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Microbiological and physicochemical characterization of dry-cured Halal goat meat. Effect of salting time and addition of olive oil and paprika covering

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ABSTRACT

The objective of this work was to define a simple technological process for dry-cured Halal goat meat elaboration. The aims of this study were to analyze physicochemical parameters and to enumerate the microbial population at the end of the different manufacturing processes (two salting times and the addition of olive oil and paprika covering) on 36 units of meat product. A total of 532 strains were isolated from several selective culture media and then identified using classical and molecular methods. In general, salt effect and the addition of olive oil and paprika were significant for all the studied microbial groups as well as on NaCl content and water activity. Molecular analysis proves that staphylococci, especially *Staphylococcus xylosus* and *Staphylococcus equorum*, were the most common naturally occurring microbiota. The best manufacturing process would be obtained with a longer salting time and the addition of the olive oil and paprika covering.

1. Introduction

Goat meat produced in Morocco is largely consumed fresh. Conversion of goat meat into a value-added Halal dry-cured product would further enhance the foreign exchange earnings. It is an intermediate moisture meat product made from different anatomical retail cuts, and the preparation method is similar to that used in dry-cured ham manufacturing. Consumers demand safe, additive-free food of high nutritional value. In this sense, a dry-cured Halal goat meat product is the best choice to enhance goat meat and produce a product without chemical additives.

Microorganisms gain access into meat from spices and other ingredients, from environment, from equipment and from handlers during processing affecting the microbiological status of the product (Sachindra, Sakhare, Yashoda, & Narasimha Rao, 2005). These microorganisms are in part responsible for the flavor and texture of the final products. They also inhibit the growth of undesirable microorganisms by decreasing the pH and competing for the nutrients (Schillinger & Lücke, 1990). But while pathogenic microorganisms would affect the safety, spoilage microorganisms would limit the shelf life of the meat product. Thus, an understanding of the microbial profile of dry-cured Halal goat meat is vital.

The two categories of bacteria that could play a significant role and are commonly found in meat products are lactic acid bacteria and Gram-positive catalase-positive cocci (Arkoudelos, Nychas, & Samaras, 1997). Lactic acid bacteria improve the safety and stability of the product, whereas Gram-positive catalase-positive cocci enhance color stability, prevent rancidity and release various aromatic substances (Coppola, Giagnacovo, Iorizzo, & Grazia, 1998). The type of microbiota that develops is closely related to the ripening technique utilized. Demeyer, Van Nevel, Teller, and Godeau (1986) suggested that with a short ripening time, there is more *Lactobacillus* from the early stages of fermentation. In contrast, with a longer maturation time, there are higher numbers of *Staphylococcaceae*.

The aims of the present study were to evaluate the physicochemical and microbiological characteristics of a dry-cured Halal goat meat product and to assess the effect of salting time and olive oil and paprika covering on salt, pH and water activity values as well as on bacterial diversity using both classical and molecular methods.

2. Materials and methods

2.1. Manufacturing and sampling process

In order to carry out this study, 12 female goats were slaughtered according to the Muslim (Halal) way by severing the throat and major blood vessels in the neck. Thirty-six pieces were manufactured: 24 limbs (shoulders and legs; 1.26 ± 0.13 kg), and 12 ribs (front and

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back; 0.85 ± 0.14 kg). Raw pieces were absolutely homogeneous in size and shape. All pieces come from native breed goats of Northern Morocco (Mouley Abdesalem). The pieces were salted with an excess of coarse salt and placed in piles formed of layer of meat and salt. The half of every group of pieces was salted for 0.4 days/kg (A) and the other half was salted for 0.6 days/kg (B); the temperature of the salting room was maintained at 2–5 °C and the relative humidity was maintained at 80–90%. After the salting stage, the pieces were removed from the pile, brushed, washed and transferred to a post-salting room where they were kept for 10 days at 2–5 °C and approximately 85–90% relative humidity. Next, the pieces were transferred to a drying–ripening room where the ribs were held for 20 days and the limbs were held for 80 days at 12 °C and 75–80% relative humidity. Each batch of limbs was divided into two smaller batches as follows: A (A1, and A2) and B (B1, and B2). Batches A1 and B1 completed their maturation, while batches A2 and B2 were covered with a mixture of olive oil and paprika (O + P) before (50 days) they completed their maturation. Sampling was carried at the end of this processing step. Samples were vacuum-packaged and stored at −80 °C until analyzed.

2.2. Microbiological analysis and preliminary physiological characterization of isolates

Twenty five grams of each sample were homogenized with 225 mL of sterile tryptone water (Oxoid, Basingstoke, UK) in a Masticator Classic (IUL Instruments, Barcelona, Spain) blender for 2 min at room temperature. Serial decimal dilutions were made in sterile tryptone water and in duplicate. Samples of appropriate dilutions were poured (1 mL) or spread (0.1 mL) onto total count and selective agar plates. The following microbiological analyses were performed: total viable counts and psychrotrophic bacteria were determined on Standard Plate Count Agar (SPCA) (Oxoid) and incubated at 30 °C for 48 h and 7 °C for 10 days, respectively; halotolerant bacteria were determined on Standard Plate Count Agar + 7.5% NaCl incubated at 37 °C for 48 h; lactic acid bacteria were determined on the Man, Rogosa, Sharpe (MRS) agar (Oxoid) incubated at 30 °C for 48 h; *Enterobacteriaceae* counts were determined on Violet Red Bile Glucose Agar (VRBGA) (Oxoid) incubated at 37 °C for 24 h; yeasts and molds were determined on Yeast and Mould Agar (Oxoid) incubated at 25 °C for 5 days; staphylococci were plated on Baird Parker Agar Base (Oxoid) supplied with an egg yolk tellurite emulsion (Oxoid), and coliforms were determined on Violet Red Agar (VRBA) (Oxoid) incubated at 37 °C for 24 h. After incubation, plates with 30–300 colonies were counted. Microbiological data were transformed into logarithms of the number of colony forming units (cfu)/g.

From each piece (limbs and ribs) and each manufacturing process (salting time and olive oil and paprika covering), five colonies from the media M17, PCA + 7.5% NaCl, MRS and Baird Parker of the highest dilution that yielded growth were randomly selected and isolated. The isolates from MRS agar were purified by four alternate subcultures in MRS agar and MRS broth (Oxoid). Isolates from SPCA agar + 7.5% NaCl were purified in Brain Heart Infusion (BHI) agar and BHI broth (Oxoid). Isolates from M17 agar and from Baird Parker agar were purified in Nutrient agar–Nutrient broth (Oxoid). The purified isolates were afterwards maintained at −80 °C using 20% of glycerol as a cryoprotector agent. All the isolates were preliminary characterized by Gram reaction and catalase activity according to the methods and criteria of Sharpe (1979) and Kandler and Weiss (1986).

2.3. Extraction of DNA from isolates

DNA extraction was carried out following the method described by Soumet, Ermel, Fach, and Colin (1994). The isolates were grown in Tryptone Soy Broth (TSB) (Oxoid) for 24 h at 37 °C; the cells were harvested by centrifugation at 12,000 ×g for 5 min and the obtained pellets were suspended in 200 μL doubly distilled sterile water. After heating at 100 °C for 15 min, the tubes were placed on ice for 5 min, and then centrifuged at 12,000 ×g for 10 min. Finally the supernatant (DNA extract) was stored at −20 °C.

2.4. Identification of isolates by PCR methods

The isolates which were preliminary classified as Gram-positive and catalase-positive were identified by means of multiplex PCR allowing the simultaneous identification of *Staphylococcus* spp., *Staphylococcus xylosus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus* and *Staphylococcus aureus* (Morot-Bizot, Talon, & Leroy, 2004). As it was not possible to identify all the isolates at the species level by multiplex PCR, *those* belonging to the *Staphylococcus* genus were subjected to a single species-specific PCR method allowing the identification of the *Staphylococcus equorum* species according to the procedure described by Blaiotta, Ercolini, Mauriello, Salzano, and Villani (2004). The primers used are listed in Table 1.

The isolates which were preliminary classified as Gram-positive and catalase-negative were identified by means of a genus-specific PCR assay, allowing the identification of *Lactobacillus* spp. using the primers lactoF/lactoR (Byun et al., 2004). Isolates belonging to the *Lactobacillus* genus were identified at the species level by using two independent PCR assays because the dissimilarity of the annealing temperature hinders the development of a multiplex PCR. Species-specific primers LbPl1 and LbPl2 were used for the identification of *Lactobacillus plantarum* (Quere, Deschamps, & Urdaci, 1997) and 16FOR and LSakeR for the identification of *Lactobacillus sakei* (Rachman et al., 2004). Isolates that could not be assigned to the *Lactobacillus* genus were subjected to an *Enterococcus* genus-specific PCR assay using primers EntF/EntR (Ke et al., 1999). Species-specific primers FK1/FK2 and Fae1/Fae2 were used for the identification of *Enterococcus faecalis* and *Enterococcus faecium*, respectively, by means of a multiplex PCR (Dutka-Malen, Evers, & Courvalin, 1995).

The size of PCR products was checked by 1% agarose gel electrophoresis in TBE buffer (40 mM Tris–borate, 89 mM boric acid, 2 mM EDTA; pH 8) using a 100 bp DNA ladder (Invitrogen, Carlsbad, CA, USA) as marker. The gel was stained with ethidium-bromide and the bands were visualized under UV light.

2.5. Physicochemical analysis

Salt content was assessed according to the recommended standard as cited by Marra, Salgado, Prieto, and Carballo (1999). The pH was measured using a pH meter Micro pH 2002 (Crison Instruments, Barcelona, Spain) after mixing 10 g of the sample with 90 mL of distilled water for 2 min in a Sorvall Omnimixer homogenizer (Omni International, Waterbury, CT, USA). Determination of water activity (a_w) was performed using a Decagon CX-1 Water Activity System apparatus (Decagon Devices, Pullman, WA, USA), according to the manufacturer's instructions.

2.6. Statistical analysis

All statistical analyses were performed using the Statistica 8.0 computer program for Windows (Statsoft Inc., Tulsa, OK, USA). Significant differences between different samples, between the two salting times and the olive oil and paprika effect were determined using analysis of variance, with a significance level $P < 0.05$, $P < 0.01$ and $P < 0.001$.

3. Results and discussion

3.1. Microbiological analysis and preliminary physiological characterization of isolates

Table 2 shows the results of microbiological analysis on different pieces (limbs and ribs) obtained at the end of the different manufacturing processes. The total viable mesophilic counts in batch A (0.4 days of salting/kg) were significantly higher ($P < 0.001$) than the values