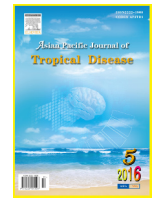




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### Role of commercial starter cultures on microbiological, physicochemical characteristics, volatile compounds and sensory properties of dry-cured foal sausage

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#### ABSTRACT

**Objective:** To assess the effect of three commercial starter cultures on microbial counts, physicochemical changes, volatile profile and sensory characteristics of dry-cured foal sausage.

**Methods:** Microbial counts (lactic acid bacteria, Enterobacteriaceae, total viable counts and yeast), proximate parameters (moisture, fat and protein), colour analysis, texture analysis (texture profile analysis test), volatile compounds (solid-phase microextraction-gas chromatography-mass spectrometer technique) and sensory analysis were evaluated in the dry-cured foal sausages using the standard food analysis techniques.

**Results:** The results revealed that the use of starter cultures increased the number of lactic acid bacteria and total viable counts, while completely reduced Enterobacteriaceae count. Started sausages presented the lowest value of pH, while CX and FL batches had the highest protein amount. In contrast, the use of starter cultures did not affect the other physicochemical parameters. According to volatile profile, there were no differences between batches in total volatile compounds, however, control batch presented the highest amount of aldehydes, derived from lipid oxidation. The sensory analysis showed low differences. Control batch presented higher flavour intensity and lower acid taste score and black pepper odour than inoculated batches.

**Conclusions:** As a general conclusion, the use of starter cultures contributed to improve the hygienic quality with low impact in physicochemical and sensory properties.

## 1. Introduction

The acceptance of horsemeat as a food for humans has changed due to changes in attitude from aversion to qualified approval of this meat[1]. From the nutritional point of view, horsemeat is excellent, since it is low in fat, rich in iron and it has a favourable dietetic fatty acid profile with a high content of unsaturated fatty acids and vitamin B[1]. “Salchichón” is a Spanish fermented dry-cured sausage. It has been reported that this kind of fermented dry sausage could contain, during processing and in the final product, some of the pathogenic bacteria which is often associated with meat products[2]. For this reason, to guarantee the safety of consumers and also the quality, maintaining the typical “salchichón” characteristics

as to colour and flavour, it is very important to use starter cultures. The final product is the result of a complex microbiological activity, which consists of a lactic fermentation and several biochemical reactions characterizing more or less prolonged ripening period[3].

Poteolysis and lipolysis reactions are responsible for the most important biochemical changes occurring during the ripening of dry fermented sausages[4]. Both reactions are catalysed by either endogenous enzymes present in the meat tissues or by those of microbial origin from added starter cultures. All volatile compounds generated during the process of a dry fermented sausage are of great importance to the aromatic character of the final product.

Given what has been said above, the aim of this work was to study the effect of different commercial starter cultures on microbial counts, chemical composition, colour and textural parameters, production of volatile compounds and on sensory characteristics of foal “salchichón”.

## 2. Materials and methods

### 2.1. Sausage production and sampling procedures

Four different batches of foal sausage were manufactured

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according to traditional techniques, one of them without starter cultures 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) CNT batch, control without starter culture; (ii) CX batch, with CXP (Cargill) [*Staphylococcus carnosus* + *Staphylococcus xylosus* (*S. xylosus*) + *Pediococcus pentosaceus*]; (iii) FL batch, with flavor start P406 (Cargill) [*Debaryomyces hansenii* (*D. hansenii*) + *S. xylosus*]; (iv) TH batch, with Iyocarni THM-17 (Sacco) (*Pediococcus pentosaceus* + *S. xylosus*). Sausage manufacture was done at 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 foal lean meat (85%), pork back fat (15%), NaCl (25.0 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 (1.0 g/kg), sodium ascorbate (0.5 g/kg), sodium nitrite (0.15 g/kg) and potassium nitrate (0.15 g/kg). The foal lean meat and the pork back fat were ground through 12- and 8-mm diameter mincing plates, respectively, and in a vacuum (Industrial Fuerpla, Mod. AO-85, Spain) mixed together with the other ingredients for 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. Microbiological analysis

Microbiological analysis was carried out following the procedure described by Lorenzo *et al.*[5]. 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[6], fat[7] and protein[8] were determined according to standards recommended by International Organization for Standardization. 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. Colour analysis

Colour parameters were measured using a portable colorimeter (Konica Minolta CM-600d, Osaka, Japan) with pulsed xenon arc lamp filtered to illuminant D65 lighting conditions, 0° viewing angle geometry and 8-mm aperture size, to estimate meat colour in the CIE L\*a\*b\* space: lightness, redness, yellowness. The colour was measured in three different points of each sample.

## 2.5. Texture analysis

Texture profile analysis was determined on “salchichón” slices of 1 cm × 1 cm × 2 cm (height, width, length) using a texture analyzer (TA.XTplus, stable micro systems, Godalming, UK). Textural parameters were measured by a 60% compression with

a compression probe of 19.85 cm<sup>2</sup> of surface contact. Force-time curves were recorded at a crosshead speed of 3.33 mm/s. Hardness, springiness, cohesiveness and chewiness values were obtained using the software TEE32 Exponent 4.0.12 (stable micro systems, Godalming, UK).

## 2.6. Volatile compound profile

The extraction of the volatile compounds was performed using solid-phase microextraction (SPME). A SPME device (Supelco, Bellefonte, USA) containing a fused silica fibre (10 mm in length) coated with a 50/30 layer of divinylbenzene/ carboxen/polydimethylsiloxane was used. Headspace SPME extraction (from 1 g of sample) and chromatography were carried out under the conditions described by Gómez and Lorenzo[9]. The results were expressed as AU (area units) × 10<sup>6</sup>/g of dry matter.

## 2.7. Sensory analysis

Sensory analysis was conducted with ten panellists selected from the Meat Technology Centre of Galicia. The panellists were trained for 2 weeks according to the attributes and scale recommended by International Organization for Standardization[10]. Thirteen sensory traits of dry-cured foal sausages, grouped as appearance (fat distribution and colour intensity), odour (odour intensity, black pepper odour and mould odour), taste (acid taste and saltiness), texture (hardness, juiciness and pastosity) and flavour (flavour intensity, cured flavour and rancid flavour), were assessed.

The casings were removed and the sausages were cut into slices approximately 4 mm thick and served at room temperature on white plastic dishes. The samples were individually labelled with three-digit random numbers. The intensity of each attribute was expressed on an unstructured scale from 0 (sensation not perceived) to 9 (the maximum sensation). The samples were evaluated by panellists in two sessions (four samples per session). During sensory evaluation, the panellists were situated in separate cubicles illuminated with red light. Water was used to clean the palates and remove residual flavours at the beginning of the session and in between samples.

## 2.8. 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 SEM. All statistical analysis was performed using international business machine SPSS statistics 19 software[11].

## 3. Results

### 3.1. Microbial counts

The effect of starter cultures on the microbial counts was shown in

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