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Bacterial communities of fresh goat meat packaged in modified atmosphere

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ABSTRACT

The objective of this work was to study the growth and development of fortuitous flora and food pathogens in fresh goat meat packaged under modified atmospheres containing two different concentrations of CO_2. Meat samples were stored at 10 °C under two different modified-atmosphere packing (MAP) conditions: treatment A had 45% CO_2 + 20% O_2 + 35% N_2 and treatment B had 20% CO_2 + 55% O_2 + 25% N_2. During 14 days of storage, counts of each bacterial group and dominant species identification by 16S rRNA gene sequencing were performed to determine the microbial diversity present. The MAP condition used for treatment A was a more effective gas mixture for increasing the shelf life of fresh goat meat, significantly reducing the total number of viable bacteria and enterobacteria counts. Members of the *Enterobacteriaceae* family were the most common contaminants, although *Hafnia alvei* was dominant in treatment A and *Serratia proteamaculans* in treatment B. Identification studies at the species level showed that different microorganisms develop under different storage conditions, reflecting the importance of gas composition in the modified atmosphere on the bacterial community. This work provides new insights into the microbial changes of goat meat storage under different MAP conditions, which will be beneficial for the meat industry.

1. Introduction

The Retinta goat is a breed from Extremadura (Spain) that is currently listed as an endangered breed. This goat is raised predominately for its meat quality, but also for milk production. The main barriers to commercialisation meat are its high water content and the abundance of important nutrients available at its surface, which rendering it one of the most perishable types of food. Deterioration of the meat, and therefore changes in its microbiological profile, depends on intrinsic and extrinsic factors such as pH, morphology of the surface, availability of O_2, contamination of meat by spoilage bacteria at different stages of the production process, the presence and colonisation of other bacteria and temperature (Ercolini et al., 2009). However, microbial contamination is the major concern and the most important factor in the deterioration process (Hernández-Macedo et al., 2011).

The normal bacterial flora of fresh meat is very heterogeneous; it is mainly composed of *Pseudomonas* spp., *Enterobacteriaceae*, *Acinetobacter* spp., *Brochotrix thermosphacta* and *Lactobacillus* spp. Depending on their number and species present, they can cause numerous alterations to the meat and in some cases cause food poisoning. Among the pathogenic bacteria, *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Escherichia coli*, *Clostridium perfringens* and occasionally *Clostridium botulinum* can be found (Huffman, 2002).

In an aerobic environment, bacteria of the genus *Pseudomonas* are the most prevalent, followed by *Acinetobacter*, *Moraxella* and *Flavobacterium*. Pseudomonas species are the main responsible for spoilage in fresh meat products stored under aerobic conditions (Ercolini et al., 2007). In meat stored under anaerobic conditions, the predominant flora consists of Gram-positive bacteria, particularly lactic acid bacteria (LAB) and *B. thermosphacta* (Doulgeraki et al., 2012).

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The microbial spoilage growth typically begins with glucose uptake and surface oxygen by *Pseudomonas* spp. *B. thermosphacta* also utilises glucose but is a weak competitor due to its slow growth rate. When a bacterial population reaches 10^8 cfu/g, amino acid consumption begins, resulting in putrefaction and generation of the rancid aromas associated with short-chain fatty acids. There is also the appearance of slime, which is a breakdown product produced by growth of *Pseudomonas* spp., *Moraxella*, *Alcaligenes*, *Aeromonas*, *Serratia* and *Acinetobacter* spp. (Jay, 2000; Li and Torres, 1993a, 1993b). Likewise, this undesired bacterial growth has been also associated to spoilage of raw goat meat (Sabow et al., 2015; Bhandare et al., 2007; Nadeem et al., 2003; Babji et al., 2000).

Meat is usually sold in small pieces that require a long shelf life. One way to increase the shelf life of meat is via cold conservation under modified atmosphere packing (MAP), in which packaging occurs under variable gas mixtures (mainly O_2, CO_2 and N_2). Each of these gases plays a specific role in the process of conservation and affects the characteristics that determine the quality of the meat (Church, 1994). MAP is inhibitory to some microorganisms and therefore increases the quality of a wide variety of foods (Cutter, 2002). According to Gill (1996), high levels of CO_2 enhance the growth of LAB and inhibit the growth of *Enterobacteriaceae* and *Pseudomonas*, but do not prevent the growth of *B. thermosphacta*. Higher concentrations of CO_2 decrease respiration and therefore the growth rate of microorganisms (Jakobsen and Bertelsen, 2000; Simpson et al., 2009). At lower temperatures CO_2 has greater inhibitory effect, probably due to the increased solubility of CO_2 (Simpson et al., 2009). However, although concentrations of CO_2 close to 100% extend the storage period by controlling spoilage bacteria, CO_2 has a negative effect on the texture, appearance and colour stability of raw meat when compared to other gas mixtures (Fernandes et al., 2014; Richardson, 2003; Viana et al., 2005). Thus, CO_2 concentrations in combination with other gases must be optimised for MAP.

The impact of MAP technology on microbial development has been widely studied in poultry, beef, pork and lamb meat (Ercolini et al., 2006; Osés et al., 2013; Säde et al., 2013). However, the microbial evolution of goat meat stored under a modified atmosphere has been poorly addressed. Identifying the impact of different gas compositions on microflora evolution will be crucial for the selection of a suitable gas composition for industrial MAP. Thus, the aim of this work was to study the development of microflora in fresh goat meat packaged in modified atmospheres containing two different concentrations of CO_2 during its storage at 10 °C.

2. Materials and methods

2.1. Sample preparation and packaging conditions

Sixteen kid goats of the Extremadura Retinta breed were used in this study. When the animals reached their optimum live weight (around 12 ± 1 kg) they were transported to the abattoir and slaughtered following standard commercial procedures. The carcasses were cooled for 24 h at 4 °C and split carefully. Leg portions were cut into chunks after removing the loose fat, fascia and connective tissue. Twenty one portions of a similar size, approximately 10–12 cm in length and 1 cm in thickness, were prepared from each animal. Three of them were used to determine the initial values for microbiological and physicochemical parameters (at 0 days). The rest of the samples obtained from the 16 animals were randomly packed into clear trays (LINPAC Plastics Ltd., UK) and covered with a film that allows an O_2 permeability of 500 $cm^3\ m^{-2}\ day^{-1}$ at 1 atm at 25 °C. Packaging was carried out in two types of modified atmospheres:

Treatment A: 45% CO_2 + 20% O_2 + 35% N_2

Treatment B: 20% CO_2 + 55% O_2 + 25% N_2

Samples from each animal were included in both treatments. The packages were kept chilled at 10 °C, and microbiological and physicochemical parameters were evaluated at 4, 9 and 14 days post-packaging from three different packages per animal and treatment condition (n = 48).

2.2. Physicochemical analysis

The moisture content of the samples was determined by dehydration at 104 °C to a constant weight as recommended by the International Organisation for Standardisation (ISO Norm R-1442, 1979). The water activity (a_w) was determined using a GBZ FA-St/1 apparatus (Scientific Instruments, Romans sur Isère, France). The pH was measured using a Crison mod. 2002 pH meter (Crison Instruments, Barcelona, Spain).

2.3. Microbial counts

For the isolates and microbial counts, 10 g of each sample was taken aseptically, transferred to sterile plastic pouches, diluted 10-fold with 1% peptone water (Pronadisa, Alcobendas, Spain), and homogenised for 120 s using a laboratory stomacher Lab-Blender 400 (Seward Lab, London, England). Serial 10-fold dilutions were then prepared and used to inoculate agar plates.

Plate count agar media (PCA, Oxoid, Hampshire, UK) was used for mesophilic aerobic bacteria counts following incubation at 30 °C for 48 h and for psychrotrophs following incubation at 7 °C for 7 days. LAB were cultured on de Man, Rogosa and Sharpe agar (MRS, Oxoid) whose pH had been adjusted to 5.6 with acetic acid (10%) and incubated at 37 °C for 48 h under anaerobic conditions. *Staphylococcus spp.* counts were determined on Baird-Parker agar (BP, Oxoid) supplemented with potassium tellurite and egg yolk emulsion following incubation at 37 °C for 48 h; black colonies were counted. To count total enterobacteria (Gram-negative and cytochrome oxidase negative), Violet Red Bile Glucose agar (VRBG, Oxoid) was inoculated, covered with a layer of the same media and incubated at 37 °C for 24 h. The colonies that were rose-coloured and surrounded by a halo of purple precipitate were counted. Violet Red Bile agar (VRBA, Oxoid) was used to count coliforms. Plates of this media were inoculated, covered with a layer of the same media and incubated at 30 °C for 48 h. Typical dark-red colonies (>0.5 mm in diameter) surrounded by a zone of precipitated bile acids were counted. Enterococci were cultured on Slanetz and Bartley (SB, Oxoid) agar, incubated at 35 °C for 48 h and the typical pink or dark red colonies with a narrow whitish border were counted.

2.4. Microbial identification

2.4.1. Identification of isolates by DNA sequencing analysis

Colonies isolated from the different types of agar media used for microbial analysis were identified by 16S rRNA gene sequencing analysis. Five colonies from the highest dilution on each type of agar plate (PCA, MRS, BP, SB, VRBG, VRBA) were isolated at random on nutrient agar plates (Oxoid). Triplicate samples were obtained from each of the 16 goats to collect a wide range of microorganisms that were representative of the microflora present. DNA was isolated and subjected to 16S rRNA gene sequencing analysis (Benito et al., 2008a, 2008b). Sequences were compared to the EMBL and GenBank databases using the BLAST algorithm. The identity of each isolate was determined based on the highest score.