mg/kg.

2.6. Free fatty acids analysis

Total lipids were extracted from 25 g of ground sample, according to Bligh and Dyer (1959) procedure. Free fatty acids were separated and transesterified according to Lorenzo et al. (2015a) procedure. Separation and quantification of the FAMES were carried out using a gas chromatograph (GC-Agilent 6890N; Agilent Technologies Spain, S.L., Madrid, Spain) equipped with a flame ionization detector following the chromatographic conditions described by Lorenzo et al. (2015a). Data regarding FAME composition were expressed in mg/100 g of fat.

2.7. Statistical analysis

A total of 80 sausages (ten sausages for each batch × four batches × two replicates) were analyzed for different parameters. The effect of different commercial starter cultures on microbial counts, free amino acids, biogenic amines and free fatty acids content was examined using a mixed-model ANOVA, where these parameters were set as dependent variables, commercial starter cultures as fixed effect, and replicate as random effect. The pairwise differences between least-square means were evaluated by Duncan's method. Differences were considered significant if $P < 0.05$. The values were given in terms of mean values and standard error (SEM). All statistical analysis were performed using IBM SPSS Statistics 19 software (IBM Corp., 2010).

3. Results and discussion

3.1. Microbial counts

The effect of starter cultures in the microbial counts of dry-cured foal sausage is shown in Table 1. Microbial counts were affected ($P < 0.05$) by the use of starter cultures. The differences in total viable counts (TVC) could be related to the addition of starter cultures, since significantly ($P < 0.001$) higher TVC values were observed in inoculated sausages compared to CO batch. This result

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