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Development of fermented sausage produced with mutton and native starter cultures

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ABSTRACT

The aims of this study were to develop and characterize a fermented sausage produced with different percentages of mutton (from adult female sheep, over four years old) and additional native starter cultures. Three treatments of fermented sausage were prepared: (T1) 30% of mutton; (T2) 60% of mutton; (T3) 90% of mutton. Starter cultures *Staphylococcus xylosus* LQ3 and *Pediococcus pentosaceus* P38 were used for the fermentation/maturation process. The parameters of a_w, pH, acidity, weight loss, and monitoring of native starter cultures were evaluated during the fermentation and maturation processes. After maturation, the sausages were assessed for texture profile, color, lipid profile, volatile compounds and sensory analysis. Based on principal components analysis (PCA), it was verified that as mutton was added, there was a reduction of unsaturated fatty acids and an increase in red tonality. T2 showed higher ester production, which possibly positively influenced the acceptance of the aroma of the sausage. Sensorial analysis demonstrated that T2 had a greater tendency to be accepted. It can be concluded that up to 60% of meat from adult sheep can be used in the preparation of fermented sausages and that the use of native cultures attributed positive and typical characteristics to fermented sausage.

1. Introduction

Global sheep production is increasing, and a 22% growth in global sheep meat supply is projected for the next 10 years (Behrendt & Weeks, 2017). Consequently, world consumption of lamb and mutton is also increasing, and in the next 10 years it is estimated that in developed countries there will be a 2% increase in consumption per person (Behrendt & Weeks, 2017).

However, consumer preference is for the consumption of meat from sheep aged up to 18 months. Other adult categories, especially those over four years old, denominated as mutton, do not achieve the same acceptance because they present a firmer texture and a more intense taste and odor, reflected in low added value (Da Silveira, Osório, & Sañudo, 2009; Nassu, Beserra, & Gonçalves, 2002). In the process of agroindustrialization of the meat it is possible to add value to the feedstock by using cuts of low added value (Nassu et al., 2002). Therefore, agroindustrialization of mutton is a necessity, because it is an economically devalued category.

A strategic alternative to improve the sensorial aspects of meat products has been the production of fermented sausages, requiring the application of starter cultures. The use of starter cultures aims to obtain standardized and safe products. Several studies indicate that native starter cultures are microorganisms adapted to the environmental and ecological conditions, and they therefore possess greater ability to grow and compete with the microbiota present in the product (Drosinos et al., 2005). In addition, they present an enzymatic profile that can give a genuine taste to sausages (Fiorentini et al., 2009).

Typical fermented meat sausages normally show water activity (a_w) below 0.92, pH between 4.8 and 5.5, weight loss between 35 and 45%, acid taste, cured sausage color (formation of nitrosomyoglobin), firm texture, formation of volatile compounds such as acids, esters, ketones, aldehydes, terpenoids, and sulfur compounds. These characteristics are associated with the participation of lactic acid bacteria (LAB) and Gram-positive catalase-positive cocci (GCC^+), belonging to *Staphylococcaceae* (Coagulase-negative staphylococci - CNS). Lactic acid bacteria's main function is to reduce the pH of the matrix, through production of lactic acid, while GCC^+ species contribute to the formation of the characteristic flavor of fermented meat products. We used two isolates from local food: *P. pentosaceus* P38 (belonging to the LAB group) and *S. xylosus* LQ3 (belonging to the CNS group).

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Therefore, the aims of this study were to develop and characterize a fermented sausage produced with different percentages of mutton and additional native starter cultures.

2. Materials and methods

2.1. Materials

Pork and pork fat, mutton from the Corriedale breed, additives, spices, and collagen casings were obtained in the local market. Lyophilized native starter cultures, *S. xylosus* LQ3 and *P. pentosaceus* P38, stored at −80 °C were used. *Staphylococcus xylosus* LQ3 was isolated from Parmesan cheese, and it was used because it presented curing and antioxidant potential in dried cured sausage (Cruxen et al., 2017). *Pediococcus pentosaceus* P38 was isolated from cooked ham and was chosen because it demonstrated tolerance to sodium chloride (6.5%) and to pH 4.0, and was able to ferment sucrose, maltose, trehalose, and galactose sugars in *in vitro* tests (unpublished data). Both isolates were identified by the sequencing of 16S gene of rDNA.

2.2. Production of fermented sausage

Initially pork, mutton and pork fat were ground in a grinder (METVISA®) using 8 mm discs. Three treatments were prepared: (T1) 30% of mutton + 60% of pork + 10% of pork fat; (T2) 60% of mutton + 30% of pork + 10% of pork fat; (T3) 90% of mutton + 10% of pork fat. A meat mixer (SKYMSEN®) was used to mix meat mass with 2.3% NaCl (Diana®), 0.7% sucrose (União®), 0.2% glucose (Synth®), 0.16% commercial cure salt (B002 Bremil), 0.5% spice (B181 Bremil®) and 0.25% antioxidant (Kraki®). After correct homogenization, the native starter cultures were added (6 Log CFU/g of *S. xylosus* LQ3 and 7 Log CFU/g of *P. pentosaceus* P38) to each treatment. Meat mass was stuffed into pre-hydrated artificial collagen casings. The sausages were then stored in a chamber for fermentation/maturation (Frilux®), with controlled relative humidity and temperature for 25 days. The temperature was set at 25 °C on the 1st day and then reduced one degree per day until 18 °C. The humidity was 95% on the 1st day, 93% for the 2nd day, 90% for the 3rd day, 85% for the 4th day, 80% for the 5th day, and 75% for the 6th day until the end of the maturation process.

Acidity, pH, a_w, weight loss, and viability of LAB and CNS analyses were performed immediately after sausage production and on the 1st, 3rd, 7th, 18th and 25th days to comprise fermentation and maturation period. The maturation period ended when the sausages reached $a_w = 0.86$ (product considered ready for consumption). After that, the sausages were vacuum packed and stored at room temperature under uncontrolled conditions (simulating market shelf conditions) and sampled on the 40th day for color, texture profile, fatty acid profile, volatile compounds, and sensory analysis.

2.3. Physicochemical analysis

2.3.1. Acidity, pH, water activity and weight loss

The titratable acidity (% lactic acid) was performed using 0.1 N sodium hydroxide and phenolphthalein as indicator, while pH was determined in a pH meter (DM-22, Digimed) according to AOAC (2005). Water activity analysis (a_w) was performed using LabMaster - a_w (Novasina®). The weight loss was defined by the difference between final weight and initial weight on semi analytical scales (Bel, M723, Piracicaba, BR).

2.3.2. Texture profile

Texture Profile Analysis (TPA) was performed in a (Stable Micro Systems, TA-XT plus, Surrey, UK) texturometer, using five samples of each treatment with thickness of 0.8 cm. The parameters used in the software configuration were according to Menegas, Pimentel, Garcia, & Prudêncio et al. (2013). The primary parameters of hardness (N), cohesiveness (dimensionless), adhesiveness (Ns), springiness (dimensionless) and the secondary parameters of chewiness (N) and gumminess were determined.

2.3.3. Color

Five samples of each treatment with thickness of 1.0 cm were used. Samples were measured for the CIE L*, a*, b* values using a (Minolta Camera Ltda, Japan) colorimeter. Hue angle (tonality) was calculated, using the following equation:

$$\text{Hue} = \tan^{-1} (b^*/a^*)$$

Approximate tonality considered for the angles: 0° = red; 90° = yellow; 180° = green; 270° = blue and 300° = purple.

2.3.4. Fatty acid profile

The total lipid fraction from three treatments (T1, T2, and T3) was extracted according to the method of Bligh and Dyer (1959). Approximately 3 g of sample were extracted with chloroform, methanol, and water, producing a biphasic system. Chloroform-lipid extracts were concentrated at 40 °C under N_2 gas flow. Then, 30 mg of the lipids were transferred to a flask and methylated leading to the formation of fatty acid methyl esters (FAMEs) (Hartman & Lago, 1973). FAMEs were analyzed in a gas chromatograph equipped with a flame ionization detector (GC-FID), Varian Star 3400CX (CA, USA), equipped with automatic sampler Varian 8200 (CA, USA). One microliter injection was performed using a split/splitless injector operating in split mode 1:20 at 250 °C. Hydrogen was used as a carrier gas at a constant pressure of 10 psi. FAMEs were separated in a polar capillary column DB-WAX of 30 m × 0.25 mm × 0.25 μm (J & W, Folson, CA, USA). The initial column temperature was 50 °C for 0.5 min, which was then increased at a rate of 15 °C/min to 180 °C, then increased to 220 °C at a rate of 2 °C/min, followed by 20 °C/min until reaching 230 °C, and remaining at this temperature for 3 min. The detector temperature was maintained at 250 °C. FAMEs were identified by comparing the retention times of the analytes with authentic standards FAME Mix-37, (P/N 47885-U), purchased from Sigma-Aldrich and produced by Supelco (PA, USA). The standards were analyzed under the same chromatographic conditions as the samples. Thus, normalized fatty acids were expressed in percentage of the total chromatographic area.

2.3.5. Analysis of volatile compounds

The volatile compounds of the fermented sausages were isolated and identified in gas chromatograph coupled to a mass spectrometer (GC/MS; SHIMADZU, QP-2010 Plus, Tokyo, Japan). The extraction was performed using the solid-phase microextraction (SPME) technique as described by Wagner and Franco (2012). Portions of 5 g of the sausage samples were ground and weighed into 20 mL flask, which was immediately sealed with a septum of an internal face of PTFE. The fiber Carboxen/PDMS (75 μm, 10 mm, Supelco, Bellefonte, PA, USA) was used for the adsorption of the volatile compounds. The fiber was exposed in the headspace of the sample for 45 min at a temperature of 40 °C.

The desorption of the compounds occurred in the split/splitless type injector, operated in split mode (1:5). Helium gas was used as a carrier gas under a constant pressure of 20 psi and a flow of 1.8 mL/min. The column CP-WAX 52 CB (30 m × 0.25 mm × 0.25 μm thickness at the stationary phase, Chrompack, USA) was used for chromatographic separation. The column temperature was set to 35 °C and maintained for 5 min, then at a gradient of 2 °C/min to reach 80 °C, followed by 5 °C/min to 230 °C, held for 5 min. The quadrupole mass analyzer was operated in the sweep mode, monitoring the masses from 35 to 350 m/z. The arbitrary areas were obtained from total ion current chromatogram (TIC).

Compounds were identified by comparing the experimental mass spectra with those provided by the National Institute of Standards and