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OXIDATIVE STABILITY AND MICROBIAL ECOLOGY OF FRESH BEEF WITH EXTREMELY LONG SHELF-LIFE

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ABSTRACT

This study was aimed at assessing the pigment and lipid stability and characterising the microbial ecology by classical methods and metagenetics in beef with an extremely long shelf-life. Bovine *longissimus thoracis et lumborum* subprimals from different origins (Australia, Brazil, Ireland, and United Kingdom), displaying a shelf-life from 35 to 140 days in vacuum packaging, were aged at -1 (subzero storage) or a -1/+4°C two-level stepwise scheme. At different times, samples were repackaged under a high-oxygen atmosphere (70/30% O_2/CO_2) and stored at 4°C for two days and then 8°C for five days to simulate retail distribution. Subzero storage inhibited the growth of total aerobic mesophilic flora and Enterobacteriaceae during ageing in vacuum ($p < 0{:}001$). During simulated retail distribution, the shelf-life was limited by metmyoglobin formation and excessive lactic acid bacteria growth. Classical microbiological methods underestimated the lactic acid bacteria count. Nonetheless, metagenetics evidenced, specifically in Australian samples, high proportions of *Carnobacterium maltaromaticum*, a lactic acid bacterium that may have contributed to the extremely long shelf-life of Australian beef.

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1. Introduction

The production of living animals is increasingly driven by a shift in diet and food consumption patterns towards livestock products [1]. Growth in the demand for meat will stem mainly from an increase in population, income, and urbanisation, especially in regions with large middle classes, such as Asia, Latin America, and the Middle East. However, beef production continues to be concentrated in a few countries, including Brazil, the United States, and Australia [2]. In this way, the meat trade becomes more globalised, forcing supply chains to get larger and more complex. One of the critical challenges for today's meat export industry is delivering a superior-quality fresh product to distant markets, considering that several weeks of chilled transportation must be anticipated to supply these markets by sea [3]. As such, fresh beef, typically a highly perishable product, is required to stay stable for extended periods.

Vacuum packaging and storage at subzero temperatures prevent the growth of oxygen-requiring spoilage bacteria and provide oxidative stability and long-term shelf-life without recourse to freezing [4]. As a result, beef-producing countries have consolidated an export market over the past decades for chilled vacuum-packaged beef, and shipping and cold storage at -1°C permitted them to achieve a shelf-life superior to 100 days for this product. Nonetheless, the arrival of chilled beef with an extremely long shelf-life in the European market has triggered intense reactions by the Belgian meat sector, which mistakenly suspected the use of methods banned by European legislation to maintain the stability of these meats.

The shelf-life of fresh meat is mainly limited due to microbial growth and oxidation phenomena. As reviewed by Coombs, Holman, Friend and Hopkins [5], an increase in the spoilage bacteria population of meat, including lactic acid bacteria (LAB), Enterobacteriaceae (EB) and *Brochothrix thermosphacta* (BT), is associated with the development of undesirable flavours and discolouration. Besides, oxidative processes are the primary nonmicrobiological factor implicated in the quality deterioration of meat during chilled storage. Lipid oxidation results in the formation of several products, leading to off-flavours and off-odours [6]. Moreover, the oxidation of myoglobin to metmyoglobin (MMb) induces a brown appearance in the meat. Hence, the meat industry's control of microbial, pigment, and lipid stability is essential to maintaining the freshness of this product.

Several studies investigated the oxidative stability of beef with long shelf-life [4, 7] and the microbiota associated with these meats, either by culture-dependent [8] or molecular methods [9]. Nevertheless, the scientific literature lacks studies combining the assessment of the oxidative stability and microbial communities by culture-independent methods in beef with extended shelf-life [10]. In this context, this study was aimed at evaluating the oxidative and microbial stability, as well as the microbial diversity by a culture-independent high-throughput sequencing method, of beef with extended shelf-life at two ageing temperatures (-1°C and two-level stepwise -1/+4°C) in two types of packaging (under vacuum and in a high-oxygen atmosphere).

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2. Material and Methods

2.1. SAMPLES. Eighteen vacuum-packaged bovine *longissimus thoracis et lumborum* (LTL) subprimals from four different origins (six from Australia, three from Brazil, three from Ireland, and six from the United Kingdom (UK)), with significant differences in shelf-life, were supplied by a Belgian food wholesaler. Throughout one year, we collected three samples from the same origin as soon as a load of imported meat was delivered to the food wholesaler. During this period, only one batch of Brazilian and Irish meat corresponding to our criteria (storage time < 2/3 of the shelf-life) was supplied. The shelf-life was labelled 140 days at <2°C for Australian, 120 days at 0°C for Brazilian, 35 days at 0-3°C for Irish, and 45 days for UK meat. The UK meat cutting plant set no storage temperature. The Belgian supplier reported storage at maximum 0°C throughout distribution.

Samples were transported to the laboratory in polystyrene isothermal boxes, and the transport time never exceeded 30 minutes. At the laboratory, they were stored at -1°C until 2/3 of their shelf-life ($SL_{2/3}$) when they were cut into six 2 to 3 cm thick steaks. Three steaks were used to evaluate meat packaged in vacuum (wholesale market simulation) and were handled as follows. One was analysed immediately after LTL was cut into steaks ($SL_{2/3}$). The other two were vacuum packaged and stored at -1 or 4°C until the end of the shelf-life ($SL_{-1°C}$ and $SL_{-1/+4°C}$, respectively) to simulate sub-zero (-1°C) and conventional storage (4°C). Vacuum bags (Cryovac, Charlotte, NC, US) were 60 µm thick, and the oxygen permeability was 13 cm^3/m^2 per day at 1 bar, 23°C, and 0% relative humidity (RH).

The three remaining steaks from each LTL were used to evaluate meat packaged in a modified atmosphere (retail display and distribution simulation). First, they were vacuum- packed, as described above. Then, at $SL_{2/3}$, $SL_{-1°C}$, and $SL_{-1/+4°C}$, they were repacked in polypropylene (PP)/ethylene-vinyl alcohol copolymer /PP trays (ES-Plastic, Hutthurm, Germany, dimensions: 187 x 137 x 50 mm, oxygen permeability: 4 cm^3/ m^2 per day at 1 bar, 23°C and 0% RH) containing a modified atmosphere of 70/30% O_2/CO_2 (Air Liquide, Brussels, Belgium), obtained with a KM 100-3 ME gas mixing and delivery system (Witt) and sealed with a polyethylene terephthalate /PP film (Wipak, Sittard, Netherlands, oxygen permeability: 8 cm^3/m^2 per day at 1 bar, 23°C and 0% RH) with a gas headspace-to-meat ratio of approximately 4: 1. Next, modified atmosphere packaged (MAP) samples were stored for seven days ($SL_{2/3/MAP}$, $SL_{-1°C/MAP}$, and $SL_{-1/+4°C/MAP}$). A temperature of 4°C was used during the first two days, and 8°C was applied during the last five days of storage. The latter temperature was chosen to simulate domestic preservation conditions, according to the *Association Française de Normalisation* (AFNOR) NF V01-003 standard [11]. Three climatic chambers (model MIR 254, Sanyo) were set at -1, 4, and 8°C, and samples were transferred from one chamber to another each time a temperature change was applied. Lightning conditions were not controlled.

In total, six treatments were applied to steaks of each origin (Figure 1), and each steak was assigned randomly to a different treatment. Analyses were performed on samples at $SL_{2/3}$, $SL_{2/3/MAP}$, $SL_{-1°C}$, $SL_{-1°C/MAP}$, $SL_{-1/+4°C}$, and $SL_{-1/+4°C/MAP}$, except for α-tocopherol content (only at $SL_{2/3}$) and metagenetics (only at $SL_{2/3}$ and $SL_{-1/+4°C}$). The corresponding time in days for the six treatments is shown in Table 1.

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2.2. PH MEASUREMENT. pH was measured using a Knick 765 pH meter equipped with a combined pH electrode (model 104063123, Ingold), according to the International Organisation for Standardisation (ISO) 2917 procedure [12]. Measurements were performed in five zones of each sample, and the values were averaged.

2.3. COLOUR MEASUREMENT AND METMYOGLOBIN PERCENTAGE. The instrumental colour of vacuum-packaged samples was evaluated at 1.5 h after removal from vacuum bags and exposure to atmospheric air at 4°C (bloom time) using a Labscan II spectrophotometer (HunterLab). The colour of samples in MAP was measured immediately after removal from trays using the same equipment. Measurement conditions were 25 mm diameter aperture, D65 illuminant and 10° observation angle. CIE LABvalues(L^*: luminance, a^*: redness, b^*: yellowness)were measured in five zones of each sample and averaged. The colour difference between samples before and after MAP display (ΔE) was calculated using the equation $\sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$. Higher Δ$E$ values mean more significant total colour changes over time.

The metmyoglobin percentage (MMb%) was determined by measuring the absorbance of an aqueous meat extract at selected wavelengths as described elsewhere [13]. Briefly, after colour measurement, samples were cut into 3 mm thick slices. Five grams of sliced meat was used for myoglobin extraction in 20 mL phosphate buffer (0.1 M, pH = 6:0). The extracts were filtered through a 0.2 µm syringe filter (Macherey-Nagel, Düren, Germany). The absorbance of the aqueous meat extracts was measured by spectrophotometry at 503, 525, 557, and 582 nm (Spectronic Genesys 2PC UV-Vis, Thermo Fisher Scientific). MMb% was calculated using the following equation:

$$\text{MMb\%} = \left[-0.159\left(\frac{A_{582}}{A_{525}}\right) - 0.085\left(\frac{A_{557}}{A_{525}}\right) + 1.262\left(\frac{A_{503}}{A_{525}}\right) - 0.520\right] \times 100 \quad (1)$$

Figure 1. *Experimental scheme. The yellow rounded squares show the six different treatments applied to samples. MAP: modified atmosphere packaging (70/30% O_2/CO_2) for 2 days at 4°C +5days at 8°C.*

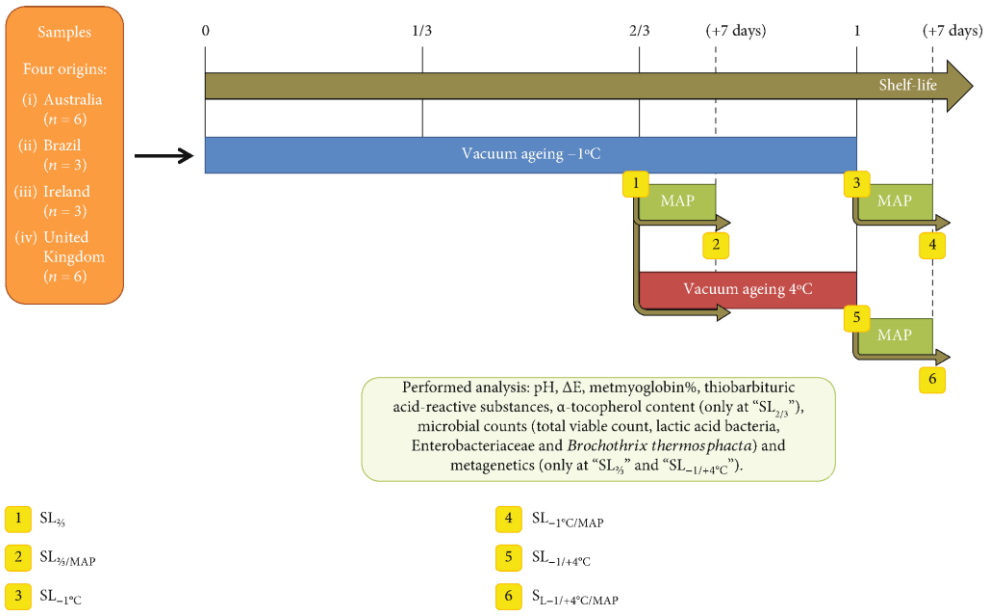

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Table 1: *Corresponding time (days after deboning and cutting) for the six treatments applied to samples of different origins.*

Treatment	Origin			
	Australia	Brazil	Ireland	United Kingdom
SL2/3	93	80	23	30
SL2/3/MAP	93+7	80+7	23+7	30+7
SL-1°C	140	120	35	45
SL-1°C/MAP	140+7	120+7	35+7	45+7
SL-1/+4°C	140	120	35	45
	140+7	120+7	35+7	45+7

$SL_{2/3}$: vacuum storage at -1°C until 2/3 of the shelf-life. $SL_{2/3/MAP}$: vacuum storage at -1°C until 2/3 of the shelf-life with subsequent modified atmosphere storage for seven days. $SL_{-1°C}$: vacuum storage at -1°C until the end of the shelf-life. $SL_{-1°C/MAP}$: vacuum storage at -1°C until the end of the shelf-life with subsequent modified atmosphere storage for seven days. $SL_{-1/+4°C}$: vacuum storage at -1°C until 2/3 of the shelf-life and 4°C until the end of the shelf-life. $SL_{-1/+4°C/MAP}$: vacuum storage at -1°C until 2/3 of the shelf-life and 4°C until the end of the shelf-life with subsequent modified atmosphere storage for seven days.

2.4. LIPID OXIDATION MEASUREMENT. To assess lipid oxidation, an aqueous acid extraction method was undertaken before the spectrophotometric measurement of the thiobarbituric acid-reactive substances (TBARS) at 530 nm [14]. This method is based on the reaction of one molecule of malondialdehyde (MDA), a significant carbonyl decomposition product of oxidised polyunsaturated lipids, with two molecules of thiobarbituric acid (TBA) to form a pink complex which can be quantified spectrophotometrically. Five grams of sample was used for the extraction in 20 mL trichloroacetic acid 5% (VWR, Leuven, Belgium). Two millilitres of TBA 80 mM (Sigma-Aldrich, Overijse, Belgium) was added to filtered meat extracts before heating at 94°C for 5 min. The absorbance was measured after cooling at 530 nm (Spectronic Genesys 2PC UV-Vis, Thermo Fisher Scientific). 1,1,3,3-Tetraethoxypropane (Sigma-Aldrich, Overijse, Belgium) was used to prepare the standard curve and determine the TBARS recovery. The results were expressed as mg MDA equivalents per kg of meat.

2.5. α-Tocopherol Content. A protocol adapted from Liu, Scheller, and Schaefer [15] was implemented to extract and quantify the α-tocopherol content in meat samples. The method consists of a saponification step followed by a double isooctane (Fischer Scientific, Merelbeke, Belgium) extraction of the saponified samples. α-Tocopherol in the extracts was separated by normal phase chromatography and quantified by fluorescence detection. High-pressure liquid chromatography analysis was performed using a model 600 E solvent delivery system equipped with a model 717 automatic injector, a Mistral oven, a 2475 fluorescence detector (excitation at 296 nm wavelength and emission at 325 nm wavelength), and a Resolve spherical silica column (3.9 X 150 mm, 5 µm) set at 15°C (all from Waters). The mobile phase was isooctane/tetrahydrofuran 96 : 4 *v*/*v*

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(Fischer Scientific, Merelbeke, Belgium) at a 1.0 mL/min flow rate, and the injection volume was 30 µL. Quantification was performed by an external standard method based on peak area.

2.6. MICROBIAL COUNTS. Twenty-five square centimetres (1 cm thick) of meat was transferred, beside a Bunsen burner, to a sterile bag with 225 mL sterile peptone water (Bio-Rad, Temse, Belgium) and homogenised for 120 s using a stomacher (primary suspension). Serial decimal dilutions were prepared using the same dilutant. Total viable count (TVC) and LAB and EB counts were performed using the TEMPO® system (BioMérieux, Schaarbeek, Belgium) for enumeration in food products. Cards containing the sample and culture media were incubated at 22°C for 48 h for TVC and LAB and 30°C for 24h for EB. *Brochothrix thermosphacta* (BT) was enumerated by plating on streptomycinsulphate, thallous-acetate, and actidione (STAA) agar with STAA-selective supplement (Oxoid, Dilbeek, Belgium) and incubating at 22°C for 48h, as per ISO 13722 [16]. The incubators were from Shel Lab (model 1535). Colonies were confirmed by oxidase tests (Sigma-Aldrich, Overijse, Belgium). Acceptability thresholds were set at LAB = 6.7 $\log_{10}$ CFU/ cm^2 [17], EB = 5.0 $\log_{10}$ CFU/cm^2 [18], and BT = 6.0 $\log_{10}$ CFU/cm^2 [3]. No threshold was applied to TVC.

2.7. METAGENETICS. Total DNA was extracted directly from each primary suspension obtained in Section 2.6, using a DNeasy blood and tissue kit for DNA extraction (Qiagen, Antwerp, Belgium) following the manufacturer's recommendations (samples lysis using proteinase K, lysate load onto a DNeasy 96 plate, centrifugation, two washing steps and DNA elution). Then, equal amounts of the extracted DNA were mixed into pools containing the DNA of three samples of each batch and origin (Australia, Brazil, Ireland, and UK) within the same treatment ($SL_{2/3}$ and $SL_{-1/+4°C}$). Thus, 12 pools were obtained, termed $AU1_{2/3}$, $AU1_{-1/+4°C}$, $AU2_{2/3}$ and $AU2_{-1/+4}$, (Australian); $BR_{2/3}$ and $BR_{-1/+4°C}$ (Brazilian); $IE_{2/3}$ and $IE_{-1/+4°C}$ (Irish); and $GB1_{2/3}$, $GB1_{-1/+4°C}$, $GB2_{2/3}$, and $GB2_{-1/+4°C}$ (UK).

16S rDNA PCR libraries targeting the V5-V6 hypervariable region were generated. Primers E783-797 and E1063- 1081 [19], specific for bacteria, were selected for their ability to generate the lowest amplification bias among the various bacterial phyla. The oligonucleotide design included titanium A or B adapters (454 Life Sciences, Branford, CT, US) and multiplex identifiers fused to the 5' end of each primer. The amplification mix contained 5 U of FastStart high fidelity polymerase, 1 X enzyme reaction buffer (Eurogentec, Seraing, Belgium), 200 µM deoxynucleotide triphosphate (dNTPs, Eurogentec, Seraing, Belgium), 0.2 µM of each primer, and 100 ng of genomic DNA, in a final volume of 100 *p*L. Thermocycling conditions consisted of a denaturation step at 94°C for 15 min, followed by 30 cycles of40 s at 94°C, 40 s at 56°C, and 1 min at 72°C, with a final elongation step at 72°C for 7 min. These amplifications were performed on an EP Mastercycler Gradient System (Eppendorf). DNA fragments were purified using an SV PCR purification kit (Promega, Leiden, Netherlands). Pyrosequencing was performed by FoodChain ID (Herstal, Belgium) with a Genome Sequencer FLX™ (Roche). The 16S rDNA sequence reads were filtered as described by Huse, Huber, Morrison, Sogin, and Welch [20], processed using the Mothur program [21] and compared to a reference dataset of aligned sequences of the corresponding region derived from the Silva database of full-length rDNA sequences [22]. The final reads were clustered into operational taxonomic units (OTU) using the nearest neighbour algorithm and Mothur with a 0.01 distance-unit cut-off. A taxonomic identity was attributed to each OTU by comparison to the Silva database using a 60% homogeneity cut-off.

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2.8. 16S RRNA GENE SEQUENCING. To identify, at the species level, the predominant bacteria present in samples, the primary suspensions prepared in Section 2.6 were inoculated on plate count agar, and the obtained colonies were selected randomly. The genomic DNA of the selected colonies obtained was extracted as described in Section 2.7 and used to amplify the entire 16S rRNA gene with the universal primers 16S1500F (5'-GAGTTTGATCMTGGCTCAG-3') and 16S1500R (5'-TACGGTTACCTTGATTACGAC-3') under the following conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of 30 s at 94°C, 30 s at 56°C, 1 min at 72°C, and a final extension step at 72°C for 5 min. All PCR reactions contained 1 U Diamond Taq polymerase buffer, 1 U Diamond Taq polymerase, 2 mM $MgCl_2$, 0.2 mM dNTPs (all from Eurogentec, Seraing Belgium), 0.4 µM of each primer and 10-100 ng DNA, in a total volume of 20 µL. PCR products were purified with a Wizard SV PCR kit (Promega, Leiden, Netherlands). The sequencing of the 16S fragments was performed by GIGA Research Centre (Liège, Belgium) using an ABI3730 sequencing machine (Thermo Fisher Scientific). The resulting sequences were assembled, and the identification was made by BLAST comparison [23].

2.9. STATISTICAL ANALYSIS. Results for physicochemical parameters (pH, Δ*E*, MMb%, and TBArS) and microbial counts (TVC, LAB, EB, and BT) measured at 2/3 of the shelf-life ($SL_{2/3}$ and $SL_{2/3/MAP}$) are presented as the mean ± standard deviation. One-way analysis of variance (ANOVA) was used to calculate the effect of origin (Australia, Brazil, Ireland, and UK) within each display time (0 and 7 days) and the effect of display time within each origin. Tukey's test was used when necessary to assess differences between test groups.

Data relating to physicochemical parameters (pH, Δ*E*, MMb%, and TBARS) and microbial counts (TVC, LAB, EB, and BT) measured at the end of the shelf-life ($SL_{-1°C}$, $SL_{-1°C/MAP}$, $SL_{-1/+4°C}$, and $SL_{-1/+4°C/MAP}$) were arranged in a split-plot design in which origin (Australia, Brazil, Ireland, and UK) was allocated in the whole plot, while the sub-plots were ageing temperature (-1 and stepwise -1/+4°C) and display time (0 and 7 days). Considering Δ*E* combines *L**, *a**, and *b** values before and after MAP display, the display time was not included in the model for *AE*. When a post hoc test was suitable, Tukey's test was performed.

All statistical analyses were performed using the R computing environment [24]. A summary of the tested effects for each group of treatments is shown in Table 2.

Table 2: *Summary of the tested effects for each group of treatments.*

Treatment	Effect		
	Origin[1]	Ageing temperature[2]	Display time[3]
$SL_{2/3}$	*X*		*X* [4]
$SL_{2/3/MAP}$	*X*		*X*[4]
$SL_{-1°C}$	*X*	*X*	*X*
$SL_{-1°C/MAP}$	*X*	*X*	*X*
$SL_{-1/+4°C}$	*X*	*X*	*X*
$SL_{-1/+4°C/MAP}$	*X*	*X*	*X*

[1]Australia, Brazil, Ireland, and the United Kingdom. [2]-1 and stepwise -1/ +4°C. [3]0 and 7 days. [4]Except for α-tocopherol content.

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3. Results and Discussion

3.1. PHYSICOCHEMICAL PARAMETERS AND OXIDATIVE STABILITY. The pH of the samples varied between 5.50 and 5.55 at $SL_{2/3}$ and between 5.45 and 5.50 at $SL_{2/3/MAP}$, respectively. No origin or display time effect was observed at 2/3 of the shelf-life (Table 3). At the end of the shelf-life ($SL_{-1°C}$, $SL_{-1°C/MAP}$, $SL_{-1/+4°C}$ and $SL_{-1/+4°C/MAP}$), the effect of origin (Australia, Brazil, Ireland, and UK), ageing temperature (-1 and stepwise -1/+4°C), display time (0 and 7 days), and all their interactions were evaluated for pH (Table 4). No origin or ageing temperature effect was observed. However, at this stage, the pH from samples decreased from 5.55 to 5.50 during display ($p < 0.05$), which may be explained by the development of homofermentative lactic acid bacteria exhibiting preferential fermentation of glucose to lactate. Kaur et al.

[25] observed that a 0.4 pH unit difference in meat affected bacterial diversity, composition, and growth rates. In addition, Hughes et al. [26] reported colour variation in meat with pH variations on the order of 0.2 units. Nonetheless, the differences in pH throughout our study did not exceed 0.05 units. It was considered that these minimal pH variations may not have influenced the other physicochemical and microbial parameters in our experiment. In all cases, beef's pH remained within normal ranges [25, 26].

The colour of meat is an essential aspect of consumer acceptability since consumers consider the bright red colour of beef as an indicator of freshness and wholesomeness. Initial colour attributes (L^*, a^*,and b^*) of beef from different origins at $SL_{2/3}$ and $SL_{2/3/MAP}$ are shown in Table 3. Irish meat presented the lowest a^* at $SL_{2/3}$, while Brazilian presented the highest L^* at $SL_{2/3/MAP}$ ($p < 0.05$). A decrease of a^* and b^* was observed for Brazilian meat during the simulated MAP display ($p < 0.05$). In addition, no effect of origin was observed for ΔE during display at 2/3 of the shelf-life (Table 3). At the end of the shelf-life, the effect of origin (Australia, Brazil, Ireland, and UK), ageing temperature (-1 and stepwise -1/+4°C), and their interactions were evaluated for ΔE (Table 4). ΔE values varied from 2.0 to 7.1 and from 2.0 to 10.7 between origins for the ageing temperatures of -1 and stepwise -1/+4°C, respectively (data not shown in tabular form). Numerically, UK meat presented the lowest and Brazilian meat the highest ΔE. Nevertheless, no statistically significant difference was observed between the samples, meaning no difference in the total change of instrumental colour during display at the end of the shelflife between origins or ageing temperatures.

In regard to MMb%, at 2/3 of the shelf-life ($SL_{2/3}$ and $SL_{2/3/MAP}$), an effect of display time (0 and 7 days) was observed in Australian, Brazilian, and UK samples ($p < 0.05$) (Table 3). At the end of the shelf-life (SL-1.C, $SL_{-1°C/MAP}$, $SL_{-1/+4°C}$, and $SL_{-1/+4°C/MAP}$), the effects of display time and the interaction origin (Australia, Brazil, Ireland, and UK) x ageing temperature (-1 and stepwise -1/+4°C) were significant ($p < 0.05$) (Table 4). Thus, a seven-day MAP display favoured pigment oxidation at 2/3 and the end of the shelf-life ($p < 0.05$) (Table 3 and Figure 2(a)). Previous authors have also reported that pigment oxidation increases as display time increases [27, 28]. According to Garner, Unruh, Hunt, Boyle, and Houser [29], the metmyoglobin reducing activity (MRA), which

represents properties that help minimise MMb, decreases over display time, contributing to an increase in MMb%. Regarding the interaction origin x ageing temperature, an ageing temperature of

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4°C during the last 1/3 of the shelf-life did not affect Australian, Brazilian, and Irish samples; however, pigment oxidation was numerically higher at -1 than at 4°C in UK samples (Figure 2(b)). Bekhit and Faustman [30] reviewed that MMb reduction by MRA may be accelerated with increased temperature, explaining the decreased pigment oxidation in UK meat at 4°C. However, it does not justify the higher pigment stability of meat from other origins at -1°C. In addition, the value of 40% MMb, reported by Greene, Hsin, and Zipser [31] as the threshold for consumers to reject meat, was not exceeded by any sample at $SL_{2/3}$, $SL_{2/3/map}$, $SL_{-1°C}$, and $SL_{-1/4°C}$. Conversely, the limit was exceeded by all samples at $SL_{-1°C/MAP}$ and $SL_{-1/4°C/MAP}$, except for UK meat previously aged at 4°C, during the last 1/3 of the shelf-life. This finding suggests that myoglobin oxidation during display is higher with increasing ageing time.

Lipid oxidation in meat can lead to quality deterioration, limiting the shelf-life from a flavour perspective. As TBARS values correlate well with sensory testing, they can be a good indicator of rancidity in meat [32]. At $SL_{2/3}$, TBARS did not exceed 0.3 mg MDA equivalent/kg, and after a seven-day simulated MAP display ($SL_{2/3/MAP}$), there was an increase in the TBARS in samples of all origins ($p < 0{:}05$), except for Brazilian (Table 3). At the end of the shelf-life, the interaction of origin (Australia, Brazil, Ireland, and UK) x display time (0 and 7 days) was significant for lipid oxidation ($p < 0{:}05$) (Table 4). At this point, before MAP display, UK meat presented the highest lipid oxidation ($p < 0{:}05$), while Brazilian and Irish meat showed TBARS values inferior to the limit of quantification (LOQ = 0:2mgMDA equivalent/ kg). After seven days of display, the lipid oxidation in Brazilian meat remained inferior to the LOQ, while Australian, Irish, and UK meat presented equivalent TBARS values (Figure 3). Campo et al. [33] proposed that the TBARS value of 2.0 mg MDA equivalent/kg in raw beef could be considered the threshold at which a rancid flavour overpowers the beef flavour when it is cooked, and, consequentially, is the maximum level for a positive sensory perception of beef.

This threshold was not exceeded in any sample for any treatment.

In addition, high-oxygen MAP is typically used for beef cuts to promote pigment oxygenation, prolonging the period before MMb is visible on the muscle surface. According to Jayasingh, Cornforth, Brennand, Carpenter, and Whittier [34], although high-oxygen atmospheres maintain redness during storage, rancidity often develops while colour is still desirable. Nonetheless, in the present study, pigment oxidation reached unacceptable levels before lipid oxidation. Myoglobin and lipid oxidation are undoubtedly interrelated; however, the exact nature of this interrelationship has not been established. One hypothesis is that myoglobin oxidation initiates before lipid oxidation. A possible mechanism was reviewed by Richards [35]: oxymyoglobin oxidation initiates the first step in a sequence of chemical reactions leading to the production of radicals, which in turn gives rise to the initiation of lipid oxidation.

Dietary vitamin E supplementation to beef cattle increases the concentration of *a* -tocopherol in skeletal muscle, allowing this antioxidant to protect membranal lipids and prevent MMb formation [36]. Table 5 shows the *a*- tocopherol content in samples at $SL_{2/3}$. Brazilian meat *a*- tocopherol content was 3.2 µg/g, the lowest among the tested samples. Australian, Irish, and UK meat presented *a*- tocopherol contents equivalent to each other, ranging from 5.0 to 6.1 µg/g. Liu et al. [37] suggest a value of 3.5 µg/g as the minimum muscle α-*Tocopherol* concentration for near maximal

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suppression of myoglobin and lipid oxidation in fresh beef. Surprisingly, only Brazilian samples, which presented high lipid stability, remained below this threshold. However, the α-*Tocopherol* content was not measured in muscles immediately after slaughter and may have decreased during storage.

Moreover, meat with a high content of polyunsaturated fatty acids requires increased endogenous antioxidants to maintain colour and lipid stability [38]. Brazilian cattle production is primarily based on *Bos indicus* breeds and crosses, whereas Bos taurus is the dominant species in Europe and Australia. Previous studies highlighted slight differences in fat content and fatty acid composition of beef from B. indicus and B. taurus cattle. However, diet is a much more important determinant of these parameters [39]. Thus, the proportion of peroxidisable lipids does not seem to be linked to the subspecies of cattle. In this way, the evaluation of other mechanisms involved in pigment and lipid oxidation, including antioxidant [40] and glycolytic [41] enzymes, which are directly influenced by genetics and feeding systems, could have provided additional clues to understand better the differences in the oxidation profiles observed.

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DOI:10.1155/2023/5319266
Status : Postprint (Author's version)

Table 3: *Physicochemical parameters and microbial counts (mean ± standard deviation) in the longissimus thoracis et lumborum cuts from different origins at 2/3 of the shelf-life before and after a 7-day modified atmosphere packaging display.*

		Origin			
Parameter	Display time (days)	Australia	Brazil	Ireland	United Kingdom
pH	0	5.50 ±0.08	5.50 ±0.02	5.55 ±0.08	5.50 ±0.03
	7	5.50 ± 0.07	5.45 ± 0.02	5.50 ± 0.02	5.50 ± 0.04
L *	0	38.2 ± 1.7	41.6 ± 0.8	38.2 ± 5.2	36.7 ± 2.6
	7	39.0 ± 2.8^{AB}	41.7 ± 0.9^{B}	36.4 ± 0.5^{A}	37.4 ± 1.9^{A}
*a**	0	24.2 ± 1.6^{A}	24.3 ± 1.4^{aA}	19.6 ± 3.1^{B}	24.8 ± 0.7^{A}
	7	22.2 ± 2.2	21.3 ± 0.6^{b}	20.2 ± 2.1	22.9 ± 2.3
*b**	0	18.9 ± 2.1	19.6 ± 0.6^{a}	15.8 ± 2.5	18.1 ± 0.7
	7	17.5 ± 1.6	17.7 ± 0.5^{b}	15.8 ± 1.3	17.1 ± 1.2
ΔE	n/a	3.7 ± 1.0	4.0 ± 1.9	5.9 ± 2.3	3.0 ± 2.1
MMb%	0	11.3 ± 9.5^{a}	2.9 ± 1.1^{a}	5.1 ± 5.3	5.0 ± 3.8^{a}
	7	37.0 ± 10.7^{b}	21.5 ± 11.0^{b}	16.5 ± 12.4	34.8 ± 12.5^{b}
TBARS[1]	0	0.3 ± 0.2^{a}	<LOQ[2]	<LOQ[2]	0.3 ± 0.2^{a}
	7	1.5 ± 0.4^{b}	<LOQ [2]	1.1 ± 0.9	1.1 ± 0.7^{b}
TVC[3]	0	5.3 ± 0.9^{aB}	5.8 ± 0.6^{aB}	3.1 ± 0.5^{aA}	5.1 ± 0.5^{aB}
	7	6.0 ± 1.1^{bA}	8.3 ± 0.5^{bB}	7.4 ± 0.4^{bAB}	7.2 ± 0.4^{bAB}
LAB[3]	0	<2.0	<2.0	<2.0	4.0 ± 1.7
	7	<2.0	5.4 ± 0.3	6.4 ± 0.1	5.8 ± 0.8
EB[3]	0	<1.0	<1.0	<1.0	2.6 ± 1.5
	7	<1.0	<1.0	2.5 ± 1.4	2.5 ± 1.4
BT[3]	0	<2.0	<2.0	<2.0	3.2 ± 1.2^{a}
	7	<2.0	<2.0	6.5 ± 0.2	5.2 ± 1.1^{b}

MMb%: metmyoglobin%; TBARS: thiobarbituric acid-reactive substances; TVC: total viable count; LAB: lactic acid bacteria; EB: Enterobacteriaceae; BT: Brochothrix thermosphacta; n/a: not applicable. 1Expressed as mg malondialdehyde equivalents/kg. 2Limit of quantification (LOQ) = 0.2 mg malondialdehyde equivalent/kg. 3Expressed as log10 CFU/cm2. abDifferent lowercase letters in a column (display time effect) within each variable indicate statistical differences ($p < 0.05$). No lowercase letter indicates no statistical difference. ABDifferent uppercase letters in a row (origin effect) indicate statistical differences ($p < 0.05$). No lowercase letter indicates no statistical difference.

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Status : Postprint (Author's version)

Table 4: *Analysis of variance (F values) on the effect of origin (Australia, Brazil, Ireland, and the United Kingdom), ageing temperature (-1 and stepwise -1/+4°C) and display time (0 and 7 days) on the pH, discolouration during modified atmosphere packaging display (ΔE), metmyoglobin percentage (MMb%), lipid oxidation (thiobarbituric acid-reactive substances (TBARS)), total viable count (TVC), lactic acid bacteria (LAB), Enterobacteriaceae (EB), and Brochothrix thermosphacta (BT) in longissimus thoracis et lumborum cuts at the end of the shelf-life. Higher F values indicate stronger effects.*

Effect	PH	*ΔE*	MMb%	TBARS	TVC	LAB	EB	BT
Origin (*O*)	0.68	0.09	**16.01*****	**7.07*****	**13.02*****	**5.91****	**6.72****	0.98
Ageing temperature (*T*)	0.32	0.02	**8.94****	1.74	**18.86*****	3.16	**16.60*****	0.75
Display time (*D*)	**4.26***	n/a	**98.27*****	**28.41*****	**13.28*****	**20.66*****	3.40	0.38
O x *T*	1.76	1.22	**3.55***	0.79	0.93	0.31	2.41	0.86
*O*x*D*	1.38	n/a	1.19	**3.09***	0.14	0.12	1.02	0.04
*T*x*D*	1.50	n/a	0.16	0.22	**7.19****	0.25	2.44	0.19
*O*x*T*x*D*	0.77	n/a	1.64	0.07	0.77	**4.04***	1.34	0.55

Significant probabilities ($p < 0.05$) are in bold. *Significant at $p < 0.05$. **Significant at $p < 0.01$. ***Significant at $p < 0.001$.

Figure 2: *(a) Effect of display and (b) interaction of origin x temperature for metmyoglobin (MMb%) in the longissimus thoracis et lumborum cuts from different origins at the end of the shelf-life. Bars represent standard error. Different letters indicate statistical differences (p < 0:05).*

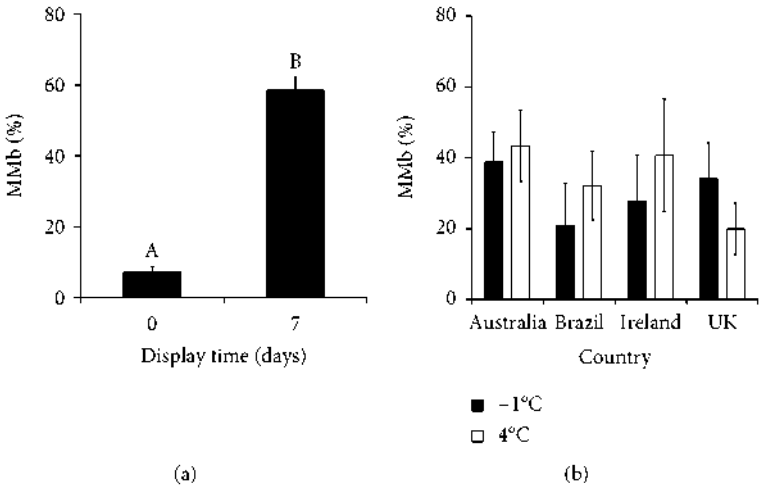

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Figure 3: *Interaction of origin x display time for lipid oxidation (thiobarbituric acid-reactive substances (TBARS)) in the longissimus thoracis et lumborum cuts from different origins at the end of the shelf-life. Bars represent standard error. Different letters indicate statistical differences (p < 0:05).*

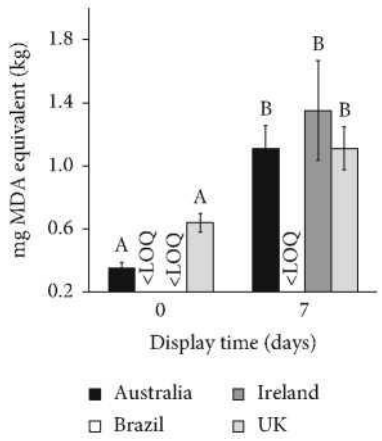

Table 5: *α-Tocopherol content (mean ± standard deviation) in the longissimus thoracis et lumborum cuts from different origins at 2/3 of the shelf-life.*

Origin	α-Tocopherol (μg/g meat)
Australia	6.1 ± 0.7^{b}
Brazil	3.2 ± 0.3^{a}
Ireland	5.1 ± 0.3^{b}
United Kingdom	5.0 ± 1.0^{b}

[ab]Different letters (origin effect) indicate statistical differences (p < 0:05).

3.2. MICROBIAL STABILITY AND MICROBIAL PROFILE. At 2/3 of the shelf-life ($SL_{2/3}$ and $SL_{2/3/MAP}$), the effect of origin (Australia, Brazil, Ireland, and UK) was significant for TVC, and the effect of display time (0 and 7 days) was observed for TVC and BT in UK meat ($p < 0:05$) (Table 3). At the end of the shelf-life ($SL_{-1°C}$, $SL_{-1°C/MAP}$, $SL_{-1/+4°C}$, and $SL_{-1/+4°C/MAP}$), the effect of origin was significant for TVC, LAB, and EB ($p < 0:05$); the effect of ageing temperature (-1 and stepwise -1/+4°C) was significant for EB ($p < 0:05$); the effect of display time was significant for LAB ($p < 0:05$); the interaction ageing temperature x display time was significant for TVC ($p < 0:05$), and the interaction origin x ageing temperature x display time was influential for LAB ($p < 0:05$) (Table 4). For practical purposes, all microbial counts will be presented as origin x ageing temperature x display time means.

The TVC ranged from 3.1 to 5.8 $\log_{10}$ CFU/cm^2 at $SL_{2/3}$ and from 6.0 to 8.3 $\log_{10}$ CFU/cm^2 at $SL_{2/3/MAP}$ (Table 3). At the end of the shelf-life ($SL_{-1°C}$, $SL_{-1°C/MAP}$, $SL_{-1/+4°C}$, and $SL_{-1/+4°C/MAP}$), Brazilian meat presented the highest while Irish meat presented the lowest TVC ($p < 0:05$), which might be solely related to their shelf-life (120 and 35 days, respectively). Furthermore, an increase in TVC was observed during MAP display for samples aged at -1°C ($p < 0:05$). This result was expected given that a high-oxygen modified atmosphere promotes bacterial growth compared to vacuum storage [42]. However, there was no difference in TVC before and after MAP display for samples having undergone a stepwise -

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DOI:10.1155/2023/5319266
Status : Postprint (Author's version)

1/+4°C ageing, likely because these meats had reached high TVC during vacuum storage at 4°C (Figure 4).

The LAB counts ranged from <2.0 to 6.4 $\log_{10}$ CFU/cm^2 at 2/3 of the shelf-life ($SL_{2/3}$ and $SL_{2/3/MAP}$), and the acceptability LAB threshold of 6.7 $\log_{10}$ CFU/cm^2 was not exceeded by any sample (Table 3). However, at the end of the shelf-life ($SL_{-1°C}$, $SL_{-1°C/MAP}$, $SL_{-1/+4°C}$, and $SL_{-1/+4°C/MAP}$), the acceptability threshold for LAB was exceeded in Brazilian and Irish samples at $SL_{-1°C/MAP}$ and in all samples at $SL_{-1/4°C/MAP}$, except for Australians. Surprisingly, LAB counts in Australian meat remained below 2.0 $\log_{10}$ CFU/cm^2 for treatments $SL_{-1°C}$, $SL_{-1/+4°C}$, and $SL_{-1/+4°C/MAP}$ (Figure 4). This result was unexpected at $SL_{-1°C}$ and $SL_{-1/+4°C}$ as LAB becomes the dominant species of the microbial flora of chilled meats when the growth of aerobic spoilage bacteria is inhibited by vacuum packaging. In addition, their presence usually ensures that shelf-life is maximal [43]. Small et al. [8] reported unusual microbial counts in beef with extremely long shelf-life. According to Pothakos, Samapundo, and Devlieghere [44], classical microbiological techniques (based on incubation at 30°C) lack the discriminatory capacity to detect psychrotrophic LAB, whose growth was probably favoured by sub-zero ageing under vacuum for extended periods. Even if an incubation temperature of 22°C was used, the psychrotrophic LAB might have been underestimated by the enumeration technique used in the present study, notably in Australian meat for treatments $SL_{-1°C}$, $SL_{-1/+4°C}$, and $SL_{-1/+4°C/MAP}$. Finally, the development of specific LAB species may have differed according to the ageing temperature, resulting in diverse ecosystems in meat. This could explain why the enumeration of LAB was higher in $SL_{-1°C/MAP}$ than in $SL_{-1/+4°C/MAP}$.

Enterobacteriaceae (EB) and BT potentially contribute to meat spoilage. Low levels of EB were found in samples at 2/3 of the shelf-life ($SL_{2/3}$ and $SL_{2/3/MAP}$) (Table 3). Surprisingly, at the end of the shelf-life ($SL_{-1°C}$, $SL_{-1°C/MAP}$, $SL_{-1/+4°C}$, and $SL_{-1/+4°C/MAP}$), Irish and UK meats, which displayed the shortest shelf-lives, presented the highest EB counts. Moreover, a stepwise -1/+4°C ageing promoted EB growth ($p < 0{:}05$) (Figure 4). This result confirms the advantage of combining subzero ageing and strict temperature control with a subsequent display in an atmosphere containing CO_2, which has an antimicrobial potential against EB [45]. In all cases, the acceptability threshold for EB was not exceeded by any sample for any treatment.

All samples presented BT counts below the threshold of acceptability at 2/3 of the shelf-life ($SL_{2/3}$ and $SL_{2/3/MAP}$), except for UK and Irish (only at $SL_{2/3/MAP}$ for Irish) (Table 3). Moreover, in Irish meat, BT counts at $SL_{-1°C/MAP}$ (6.1 $\log_{10}$ CFU/cm^2) and $SL_{-1°C/4/MAP}$ (<2.0 $\log_{10}$ CFU/cm^2) were lower than those at $SL_{2/3/MAP}$ (6.5 $\log_{10}$ CFU/cm^2) ($p < 0{:}05$). As vacuum packaging limits the growth of BT in meat [46], the fact that UK meat presented a higher BT count at $SL_{2/3}$ than the other samples leads to the hypothesis of delayed packaging or the presence of residual oxygen in vacuum bags. However, since the initial handling conditions of the samples are unknown, this hypothesis could not be verified. Russo, Ercolini, Mauriello, & Villani [47] evidenced a decrease in the growth of BT in the presence of LAB *in vitro*, which could explain the reduction of BT in Irish samples over time. Furthermore, BT is psychrotolerant [48] and may have been selected during ageing at -1°C, which may explain the differences in BT count between $SL_{-1°C/MAP}$ and $SL_{-1°C/4/MAP}$.

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DOI:10.1155/2023/5319266
Status : Postprint (Author's version)

Figure 5 presents the microbial diversity of samples evaluated by metagenetics. At first glance, Australian beef presented a lower bacterial diversity when compared to beef from other origins. Brazilian and Irish meats presented high bacterial diversity, evidenced by the bars' size representing genus counting for less than 5% of relative abundance ("others"). In all cases, ageing time contributed to the selection of fewer genera in the beef microbial environment. The microbial ecology of raw meat is very complex, and its storage conditions will influence how different groups of organisms will colonise and eventually dominate the meat microbial ecosystem [49]. When meat is packaged under vacuum, psychrotrophic LAB become the dominant bacterial group, which is confirmed by the significant proportions of Lactobacillales, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, and *Leuconostoc* found at the end of the shelf-life ($SL_{-1/+4°C}$).

Moreover, members of the EB in certain meats, including the genus *Serratia*, may have contributed to meat spoilage. Surprisingly, *Pseudomonas*, often associated with meat spoilage under aerobic conditions, was detected in Brazilian and Irish beef at the end of the shelf-life. However, Motoyama, Kobayashi, Sasaki, Nomura, and Mitsumoto [50] observed that *Pseudomonas fragi* could convert MMb into deoxymyoglobin and inhibit meat lipid oxidation. In this way, the interaction between the different bacterial groups found in meat and their effect on oxidative stability should be further investigated.

The relative abundance of *Carnobacterium*, specifically in Australian samples, reached up to 98%, and their presence in food is frequently underestimated with culture-depend methods [51]. It is known that factors such as the presence of acetate or low pH values can inhibit the growth of *Carnobacterium* [52]. However, the composition of the media used in the TEMPO® system for automated enumeration is not available. Therefore, it was not possible to verify if the formulation of TEMPO® LAB test kits could explain the extremely low results obtained for LAB counts in Australian meat.

Sequencing the 16S rRNA genes of 11 isolates from different Australian meat samples revealed that all isolates belonged to the species *Carnobacterium maltaromaticum*. These LAB have been extensively studied as protective cultures in foodstuff, and some strains can inhibit the growth of spoilage bacteria, including EB, and pathogenic bacteria, such as *Listeria monocytogenes* [53]. In this manner, the high proportions of *C. maltaromaticum* in Australian samples may have contributed positively to the extremely long shelf-life of these meats.

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Figure 4: *Interaction of origin x temperature x display time for (a) total viable count, (b) lactic acid bacteria, (c) Enterobacteriaceae, and (d) Brochothrix thermosphacta in the longissimus thoracis et lumborum cuts from different origins at the end of the shelf-life. Bars represent standard error. The dashed lines represent the thresholds of acceptability. No threshold was applied to the total viable count. Different letters indicate statistical differences (p < 0:05).*

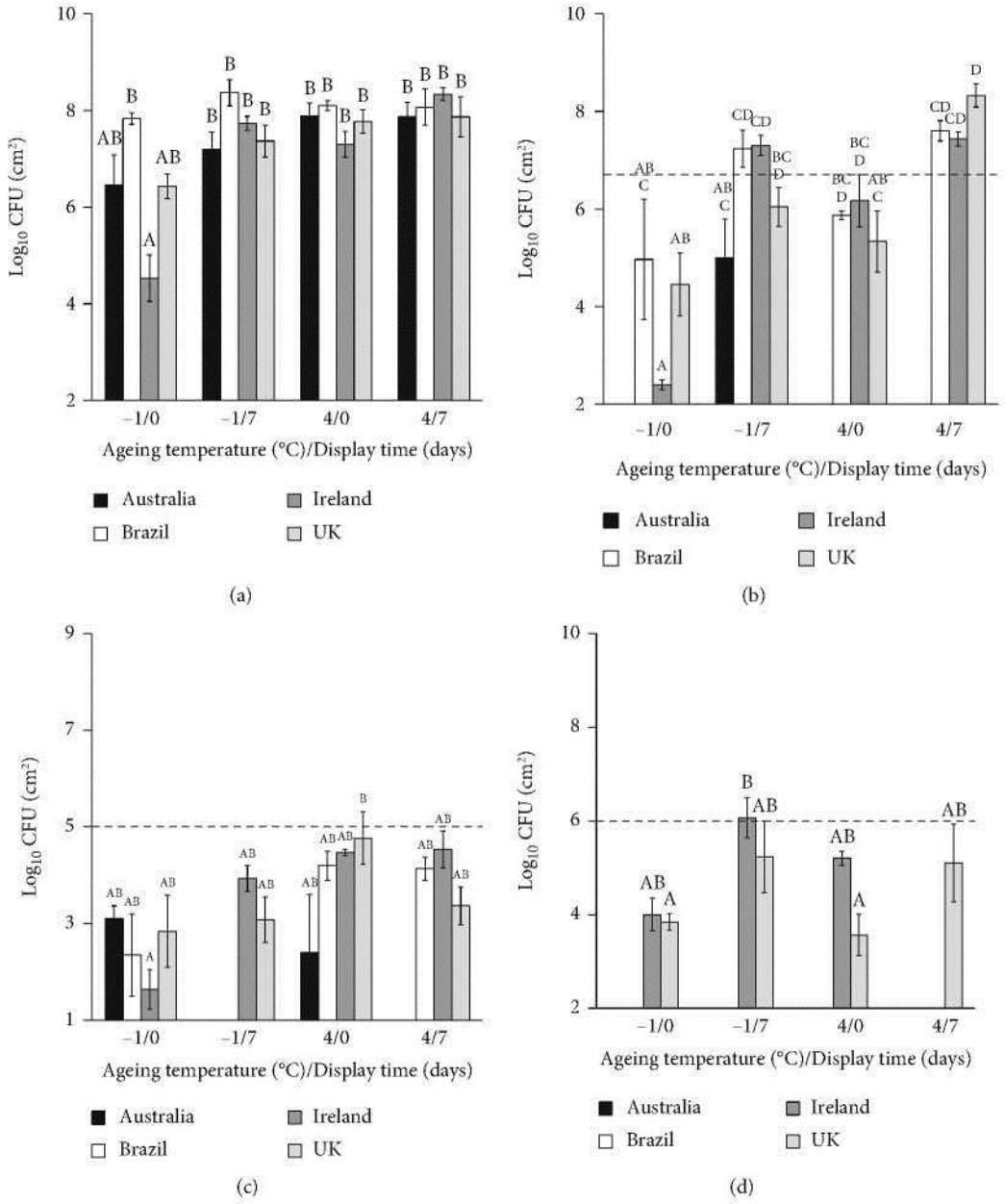

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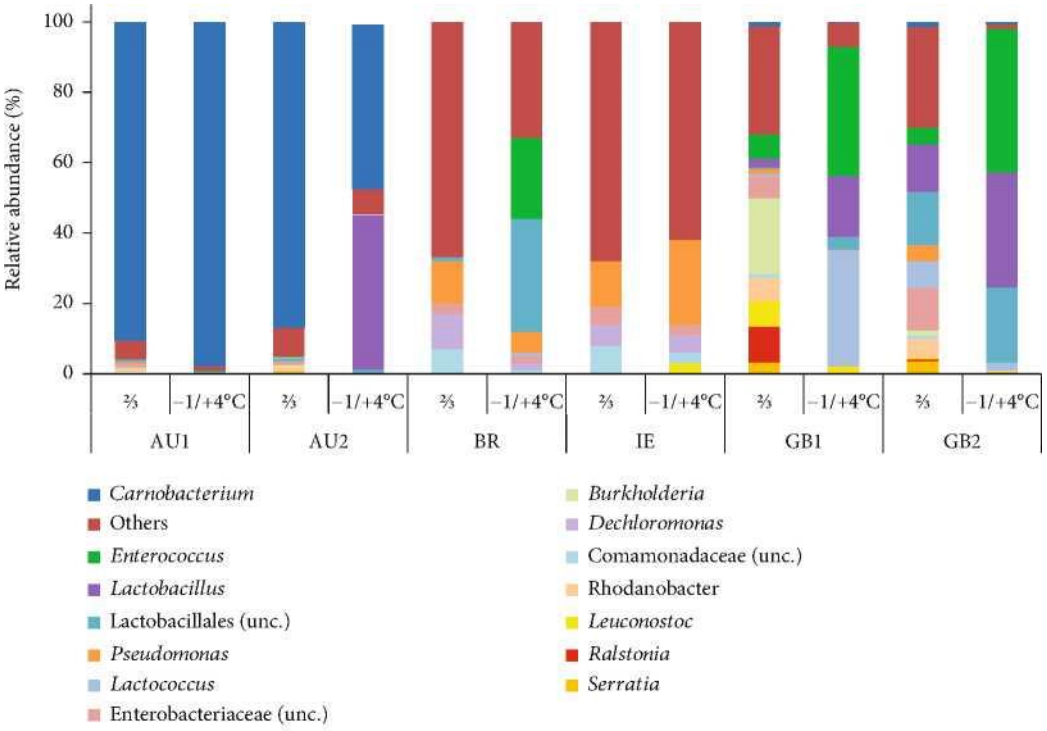
Figure 5: *Distribution of reads percentages for major bacterial genera in beef from different origins at 2/3 and at the end of the shelf-life (for a stepwise -1/+4°C ageing). Other genera represented, individually, less than 5% relative abundance in all samples. AU: Australia; BR: Brazil; IE: Ireland; GB: the United Kingdom; unc.: unclassified genus.*



4. Conclusions

Using subzero temperatures during vacuum storage is helpful in meat shelf-life extension because it prevents myoglobin oxidation and TVC and EB growth. However, ageing at -1°C did not prevent MMb formation and bacterial growth in meat once it was repacked in a high-oxygen atmosphere (70/30% O_2/CO_2) at the end of the shelf-life, suggesting that extended beef ageing is more suitable for out of home operators, for whom high-oxygen MAP is not used. However, the differences in physicochemical and microbial parameters highlighted between origins should be considered carefully due to the low number of samples used in this research. Moreover, the lack of information related to the background of the samples hinders the further evaluation of the possible effect of production conditions (e.g., finishing system, fat score, and carcase handling) on the quality of meat.

Metagenetics proved to be a powerful and revolutionary tool for evaluating the bacterial diversity of fresh meat, as it could detect the presence of bacteria, notably psychrotrophic LAB, which are usually missed or underestimated by culture-dependent method assays.

Carnobacterium maltaromaticum was the dominant bacteria in Australian meat with 140 days of shelf-life. This species has been used for bioprotection and may have played an essential role in the extremely long shelf-life of these samples. The next step of this study will characterise the obtained *C. maltaromaticum* isolates to better understand their role in food preservation.

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DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author, PH Imazaki, upon reasonable request.

ADDITIONAL POINTS

Novelty Impact Statement. Subzero chilling ensures the optimal shelf-life of vacuum-packed beef; however, preserving meat for extended periods is not recommended before retail display under a high-oxygen atmosphere. Culturedependent methods underestimate psychrotrophic lactic acid bacteria counts; thus, culture-independent high-throughput sequencing methods, including metagenetics, are powerful tools to evaluate the microbial ecosystem of meat. Furthermore, molecular methods evidenced high proportions of *Carnobacterium maltaromaticum* in fresh beef with an extremely long shelf-life. This bacterium can grow at temperatures as low as -1.5°C, whether the products have been stored aerobically, vacuum packaged, or under a modified atmosphere. Therefore, they may have been advantaged by storage conditions and contributed positively to the extremely long shelf-life of the meats in which they were found.

DISCLOSURE

An earlier version of this paper has been presented as a doctoral thesis [54].

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Pedro Henrique Imazaki first conceived and designed the study, collected, analysed, and interpreted the data and wrote the article. Bernard Taminiau and Papa Abdoulaye Fall assisted with collecting data and analysing metagenetics and 16S rRNA gene sequencing results. Mahmoud Elansary assisted with statistical analyses. Caroline Douny, Marie- Louise Scippo, Georges Daube, and Antoine Clinquart

assisted with refining the concept and design and critically revised the article for valuable intellectual content. Marie-Louise Scippo, Georges Daube, and Antoine Clinquart supervised the study.

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DOI:10.1155/2023/5319266
Status : Postprint (Author's version)

References

[1] T. Whitnall and N. Pitts, "Global trends in meat consumption," *Agricultural Commodities,* vol. 9, pp. 96-99, 2019.

[2] OECD & FAO, "Meat," in *OECD-FAO Agricultural Outlook 2021-2030,* OECD and FAO, Eds., OECD Publishing, Paris, 2021.

[3] J. Mills, A. Donnison, and G. Brightwell, "Factors affecting microbial spoilage and shelf-life of chilled vacuum-packed lamb transported to distant markets: a review," *Meat Science*, vol. 98, no. 1, pp. 71-80, 2014.

[4] L. E. Jeremiah and L. L. Gibson, "The influence of storage temperature and storage time on color stability, retail properties and case-life of retail-ready beef," *Food Research International*, vol. 34, no. 9, pp. 815-826, 2001.

[5] C. E. O. Coombs, B. W. B. Holman, M. A. Friend, and D. L. Hopkins, "Long-term red meat preservation using chilled and frozen storage combinations: a review," *Meat Science*, vol. 125, pp. 84-94, 2017.

[6] F. Shahidi, "Oxidative stability and shelf life of meat and meat products," in *Oxidative Stability and Shelf Life of Foods Containing Oils and Fats*, M. Hu and C. Jacobsen, Eds., pp. 373389, AOCS Press, London, 2016.

[7] A. Rodas-González, C. Narváez-Bravo, M. M. Brashears et al., "Evaluation of the storage life of vacuum packaged Australian beef," *Meat Science*, vol. 88, no. 1, pp. 128-138, 2011.

[8] A. H. Small, I. Jenson, A. Kiermeier, and J. Sumner, "Vacuum- packed beef primals with extremely long shelf life have unusual microbiological counts," *Journal of Food Protection*, vol. 75, no. 8, pp. 1524-1527, 2012.

[9] M. K. Youssef, C. O. Gill, and X. Yang, "Storage life at 2 ˚Cor -1.5 ˚C of vacuum-packaged boneless and bone-in cuts from decontaminated beef carcasses," *Journal of the Science of Food and Agriculture*, vol. 94, no. 15, pp. 3118-3124, 2014.

[10] X. Chen, L. Zhu, R. Liang et al., "Shelf-life and bacterial community dynamics of vacuum packaged beef during long-term super-chilled storage sourced from two Chinese abattoirs," *Food Research International*, vol. 130, article 108937, 2020.

[11] AFNOR, "Hygiène et sécurité des produits alimentaire- s—Lignes directrices pour l'élaboration d'un protocole de test de vieillissement pour la validation de la durée de vie microbiologique—Denrées périssables, réfrigérées (2004), NF V01-003," Association Française de Normalisation, La Plaine Saint-Denis, 2004.

[12] ISO, *Viande et produits à base de viande—Mesurage du pH—Méthode de référence, ISO 2917*, Organisation internationale de normalisation, Genève, 1999.

[13] J. Tang, C. Faustman, and T. A. Hoagland, "Krzywicki revisited: equations for spectrophotometric determination of myoglobin redox forms in aqueous meat extracts," *Journal of Food Science,* vol. 69, no. 9, pp. C717-C720, 2004.

[14] S. Raharjo, J. N. Sofos, and G. R. Schmidt, "Improved speed, specificity, and limit of determination of an aqueous acid extraction thiobarbituric acid-C_{18} method for measuring lipid peroxidation in beef," *Journal of Agricultural and Food Chemistry,* vol. 40, no. 11, pp. 2182-2185, 1992.

[15] Q. Liu, K. K. Scheller, and D. M. Schaefer, "Technical note: a simplified procedure for vitamin E determination in beef muscle," *Journal of Animal Science*, vol. 74, no. 10, pp. 2406-2410, 1996.

Published in : Journal of Food Processing and Preservation (2023), article ID 5319266, 13 pages
DOI:10.1155/2023/5319266
Status : Postprint (Author's version)

[16] ISO, *Viande et produits à base de viande—Dénombrement de Brochothrix thermosphacta—Technique par comptage des colonies obtenues, ISO 13722*, Organisation internationale de normalisation, Genève, 1996.

[17] L. Picgirard, "Comparaison de muscles d'origine française et sud-américaine," *Viandes et Produits Carnés*, vol. 27, pp. 171-177, 2009.

[18] FCD, *Critères microbiologiques applicables à partir de 2015 aux marques de distributeurs, marques premiers prix et matières premières dans leur conditionnement initial industriel*, Fédera- tion du Commerce et de la Distribution, Paris (France), 2016.

[19] J. Brosius, T. J. Dull, D. D. Sleeter, and H. F. Noller, "Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*," *Journal of Molecular Biology*, vol. 148, no. 2, pp. 107-127, 1981.

[20] S. M. Huse, J. A. Huber, H. G. Morrison, M. L. Sogin, and D. Welch, "Accuracy and quality of massively parallel DNA pyrosequencing," *Genome Biology*, vol. 8, no. 7, p. R143, 2007.

[21] P. D. Schloss, S. L. Westcott, T. Ryabin et al., "Introducing mothur: open-source, platform-independent, community- supported software for describing and comparing microbial communities," *Applied and Environmental Microbiology*, vol. 75, no. 23, pp. 7537-7541, 2009.

[22] E. Pruesse, C. Quast, K. Knittel et al., "SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB," *Nucleic Acids Research*, vol. 35, no. 21, pp. 7188-7196, 2007.

[23] S. F. Altschul, W. Gish, W. Miller, E. W. Myers, and D. J. Lipman, "Basic local alignment search tool," *Journal of Molecular Biology*, vol. 215, no. 3, pp. 403-410, 1990.

[24] R Core Team, *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, Austria, 2016, https://www.R-project.org/.

[25] M. Kaur, M. Williams, A. Bissett, T. Ross, and J. P. Bowman, "Effect of abattoir, livestock species and storage temperature on bacterial community dynamics and sensory properties of vacuum packaged red meat," *Food Microbiology*, vol. 94, article 103648, 2021.

[26] J. M. Hughes, G. Kearney, and R. D. Warner, "Improving beef meat colour scores at carcass grading," *Animal Production Science*, vol. 54, no. 4, pp. 422-429, 2014.

[27] P. H. Imazaki, M. Elansary, M.-L. Scippo, G. Daube, and A. Clinquart, "Effect of sex and sub-zero storage temperature on the microbial and oxidative stability of beef packed in a high-oxygen atmosphere after different vacuum ageing times," *Meat Science*, vol. 148, pp. 198-205, 2019.

[28] R. A. Mancini and R. Ramanathan, "Effects of postmortem storage time on color and mitochondria in beef," *Meat Science*, vol. 98, no. 1, pp. 65-70, 2014.

[29] C. M. Garner, J. A. Unruh, M. C. Hunt, E. A. E. Boyle, and T. A. Houser, "Effects of subprimal type, quality grade, and aging time on display color of ground beef patties," *Meat Sci*ence, vol. 98, no. 2, pp. 301-309, 2014.

[30] A. E. D. Bekhit and C. Faustman, "Metmyoglobin reducing activity," *Meat Science*, vol. 71, no. 3, pp. 407-439, 2005.

[31] B. E. Greene, I. M. Hsin, and M. Y. W. Zipser, "Retardation of oxidative color changes in raw ground beef," *Journal of Food Science*, vol. 36, no. 6, pp. 940-942, 1971.

[32] R. Domínguez, M. Pateiro, M. Gagaoua, F. J. Barba, W. Zhang, and J. M. Lorenzo, "A comprehensive review on lipid oxidation in meat and meat products," *Antioxidants*, vol. 8, no. 10, p. 429, 2019.

[33] M. M. Campo, G. R. Nute, S. I. Hughes, M. Enser, J. D. Wood, and R. I. Richardson, "Flavour perception of oxidation in beef," *Meat Science*, vol. 72, no. 2, pp. 303-311, 2006.

[34] P. Jayasingh, D. P. Cornforth, C. P. Brennand, C. E. Carpenter, and D. R. Whittier, "Sensory evaluation of ground beef stored in high-oxygen modified atmosphere packaging," *Journal of Food Science*, vol. 67, no. 9, pp. 3493-3496, 2002.

[35] M. P. Richards, "Redox reactions of myoglobin," *Antioxidants & Redox Signaling*, vol. 18, no. 17, pp. 2342-2351, 2013.

[36] P. Gatellier, C. Hamelin, Y. Durand, and M. Renerre, "Effect of a dietary vitamin E supplementation on colour stability and lipid oxidation of air- and modified atmosphere-packaged beef," *Meat Science*, vol. 59, no. 2, pp. 133-140, 2001.

[37] Q. Liu, M. C. Lanari, and D. M. Schaefer, "A review of dietary vitamin E supplementation for improvement of beef quality," *Journal of Animal Science*, vol. 73, no. 10, pp. 3131-3140, 1995.

[38] A. Yang, M. C. Lanari, M. Brewster, and R. K. Tume, "Lipid stability and meat colour of beef from pasture- and grain-fed cattle with or without vitamin E supplement," *Meat Science*, vol. 60, no. 1, pp. 41-50, 2002.

[39] M. C. Bressan, L. V. Rossato, E. C. Rodrigues et al., "Genotype x environment interactions for fatty acid profiles in Bos indi- cus and Bos taurus finished on pasture or grain," *Journal of Animal Science*, vol. 89, no. 1, pp. 221-232, 2011.

[40] P. H. Imazaki, C. Douny, M. Elansary, M.-L. Scippo, and A. Clinquart, "Effect of muscle type, aging technique, and aging time on oxidative stability and antioxidant capacity of beef packed in high-oxygen atmosphere," *Journal of Food Processing and Preservation*, vol. 42, no. 5, article e13603, 2018.

[41] A. C. V. C. S. Canto, S. P. Suman, M. N. Nair et al., "Differential abundance of sarcoplasmic proteome explains animal effect on beef *Longissimus lumborum* color stability," *Meat Science*, vol. 102, pp. 90-98, 2015.

[42] M. N. Lund, R. Lametsch, M. S. Hviid, O. N. Jensen, and L. H. Skibsted, "High-oxygen packaging atmosphere influences protein oxidation and tenderness of porcine *longissimus dorsi* during chill storage," *Meat Science*, vol. 77, no. 3, pp. 295-303, 2007.

[43] D. Arrioja-Bretón, E. Mani-López, E. Palou, and A. López- Malo, "Antimicrobial activity and storage stability of cell- free supernatants from lactic acid bacteria and their applications with fresh beef," *Food Control*, vol. 115, article 107286, 2020.

[44] V. Pothakos, S. Samapundo, and F. Devlieghere, "Total meso- philic counts underestimate in many cases the contamination levels of psychrotrophic lactic acid bacteria (LAB) in chilled- stored food products at the end of their shelf-life," *Food Microbiology*, vol. 32, no. 2, pp. 437-443, 2012.

[45] M. Milijasevic, J. Babic, and S. Veskovic-Moracanin, "Effect of vacuum and modified atmosphere on Enterobacteriaceae count determined in rainbow trout (Oncorhynchus Mykiss) and carp (Cyprinus Carpio) steaks," Procedia Food Science, vol. 5, pp. 195-198, 2015.

[46] C. Pennacchia, D. Ercolini, and F. Villani, "Spoilage-related microbiota associated with chilled beef stored in air or vacuum pack," Food Microbiology, vol. 28, no. 1, pp. 84-93, 2011.

[47] F. Russo, D. Ercolini, G. Mauriello, and F. Villani, "Behaviour of Brochothrix thermosphacta in presence of other meat spoilage microbial groups," Food Microbiology, vol. 23, no. 8, pp. 797-802, 2006.

[48] F. Leroi, P. A. Fall, M. F. Pilet, F. Chevalier, and R. Baron, "Influence of temperature, pH and NaCl concentration on the maximal growth rate of Brochothrix thermosphacta and a bioprotective bacteria Lactococcus piscium CNCM I-4031," Food Microbiology, vol. 31, no. 2, pp. 222-228, 2012.

[49] D. Ercolini, F. Russo, E. Torrieri, P. Masi, and F. Villani, "Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions," Applied and Environmental Microbiology, vol. 72, no. 7, pp. 4663-4671, 2006.

[50] M. Motoyama, M. Kobayashi, K. Sasaki, M. Nomura, and M. Mitsumoto, "Pseudomonas spp. convert metmyoglobin into deoxymyoglobin," Meat Science, vol. 84, no. 1, pp. 202207, 2010.

[51] G. Huys, J. Leisner, and J. Björkroth, "The lesser LAB gods: Pediococcus, Leuconostoc, Weissella, Carnobacterium and affiliated genera," in Lactic Acid Bacteria: Microbiological and Functional Aspects, S. Lahtinen, A. C. Ouwehand, S. Salminen, and A. Wright, Eds., pp. 93-122, CRC Press, Boca Raton, 2012.

[52] H. C. Edima, C. Cailliez-Grimal, A.-M. Revol-Junelles, L. Tonti, M. Linder, and J.-B. Millière, "A selective enumeration medium for Carnobacterium maltaromaticum," Journal of Microbiological Methods, vol. 68, no. 3, pp. 516-521, 2007.

[53] G. M. Danielski, P. H. Imazaki, C. M. de Andrade Cavalari, G. Daube, A. Clinquart, and R. E. F. de Macedo, "Carnobacter- ium maltaromaticum as bioprotective culture in vitro and in cooked ham," Meat Science, vol. 162, article 108035, 2020.

[54] P. H. Imazaki, "Study of Microbial and Physicochemical Factors Determining the Long-Term Preservation of Chilled Fresh Beef at Subzero Temperatures (Doctoral Thesis)," University of Liège, Belgium, 2018.