Final Program

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FOOD_INNOV_07_ONL
In Vitro Fermentation Kinetics of an Antarctic Yeast and Suitability for Beer Production
Juan Manuel Cevallos-Cevallos, Christian Guarco and Andrea Morales, ESPOL, CIBE, Campus Gustavo Galindo, Guayaquil, Ecuador

FOOD_INNOV_14_ONL
Cold Plasma Treatment as a Processing Technology to modify Peanut Oil
Ximena V Yepez and Haci Baykara, Facultad de Facultad de Ingenieria, Mecanica y Ciencias de la Produccion, ESPOL, Guayaquil, Ecuador and Sarah Cady, Chemical Instrumentation Facility, Iowa State University, Ames, IA, USA and Kevin M. Keener, College of Engin. & Physical Sci., Univ. of Guelph, Guelph, Canada

ROOM B – M228 - 15.00-17.30 FOOD SAFETY
FOOD SAFETY II – ENVIRONMENTAL RISK ASSESSMENT I

FOOD_RISK_01_ONL
A Quantitative Risk Assessment of E. coli O157:H7 on Ready to Eat Foods following the Application of Biomaterials on Land
Rajat Nag and Enda Cummins, UCD School of Biosystems and Food Engineering and Annetta Zintl, Bryan K Markey and Paul Whyte, UCD School of Veterinary Medicine, Belfield, Dublin, and Vincent O'Flaherty, National University of Ireland Galway, School of Natural Sciences, Galway, and Declan Bolton, Teagasc, Ashtown Food Research Centre, Ashtown, Dublin, and Owen Fenton and Karl G. Richards Teagasc, Environment Research Centre, Johnstown Castle, County, Wexford, Ireland

FOOD_SAFE_01
Microbial Deterioration of Lamb Meat of Portuguese Origin as affected by Its Intrinsic Properties
Vasco A. P. Cadavez, Ursula Gonzales-Barron, Diogo Félix-Oliveira, Sara Coelho-Fernandes and Gisela Santos-Rodrigues, Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal and José M. Lorenzo and Roberto Bermúdez Piedra, Fundación Centro Tecnolóxico da Carne, Parque Tecnolóxico de Galicia, San Cibrao das Vióas, Spain

FOOD_SAFE_02
Meta-Regression Models describing The Effects of Added Lactic Acid Bacteria on Pathogen Inactivation in Milk and Cheese
Beatriz Nunes Silva and José António Teixeira, Centre of Biological Engineering (CEB), Univ. of Minho, Braga and Ursula Gonzales-Barron and Vasco Cadavez, Centro de Investigação da Montanha (CIMO), IPB, Bragança, Portugal

MICROBIAL DETERIORATION OF LAMB MEAT OF PORTUGUESE ORIGIN AS AFFECTED BY ITS INTRINSIC PROPERTIES

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KEYWORDS
Sheep, local breeds, proximate composition, pH, lactic acid bacteria, *Pseudomonas*, psychrotrophic bacteria, shelf-life

ABSTRACT

In Portugal, sheep and goat meat production constitutes 2.8% of the total meat production, with a self-sufficiency of ~82%. The main autochthonous sheep breeds exploited for meat production are Churra-Galega-Bragançana (CGB) and Bordaleira-de-Entre-Douro-e-Minho (BEDM), whose quality must be optimised in order to ensure adequate income levels for sheep producers. The study aimed to characterise the evolution of spoilage microorganisms in refrigerated vacuum-packed (VP) lamb meat from BEDM and CGB breeds; and elucidate how intrinsic properties of meat can affect its microbial spoilage. Meat from BEDM breed presented higher ($p<.0001$) 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 ($p<.0001$). While water activity and protein content were not found to modulate microbial deterioration ($p>0.05$), the growth of spoilage bacteria was found to be exacerbated by higher moisture ($p<.0001$) and higher ash content ($p<0.001$). By contrast, a higher fat content retarded ($p<.0001$) the growth of spoilage bacteria in VP lamb meat. In order to extend the shelf-life of Portuguese-origin lamb meat, animal handling must be enhanced to minimise pre-slaughter stress, and a carcass classification system should be adopted towards the selection of fatter animals and chilled carcasses of optimal ultimate pH.

INTRODUCTION

Sheep farming is an activity of high economic importance as these animals are producers of meat, milk, wool and fur. In Portugal, sheep and goat meat production constitutes 2.8% of the country's meat production (INE, 2016), with a level of self-sufficiency of 82% (Santos-Rodrigues et al. 2019). 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.

Portuguese sheep production is featured by a large diversity of production systems, all based on the use of natural pastures and agricultural residues (Cruz et al. 2019). These animal production grazing systems are characterised by great heterogeneity and have contributed to the maintenance of the ecological balance of the natural pastures, and the environmental and socio-cultural richness of the landscapes of Portugal. The sixteen national indigenous sheep breeds, in addition to contributing to the diversity of production systems, are an important genetic heritage that must be preserved. Some of these breeds, classified as threatened, have small body size and good adaptation to adverse environments (climate and orography), which makes them particularly well suited to the use and enhancement of natural pastures. Two of the autochthonous sheep breeds exploited for meat production are Churra-Galega-Bragançana (CGB) and Bordaleira-de-Entre-Douro-e-Minho (BEDM), from the Mediterranean bioregion and the Atlantic bioregion, respectively (Cruz et al. 2019).

Today's consumer is sensitive to management practices capable of improving animal welfare, and is available to pay a higher price for quality certified meat. Enhancing the quality of meat of autochthonous breeds could contribute to the preservation of the rural world and its diversity, as well as increasing the profitability of these production systems. In this way, we will be able to ensure the conservation of endangered breeds and improve the living standards of the sheep farmers that remain in the rural areas of Portugal.

However, meat quality is a multifactorial concept regulated by factors that are intrinsic and extrinsic to the animal. On the one hand, quality is perceived by the consumer through good sensorial properties – optimised through cold maturation; while, on other hand, the producer must not only meet such organoleptic demands, but must also ensure that the product remains safe during its life time, which should aim to be the longest possible to avoid economic losses (Mills et al, 2014).

In the case of lamb meat, after 7 days of maturation ~80% of its maximum tenderness potential is reached (Prates, 2000). However, during this maturation, microbial deterioration takes place due to the proliferation of psychrotrophic bacteria, lactic acid bacteria, *Pseudomonas* spp., *Clostridium* spp., etc. (Clemens et al. 2010). 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 (mesophiles, psychrotrophic, lactic acid bacteria and *Pseudomonas* spp) in refrigerated vacuum-packed lamb meat from two Portuguese breeds, BEDM and CGB; and (ii) to elucidate, by means of mixed models, any interrelationship between meat's intrinsic properties (i.e., pH, water activity, moisture content, fat content, protein content and ashes) and microbial growth.

METHODOLOGY

Lamb rearing and feeding

In the Mediterranean region, located in Bragança, CGB lambs were raised on the holding of the School of Agriculture of the Polytechnic Institute of Bragança. In the Atlantic bioregion, located in Ponte de Lima, BEDM lambs were raised on the holding of the Ponte de Lima Agrarian School. In both bioregions, the production system used was the semi-intensive one, whose feeding was based on grazing on natural pastures. The hours of grazing varied according to hours of light, heat and herd size. In winter, the flocks would be released in the morning to graze all day until dark. In the summer, the herds would leave at dawn and graze until midmorning, then they would be put in a stable under shade, and would come out when the heat had subsided. Once on the premises, lambs had access to meadow hay, and supplemented with protein and mineral-rich concentrates. In the stable, the lambs had water *ad libitum*. The lambs were not weaned, and were reared in the fall of 2018 and spring 2019. For this investigation, 15 BEDM and 15 CGB lambs were reared in 2018 and 15 BEDM and 15 CGB lambs were reared in 2019.

Preparation of meat samples

The slaughter and therefore the analyses were carried out in batches in the same abattoir: in 2018, three batches of 5 CGB lambs each, and three batches of 5 BEDM lambs of 5 each were slaughtered; and in 2019 two batches of 7 and 8 CGB lambs each, and two batches of 7 and 8 BEDM lambs each were slaughtered.

All lambs were four months old when slaughtered, and the carcasses obtained were chilled at 4 ºC for 24 hours. After carcass splitting, the *Longissimus dorsi* muscle was removed from the 6th to the 13th vertebra under aseptic conditions. The left side was divided into three parts. Each of them was vacuum packed (Silvercrest SFS 110 B2, Germany); labelled with the number 3, 9 or 15, corresponding to the day of microbiological analysis; and stored at 4±0.5 ° C (Portiso ECB-3000, UK). Concentrations of total viable counts, psychrotrophic bacteria, lactic acid bacteria and *Pseudomonas* spp. were determined at each time point in duplicate.

The right half of the *L. dorsi* muscle was kept for the physicochemical analyses – pH, moisture and dry matter, fat content, protein content and ash content – which were carried out on day 1 after slaughter. Only water activity was measured on day 3 post-slaughter. Unlike microbiological analyses, the *intrinsic properties of meat* (physicochemical analyses) were essayed right at the beginning of the cold maturation.

Microbiological and physicochemical analyses

For the microbiological analyses, twenty-five g of meat were homogenised in 225 ml of buffered peptone water (611014 Liofilchem, Roseto degli Abruzzi, Italy) for 1 min (Interscience Bag Mixer 400, France). One-ml aliquots from decimal dilutions were inoculated on Aerobic Count Plate petrifilms (3M, MN, USA) for counting of mesophiles, and on Lactic Acid Count Plate petrifilms (3M, MN, USA) for counting of lactic acid bacteria. One-ml aliquots were plated by incorporation in Plate Count Agar (610040 Liofilchem, Roseto degli Abruzzi, Italy) for the determination of psychrotrophic bacteria, while, for the quantification of *Pseudomonas*, 0.5-ml aliquots were spread onto *Pseudomonas* Agar Base (CM0559 Oxoid, Thermo Fisher Scientific, UK), added with 1% v/v glycerol and supplemented with cetrimide-fucidine-cephalosporin (610071 Liofilchem, Roseto degli Abruzzi, Italy). The mesophilic and lactic acid bacteria plates were incubated at 35±0.5ºC for 48 h; the psychrotrophic bacteria plates at 7±0.5ºC for 11 days; and the *Pseudomonas* plates at 25±0.5ºC for 24 h. Plating was done in duplicate, and colony counts were transformed to log CFU/g.

The meat's intrinsic properties measured were pH, aw and proximate composition. The pH measurement was carried out according to Franco (2009), using a pH meter (HI 99163, Hanna Instruments, Eibar, Spain) equipped with a 232D glass penetration probe. To measure aw, beef steaks were cut to exactly fit in the cuvette of the Aqualab meter (4TE Decagon, USA). Aw was recorded after measurement stabilisation. Moisture, fat, protein and ashes contents were determined according to ISO (1997), AOCS (2005), ISO (1978) and ISO (1998), respectively. Determinations were made in triplicate per meat sample. Contents of fat, protein and ashes were expressed in dry basis.

Mixed-effects models

The statistical analysis aimed to understand to what extent the intrinsic properties of meat (i.e., animal breed, pH, aw and proximal 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.

A general mixed-effects model of the type,

$$Y_{rj} = \beta_{0j} + \beta_{1r} + \beta_2 Day + \beta_3 (X)(Day) + \beta_4 Day^2 + \varepsilon_{rj}$$

$$\beta_{0j} = \beta_0 + v_j \qquad \text{…(Eq 1)}$$

was adjusted to each of the microbial groups (Y) to assess the effect of breed, time of maturation and each of the intrinsic properties of meat (X), in separate. The response variable Y_{rj} is the microbial concentration in the meat sample from lamb *j* of breed *r*, measured after a maturation

time *Day*. The model intercept β_0 is affected by random shifts v_j caused by the different lambs *j*. These random effects were removed from the model assuming they follow a normal distribution. The parameter β_{1r} is the fixed effect of lamb breed, while β_2 the linear effect of maturation time. It is allowed that the effect of maturation time could be modulated by the intrinsic property *X*, so the parameter β_3 assesses this interaction. A quadratic effect for *Day* (β_4) was added given its significance in all models. The residuals ε_{rj} are assumed to follow a normal distribution.

Equation (1) was adjusted separately to each of the four microbial groups (***Y***), and in each of the adjustments, the independent variable ***X*** represents pH, aw, humidity, protein, fat or ashes content. Thus, in total 24 models were fitted. The results of the mixed linear models presented in the following Section include: (i) parameter estimates along with standard errors; and (ii) the significance of the sources of variation tested in analysis of variance (ANOVA) by the F-test. The models were adjusted in the R software (The R Core Team 2019).

RESULTS AND DISCUSSION

If lamb is produced under good manufacturing practices, the initial counts of microorganisms on the meat surface is likely to be ~10^3/cm^2 or lower (Mills et al. 2014). In our experiments, the abattoir's controlled process hygiene ensured that the bacterial counts in vacuum-packed meat were relatively low still on the third day after slaughter, at mean values of 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 (Figure 1). By contrast, the mesophiles level determined by Wang et al (2019) in VP lamb meat on the third day of storage was much higher at 4.95 log CFU/g despite the comparable storage temperature of 4-6ºC. In our work, 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 (2014) explained that, after vacuum-packaging, the population of LAB is generally low (10 LAB/cm^2) but it increases during storage until growth stops due to substrate depletion. At -1.5ºC, growing LAB populations have been shown to be displaced by succeeding populations without a decline in observable LAB numbers (Jones 2004).

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 maturation took place (p<.0001 for the terms Day and Day^2 in Tables 1-4), the microbial growth trends were clearly different between lamb breeds (Figure 1), which was corroborated by the significant effect of Breed on mesophiles counts (p<.0001; Table 1), LAB counts (p<.0001; Table 2), *Pseudomonas* counts (p between 0.004 and 0.006; Table 3) and psychrotrophic bacteria counts (p between 0.009 and 0.017; Table 4). In addition, for the four bacterial groups, the increase in population appeared less pronounced in VP meat from CGB lambs (Figure 1), which was also suggested by the negative sign of the model term "Breed - CGB", displayed in Tables 1-4. The fact that the microbial populations of CGB lamb meat were for all microbial groups significantly lower than those of BEDM lamb meat are linked to the lower ultimate pH of the CGB lamb meat (5.58 for CGB versus 5.77 for BEDM; Figure 2).

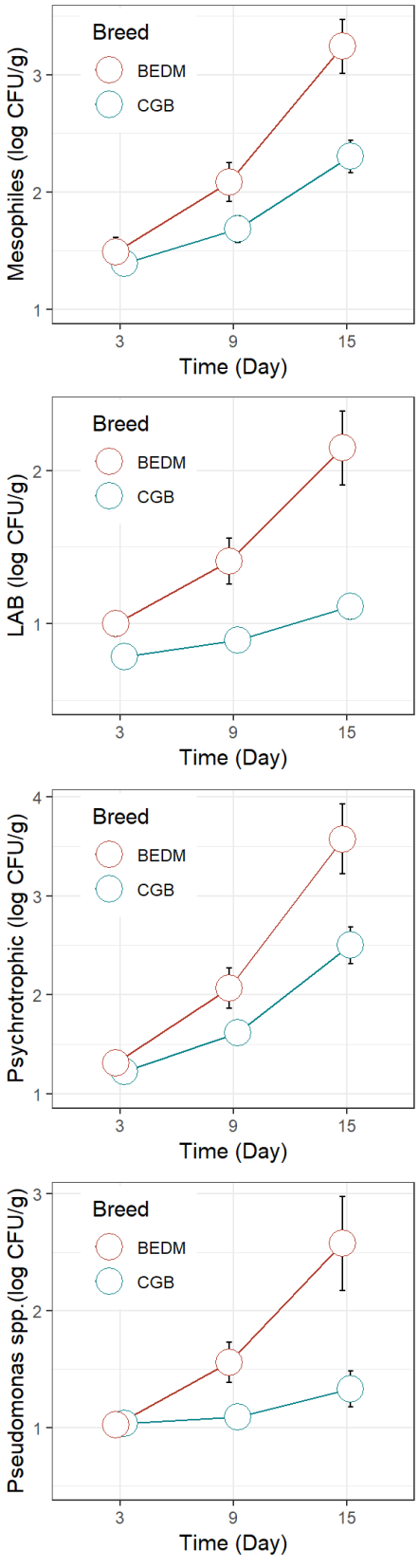

Figure 1: Increase in Microbial Populations in Vacuum-Packed Lamb Meat Stored at 4ºC During 15 Days, by Breed Churra-Galega-Bragançana (CGB) and Bordaleira-entre-Douro-e-Minho (BEDM)

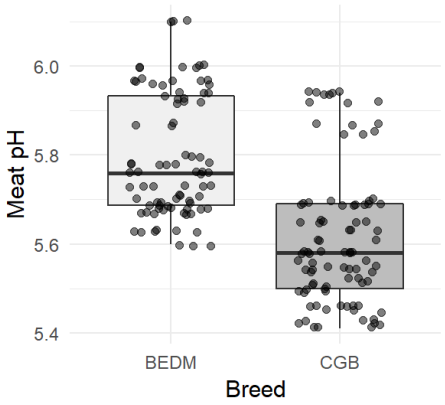

Figure 2: Box Plots of Lamb *Longissimus dorsi* pH Measured at 24 hours Post-Slaughter by Breed, Churra-Galega-Bragançana (CGB) and Bordaleira-entre-Douro-e-Minho (BEDM)

Table 1: Effects of Lamb Breed and Initial Intrinsic Factors of Meat on the Concentration of **Mesophilic Bacteria** in Refrigerated Vacuum-Packed Meat as Quantified by Six Separate Linear Mixed Models (F-Values and Associated p-Values from ANOVA are Shown; Quadratic Effect of Day is Significant in All Models but Not Shown)

Model	Term	Estimate (SE)	F (p-value)
pH	Intercept	1.576 (0.337)	533.8 (<.0001)
	Breed - CGB	-0.262 (0.192)	7.503 (0.008)
	Day	-3.598 (1.249)	168.4 (<.0001)
	pH×Day	0.592 (0.209)	7.951 (**0.006**)
aw	Intercept	1.689 (0.343)	516.5 (<.0001)
	Breed - CGB	-0.480 (0.178)	7.259 (0.009)
	Day	2.928 (14.04)	159.9 (<.0001)
	aw×Day	-3.175 (14.14)	0.050 (0.823)
Moisture (%)	Intercept	1.543 (0.329)	723.0 (<.0001)
	Breed - CGB	-0.196 (0.160)	10.16 (0.002)
	Day	-9.793 (1.813)	169.5 (<.0001)
	Moisture×Day	0.125 (0.023)	28.97 (<**.0001**)
Fat (% db)	Intercept	1.469 (0.319)	662.8 (<.0001)
	Breed - CGB	-0.055 (0.174)	9.314 (0.004)
	Day	0.133 (0.345)	184.67 (<.0001)
	Fat×Day	-0.066 (0.012)	33.30 (<**.0001**)
Protein (% db)	Intercept	1.655 (0.342)	515.4 (<.0001)
	Breed - CGB	-0.414 (0.187)	7.243 (0.009)
	Day	-1.804 (1.388)	161.9 (<.0001)
	Protein×Day	0.018 (0.015)	1.392 (0.241)
Ashes (% db)	Intercept	1.578 (0.328)	643.8 (<.0001)
	Breed - CGB	-0.264 (0.166)	9.047 (0.004)
	Day	-1.427 (0.433)	172.0 (<.0001)
	Ashes×Day	0.222 (0.047)	22.25 (<**.0001**)

The effect of pH on the steeper or slower microbial growth can be also deduced from the interaction term pH×Day, which was significant for the four bacterial groups, namely mesophiles (p=0.006 in Table 1), LAB (p=0.001 in Table 2), *Pseudomonas* (p=0.050 in Table 3) and psychrotrophic bacteria (p=0.010 in Table 4). The positive estimates for the interaction pH×Day imply that a higher ultimate pH of meat tends to accelerate the microbial growth. Thus, the rate of bacterial population growth is regulated by the ultimate pH.

Table 2: Effects of Lamb Breed and Initial Intrinsic Factors of Meat on the Concentration of **Lactic Acid Bacteria** in Refrigerated Vacuum-Packed Meat as Quantified by Six Separate Linear Mixed Models (F-Values and Associated p-Values from ANOVA are Shown; Quadratic Effect of Day is Significant in All Models but Not Shown)

Model	Term	Estimate (SE)	F (p-value)
pH	Intercept	1.040 (0.311)	300.5 (<.0001)
	Breed - CGB	-0.355 (0.155)	17.70 (<.0001)
	Day	-3.692 (1.085)	56.99 (<.0001)
	pH×Day	0.633 (0.018)	12.24 (**0.001**)
aw	Intercept	1.162 (0.325)	311.1 (<.0001)
	Breed - CGB	-0.588 (0.137)	18.32 (<.0001)
	Day	5.347 (11.80)	50.79 (<.0001)
	aw×Day	-5.466 (11.88)	0.211 (0.646)
Moisture (%)	Intercept	1.075 (0.316)	340.0 (<.0001)
	Breed - CGB	-0.421 (0.140)	20.02 (<.0001)
	Day	-5.735 (1.653)	53.86 (<.0001)
	Moisture×Day	0.074 (0.021)	12.23 (<**.0001**)
Fat (% db)	Intercept	1.016 (0.309)	335.4 (<.0001)
	Breed - CGB	-0.308 (0.148)	19.75 (<.0001)
	Day	0.157 (0.340)	56.73 (<.0001)
	Fat×Day	-0.044 (0.010)	17.84 (<**.0001**)
Protein (% db)	Intercept	1.115 (0.322)	311.0 (<.0001)
	Breed - CGB	-0.499 (0.145)	18.32 (<.0001)
	Day	-2.233 (1.186)	52.31 (<.0001)
	Protein×Day	0.025 (0.013)	3.612 (0.060)
Ashes (% db)	Intercept	1.081 (0.309)	318.3 (<.0001)
	Breed - CGB	-0.432 (0.142)	18.74 (<.0001)
	Day	-0.949 (0.405)	56.67 (<.0001)
	Ashes×Day	0.161 (0.042)	14.67 (**0.001**)

Meat pH has been shown to affect the growth of bacteria in two ways (Gill 2004). Firstly, the growth of some bacteria is reduced, or inhibited completely, when the pH falls below a certain level. Secondly, the glycolytic processes that determine the ultimate pH also determine the concentration of residual glucose in the meat, and therefore the point at which this preferred growth substrate becomes exhausted and aminocids start to be metabolised by LAB and *Enterobacteriaceae*, thereby resulting in spoilage. For instance, it is generally accepted that *Enterobacteriaceae* and *Bacillus thermosphacta* are inhibited by pH values lower than 5.8 (Bell 2001).

Although, generally, lamb meat has a higher mean ultimate pH (5.6 – 5.7) than beef meat (pH = 5.5), still the significant effect of Breed in all models (Tables 1-4) suggest 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 – as already explained – ending up ultimately (and unavoidably) in a reduction of shelf-life in refrigerated conditions even when vacuum-packaging is applied.

Table 3: Effects of Lamb Breed and Initial Intrinsic Factors of Meat on the Concentration of ***Pseudomonas* pp.** in Refrigerated Vacuum-Packed Meat as Quantified by Six Separate Linear Mixed Models (F-Values and Associated p-Values from ANOVA are Shown; Quadratic Effect of Day is Significant in All Models but Not Shown)

Model	Term	Estimate (SE)	F (p-value)
pH	Intercept	1.255 (0.510)	206.3 (<.0001)
	Breed - CGB	-0.369 (0.222)	8.147 (0.006)
	Day	-3.239 (1.657)	32.15 (<.0001)
	pH×Day	0.533 (0.270)	3.795 (**0.050**)
aw	Intercept	1.356 (0.513)	215.7 (<.0001)
	Breed - CGB	-0.567 (0.194)	8.520 (0.005)
	Day	-30.48 (17.26)	31.28 (<.0001)
	aw×Day	30.50 (17.38)	3.082 (0.082)
Moisture (%)	Intercept	1.188 (0.485)	224.2 (<.0001)
	Breed - CGB	-0.242 (0.203)	8.854 (0.004)
	Day	-11.15 (2.448)	35.53 (<.0001)
	Moisture×Day	0.143 (0.031)	21.00 (<.**0001**)
Fat (% db)	Intercept	1.115 (0.483)	221.7 (<.0001)
	Breed - CGB	-0.100 (0.215)	8.754 (0.005)
	Day	0.191 (0.534)	36.00 (<.0001)
	Fat×Day	-0.073 (0.015)	22.01 (<.**0001**)
Protein (% db)	Intercept	1.303 (0.513)	207.5 (<.0001)
	Breed - CGB	-0.464 (0.210)	8.196 (0.006)
	Day	-2.646 (1.785)	31.59 (<.0001)
	Protein×Day	0.028 (0.019)	2.085 (0.151)
Ashes (% db)	Intercept	1.255 (0.501)	217.5 (<.0001)
	Breed - CGB	-0.371 (0.203)	8.591 (0.005)
	Day	-1.288 (0.646)	33.11 (<.0001)
	Ashes×Day	0.201 (0.063)	10.32 (**0.002**)

In relation to meat aw, this intrinsic property was not found to modulate the growth of any microbial group, as deduced by the non-significance of the interaction term aw×Day (p=0.823 for mesophiles; p=0.646 for LAB; p=0.082 for *Pseudomonas* and p=0.315 for psychrotrophic bacteria in Tables 1 to 4, respectively). The lack of effect of aw is not surprising since vacuum packaging prevents drying at the meat surface, and moisture from within the meat allows the surface aw to equilibrate to above 0.98. Consequently, there is no inhibitory effect on bacteria once the meat has been packed and stored (Bell 2001). The mean aw of VP meat measured on the third day after slaughter was the same, 0.9927, for both CGB and BEDM breeds.

Unlike aw, the growth of spoilage bacteria was found to be exacerbated by the moisture content of meat, as implied by the interaction Moisture×Day that was significant in all bacterial groups (p<.0001 in Tables 1-4). The positive sign of 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 hours in a chilling room at 90% RH with loadings that varied from batch to batch. Under these conditions, moisture loss from lamb carcasses have been reported to be up to 2.2% (Brown et al. 1993). 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%).

Table 4: Effects of Lamb Breed and Initial Intrinsic Factors of Meat on the Concentration of **Psychrotrophic Bacteria** in Refrigerated Vacuum-Packed Meat as Quantified by Six Separate Linear Mixed Models (F-Values and Associated p-Values from ANOVA are Shown; Quadratic Effect of Day is Significant in All Models but Not Shown)

Model	Term	Estimate (SE)	F (p-value)
pH	Intercept	1.463 (0.455)	319.6 (<.0001)
	Breed - CGB	-0.251 (0.251)	6.100 (0.017)
	Day	-4.615 (1.686)	159.1 (<.0001)
	pH×Day	0.741 (0.282)	6.893 (**0.010**)
aw	Intercept	1.598 (0.464)	330.9 (<.0001)
	Breed - CGB	-0.531 (0.223)	6.308 (0.015)
	Day	18.03 (18.23)	148.7 (<.0001)
	aw×Day	-18.54 (18.36)	1.019 (0.315)
Moisture (%)	Intercept	1.387 (0.427)	374.4 (<.0001)
	Breed - CGB	-0.141 (0.221)	7.141 (0.009)
	Day	-13.70 (2.447)	178.5 (<.0001)
	Moisture×Day	0.175 (0.031)	30.76 (<.**0001**)
Fat (% db)	Intercept	1.288 (0.422)	365.3 (<.0001)
	Breed - CGB	0.045 (0.233)	6.973 (0.011)
	Day	0.134 (0.456)	185.5 (<.0001)
	Fat×Day	-0.091 (0.016)	34.51 (<.**0001**)
Protein (% db)	Intercept	1.532 (0.462)	324.7 (<.0001)
	Breed - CGB	-0.413 (0.237)	6.192 (0.016)
	Day	-3.261 (1.866)	152.2 (<.0001)
	Protein×Day	0.033 (0.021)	2.576 (0.111)
Ashes (% db)	Intercept	1.468 (0.442)	347.5 (<.0001)
	Breed - CGB	-0.274 (0.226)	6.629 (0.013)
	Day	-1.800 (0.587)	166.1 (<.0001)
	Ashes×Day	0.263 (0.064)	16.87 (**0.001**)

Interestingly, lamb meat samples of higher fat content tended to have a slower microbial deterioration, as suggested by the negative interaction Fat×Day that was significant for all microbial groups (p<.0001 in Tables 1-4). On the contrary, lamb meat samples of higher metal salts and trace minerals

(ashes) tended to have a faster microbial deterioration (p from 0.001 until <.0001 in Tables 1-4). Despite protein content of lamb meat presented a wide range of variation, between 80.97 to 91.34% (db), protein content could not be found to regulate the growth of spoilage bacteria in VP lamb meat. Notice that the interaction term Protein×Day was non-significant ($p>0.05$) in all bacterial groups (Table 1-4).

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 products sought by consumers, it is necessary to understand how the intrinsic properties of carcass and meat affect or 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 was high moisture and ash content. By contrary, lamb meat samples with higher total fat content tended to have slower 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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