

Microbial Deterioration of Portuguese Lamb Meat as Affected by Its Intrinsic Properties[†]

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Abstract: In Portugal, the main autochthonous sheep breeds exploited for meat production are Churra-Galega-Bragançana (CGB) and Bordaleira-de-Entre-Douro-e-Minho (BEDM). The study aimed to characterise the evolution of spoilage microorganisms in refrigerated vacuum-packed (VP) lamb meat from BEDM and CGB breeds; and to elucidate how intrinsic properties of meat can affect its microbial spoilage. Meat from BEDM breed presented higher ($p < 0.0001$) populations of mesophiles, lactic acid bacteria, *Pseudomonas* spp. and psychrotrophic bacteria, since its higher ultimate pH (means: 5.77 for BEDM vs. 5.58 for CGB) accelerated spoilage rate ($p < 0.0001$). While water activity and protein content were not found to regulate microbial deterioration ($p > 0.05$), the growth of spoilage bacteria was exacerbated by higher moisture ($p < 0.0001$) and higher ash content ($p < 0.001$). On the contrary, a higher intramuscular fat content slowed down ($p < 0.0001$) the growth of spoilage bacteria in VP lamb meat.

Keywords: Sheep; local breeds; proximate composition; lactic acid bacteria; *Pseudomonas*; psychrotrophic bacteria; shelf-life.

1. Introduction

In Portugal, sheep and goat meat production constitutes 2.8% of the country's meat production with a level of self-sufficiency of 82% [1]. Therefore, increasing the production of sheep meat and optimising its quality, making it more attractive to consumers, is essential to ensure a good income level for sheep producers. In the case of lamb meat, after 7 days of maturation ~80% of its maximum tenderness potential is reached [2]. However, during this maturation, microbial deterioration takes place due to the proliferation of psychrotrophic bacteria, lactic acid bacteria, *Pseudomonas* spp., *Clostridium* spp., etc. [3]. One of the well-known strategies to prolong the life of the meat is vacuum packaging, which can moderately retard microbial deterioration. However, the extent of such retardation depends on chilling system/profile, initial microbial contamination and the physicochemical or intrinsic properties of meat.

Therefore, the objective of this study was twofold: (i) to evaluate the evolution of spoilage indicator microorganisms in refrigerated vacuum-packed (VP) lamb meat from two Portuguese breeds, Churra-Galega-Bragançana (CGB) and Bordaleira-de-Entre-Douro-e-Minho (BEDM); and (ii) to elucidate, by means of mixed models, any interrelationship between meat's intrinsic properties (i.e., pH, water activity and proximate composition) and microbial growth.

2. Materials and Methods

CGB and BEDM lambs were raised in semi-extensive systems on the holdings of the School of Agriculture of the Polytechnic Institute of Bragança and Ponte de Lima Agrarian School, respectively. They were reared in the fall 2018 and spring 2019, and a total of 30 CGB and 30 BEDM lambs were used in this investigation. All lambs were four months old when slaughtered, and the carcasses obtained were chilled at 4 °C for 24 h. After carcass splitting, the left side of the *Longissimus dorsi* muscle was removed from the 6th to the 13th vertebra under aseptic conditions, and was vacuum-packed for microbiological analysis on days 3, 9 and 15. The right half of the muscle was kept for the physicochemical analyses—pH, water activity and proximate composition—which were carried out on day 1 after slaughter.

3.1. Physicochemical analysis

The meat's intrinsic properties measured were pH, water activity (a_w) and proximate composition. The pH measurement was carried out using a pH meter (HI 99163, Hanna Instruments, Eibar, Spain) equipped with a 232D glass penetration probe. To measure a_w , beef steaks were cut to exactly fit in the cuvette of the Aqualab meter (4TE Decagon, City, USA). a_w was recorded after measurement stabilisation. Moisture, fat, protein and ash contents were determined according to international standards [4–7]. Determinations were made in triplicate per meat sample. Contents of fat, protein and ashes were expressed in dry basis for statistical analysis.

3.2. Microbiological analysis

Concentrations of total viable counts, psychrotrophic bacteria, lactic acid bacteria and *Pseudomonas* spp. were determined at each time point in duplicate. Total mesophiles and psychrotrophic bacteria were counted on Plate Count Agar (610040, Liofilchem, City, Italy), while lactic acid bacteria (LAB) were counted on MRS agar, and confirmed by microscopy and biochemical tests [8]. *Pseudomonas* Agar Base (CM0559 Oxoid, Thermo Fisher Scientific, City, UK), added with 1% *v/v* glycerol and supplemented with cetrimide-fucidine-cephalosporin (610071 Liofilchem, Italy), was used for the quantification of *Pseudomonas* spp.

3.3. Statistical analysis

The statistical analysis aimed to understand to what extent the intrinsic or physicochemical properties of meat (i.e., pH, a_w and proximate composition) can affect or modulate its microbial spoilage, as characterised by the change in the populations of mesophiles, lactic acid bacteria, psychrotrophic bacteria and *Pseudomonas* spp. This was done by fitting a series of mixed-effects models with each of the microbial groups as the dependent variable (Y), and each of the intrinsic properties of meat as the independent variable (X). Additionally, breed, time, time² and the interaction X × time were added as independent variables in all models. The subject of variation of the random effects was the lambs. Models were evaluated in the R software (version 3.6.2, R Foundation for Statistical Computing, Vienna, Austria).

3. Results and Discussion

It was evident that the abattoir's controlled process hygiene ensured that the bacterial counts in meat were relatively low, since still on the third day after slaughter, the mean microbial concentrations were at 1.45 log CFU/g for mesophiles, 0.899 log CFU/g for LAB, 1.27 log CFU/g for psychrotrophic bacteria and 1.02 log CFU/g for *Pseudomonas* spp (Figures 1 and 2). The lowest initial microbial populations were found for *Pseudomonas* and LAB. Since vacuum packaging excludes oxygen, the strictly aerobic rapidly-growing *Pseudomonas* are inhibited. Mills et al. [9] pointed out that, after vacuum-packaging, the population of LAB is generally low (~10 LAB/cm²) but it increases during storage until growth stops due to substrate depletion. At -1.5 °C, LAB populations become displaced by succeeding populations without a decline in observable LAB numbers [10].

The difference in bacterial population size between lamb breeds was a recurrent fact in the analysed slaughter batches from both years, 2018 and 2019. While deteriorating bacteria increased as meat maturation took place ($p < .0001$ for the term Day in Table 1), the microbial growth trends were clearly different between lamb breeds (Figures 1 and 2), which was corroborated by the significant effect of Breed on mesophiles, LAB, *Pseudomonas* and psychrotrophic bacteria counts (p between 0.009 and 0.017 in Table 1). The fact that the microbial populations of CGB lamb meat were for all microbial groups significantly lower than those of BEDM lamb meat (Figures 1 and 2) are linked to the lower ultimate pH of the CGB lamb meat (5.58 for CGB versus 5.77 for BEDM; Figure 3).

The effect of pH on the steeper or slower microbial growth can be also deduced from the interaction term $pH \times Day$, which was significant for the four bacterial groups, namely mesophiles ($p = 0.006$), LAB ($p = 0.001$), *Pseudomonas* ($p = 0.050$) and psychrotrophic bacteria ($p = 0.010$; Table 1). This interaction implies that a higher ultimate pH of meat tends to accelerate microbial growth. Thus, the rate of bacterial population growth is regulated by the ultimate pH. Although, generally, lamb meat has a higher mean ultimate pH (5.6–5.7) than beef meat (pH = 5.5) [11], still the significant effect of Breed in all models (Table 1) suggests that BEDM lambs may have been more susceptible to pre-mortem stress than CGB lambs. The rapid depletion of glycogen levels, prompted by stress, prevents the normal drop in pH to optimal levels. As a result, meat of higher pH (>5.7) has better conditions for microbial growth, ultimately leading to a reduction of shelf-life in refrigerated conditions even when vacuum-packaging is applied.

Table 1. Effects of lamb breed and initial intrinsic factors of meat on the concentration of spoilage microorganisms in refrigerated vacuum-packed meat as quantified by mixed models (F-values and p-values from analysis of variance are shown).

| Model | Variable | Mesophiles | LAB | <i>Pseudomonas</i> | Psychrotrophic |
|----------|--------------|------------------------|------------------------|------------------------|------------------------|
| pH | Breed | 7.503 (0.008) | 17.70 (<0.0001) | 8.147 (0.006) | 6.100 (0.017) |
| | Day | 168.4 (<0.0001) | 56.99 (<0.0001) | 32.15 (<0.0001) | 159.1 (<0.0001) |
| | pH×Day | 7.951 (0.006) | 12.24 (0.001) | 3.795 (0.050) | 6.893 (0.010) |
| aw | Breed | 7.259 (0.009) | 18.32 (<0.0001) | 8.520 (0.005) | 6.308 (0.015) |
| | Day | 159.9 (<0.0001) | 50.79 (<0.0001) | 31.28 (<0.0001) | 148.7 (<0.0001) |
| | aw ×Day | 0.050 (0.823) | 0.211 (0.646) | 3.082 (0.082) | 1.019 (0.315) |
| Moisture | Breed | 10.16 (0.002) | 20.02 (<0.0001) | 8.854 (0.004) | 7.141 (0.009) |
| | Day | 169.5 (<0.0001) | 53.86 (<0.0001) | 35.53 (<0.0001) | 178.5 (<0.0001) |
| | Moisture×Day | 28.97 (<0.0001) | 12.23 (<0.0001) | 21.00 (<0.0001) | 30.76 (<0.0001) |
| Fat | Breed | 9.314 (0.004) | 19.75 (<0.0001) | 8.754 (0.005) | 6.973 (0.011) |
| | Day | 184.67 (<0.0001) | 56.73 (<0.0001) | 36.00 (<0.0001) | 185.5 (<0.0001) |
| | Fat×Day | 33.30 (<0.0001) | 17.84 (<0.0001) | 22.01 (<0.0001) | 34.51 (<0.0001) |
| Protein | Breed | 7.243 (0.009) | 18.32 (<0.0001) | 8.196 (0.006) | 6.192 (0.016) |
| | Day | 161.9 (<0.0001) | 52.31 (<0.0001) | 31.59 (<0.0001) | 152.2 (<0.0001) |
| | Protein×Day | 1.392 (0.241) | 3.612 (0.060) | 2.085 (0.151) | 2.576 (0.111) |
| Ashes | Breed | 9.047 (0.004) | 18.74 (<0.0001) | 8.591 (0.005) | 6.629 (0.013) |
| | Day | 172.0 (<0.0001) | 56.67 (<0.0001) | 33.11 (<0.0001) | 166.1 (<0.0001) |
| | Ash×Day | 22.25 (<0.0001) | 14.67 (0.001) | 10.32 (0.002) | 16.87 (0.001) |

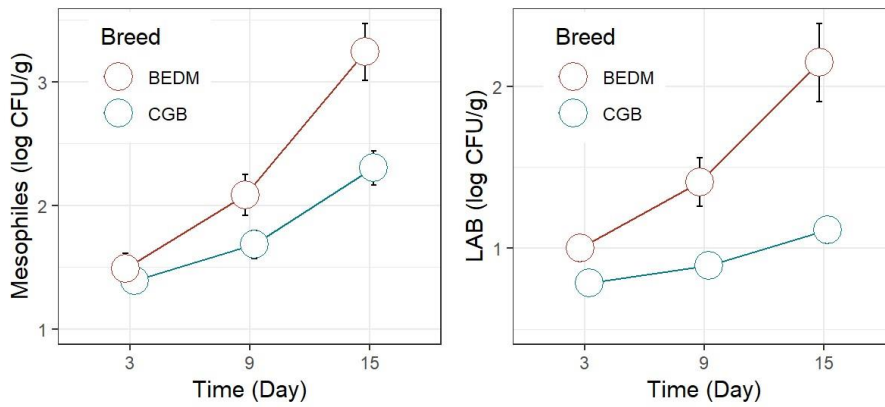


Figure 1. Increase in mesophiles and lactic acid bacteria in vacuum-packed lamb meat, by breed Churra-Galega-Bragançana (CGB) and Bordaleira-entre-Douro-e-Minho (BEDM).

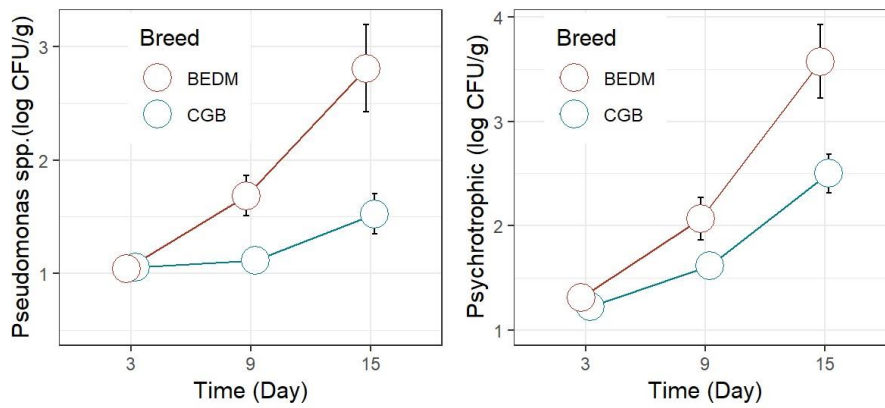


Figure 2. Increase in *Pseudomonas* spp. and psychrotrophic bacteria in vacuum-packed lamb meat, by breed Churra-Galega-Bragançana (CGB) and Bordaleira-entre-Douro-e-Minho (BEDM).

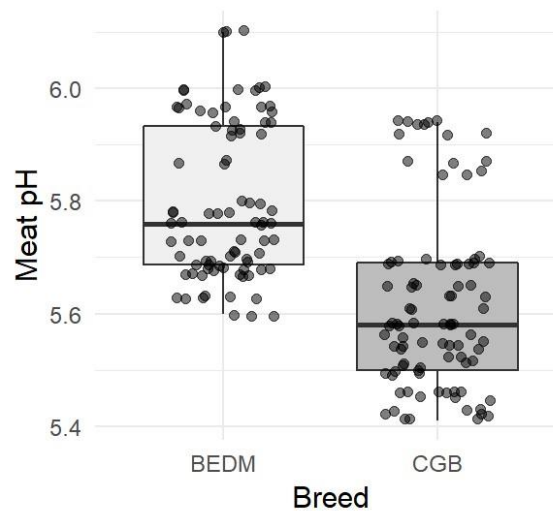


Figure 3. Box plots of lamb *Longissimus dorsi* pH measured 24 hours post-slaughter, by breed, Churra-Galega-Bragançana (CGB) and Bordaleira-entre-Douro-e-Minho (BEDM).

With regards to meat a_w , this intrinsic property was not found to modulate the growth of any microbial group, as deduced by the non-significance of the interaction term $a_w \times \text{Day}$ (p values from 0.082 to 0.823 in Table 1). The lack of effect of a_w is not unexpected since vacuum packaging prevents drying at the meat surface, and moisture from within the meat allows the surface a_w to equilibrate to above 0.98. Consequently, there is no inhibitory effect on bacteria once the meat has been packed and stored [12]. The mean a_w of VP meat measured on the third day after slaughter was the same, 0.9927, for both CGB and BEDM breeds.

Unlike a_w , the development of deteriorating bacteria was found to be exacerbated by the moisture content of meat, as implied by the interaction $\text{Moisture} \times \text{Day}$ that was significant in all bacterial groups ($p < 0.0001$ in Table 1). This interaction suggests that a higher moisture content in VP lamb meat prompted a faster increase in spoilage bacterial numbers. Meat samples may have had different levels of moisture, since lamb carcasses were held for 24 h in a chilling room at 90% RH with loadings that varied from batch to batch. Under these conditions, moisture loss from lamb carcasses has been reported to be up to 2.2% [13]. The mean moisture content of lamb meat originating from the BEDM breed was 77.03% (SD = 1.517%) whereas that of lamb meat originating from the CGB breed was 75.89% (SD = 0.962%).

Despite protein content of lamb meat presented a wide range of variation, between 80.97 to 91.34% (db), protein content was not found to regulate the growth of spoilage bacteria in VP lamb meat. Notice that the interaction term $\text{Protein} \times \text{Day}$ was non-significant ($p > 0.05$) in all bacterial groups (Table 1). Interestingly, lamb meat samples of higher intramuscular fat content underwent a slower microbial deterioration, as implied by the negative interaction $\text{Fat} \times \text{Day}$ (not shown) that was significant for all microbial groups ($p < 0.0001$ in Table 1). Fat has therefore an important role in delaying microbial spoilage, which could arise from the fact that meat of greater intramuscular fat originates from heavier animals (carcasses) having thicker fat cover. Fat cover has been demonstrated to protect carcasses against contamination and proliferation of bacteria. Sauter et al. [14] found that lamb carcasses having 0.36 cm or less of fat cover presented significantly higher psychrotrophic counts on the surface; concluding that the population size of microorganisms up to 7 days post-slaughter was related to the thickness of fat cover.

On the contrary, lamb meat samples of higher content of metal salts and trace minerals (ashes) tended to have a faster microbial spoilage (p of at least 0.001 in Table 1). As Gadd et al. [15] explained, metals such as Na, K, Cu, Zn, Co, Ca, Mg, Mn and Fe are essential for life, and are directly and/or indirectly involved in all aspects of microbial growth, metabolism and differentiation. Furthermore, many important microbial processes can be influenced by minerals, including energy generation, nutrient acquisition, cell adhesion and biofilm formation.

4. Conclusion

In order for native-breed lamb meat producers to optimise and meet the challenges posed by the heterogeneity of production chain actors, as well as the challenges of homogeneous quality meat sought by consumers, it is necessary to understand how the intrinsic properties of carcass and meat regulate meat microbiological attributes. This study showed that populations of spoilage bacterial groups were higher in vacuum-packed lamb meat originating from BEDM breed than in that of CGB breed, since ultimate pH was significantly higher in BEDM lamb meat. In addition, a high ultimate pH was demonstrated to increase the rate of microbial deterioration. Other meat's intrinsic properties that increased the rate of microbial spoilage were high moisture and ash content. By contrary, a higher intramuscular fat content tended to retard its microbial spoilage. In order to extend the shelf-life of Portuguese-origin lamb meat, animal handling and carcass classification can be improved towards the selection of fatter animals and chilled carcasses of optimal ultimate pH.

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Conflicts of Interest: The authors declare no conflict of interest.

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