



## Impact of Aging Methods and Frozen Storage on Beef Quality Attributes from Different Finishing Diets

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**Abstract:** The effects of finishing diet (pasture or grain) and meat preservation method on beef's physicochemical, microbiological, and sensory attributes were evaluated. The preservation methods assessed were dry aging in bag (DAb) and wet aging (WA) for 40 d, and then frozen storage (Fr) ([DAb+Fr] and [WA+Fr]) for 180 d. Sixty striploins (*Longissimus lumborum*) from British breed steers ( $n = 15$  from pasture and  $n = 15$  from grain-based diet) were used. Lightness ( $L^*$ ) was only affected by finishing diet where meat from grain-fed steers was lighter than those fed on pasture ( $P < 0.01$ ). DAb meat had higher pH ( $P < 0.01$ ) and lower cooking losses ( $P < 0.01$ ) than WA. DAb+Fr had the highest *Psychrotrophic* bacteria counts compared to WA+Fr, DAb and WA ( $P < 0.01$ ). DAb and DAb+Fr increased *Enterobacteriaceae* bacteria counts ( $P < 0.01$ ) compared to WA and WA+Fr. DAb+Fr samples had the lowest  $L^*$ ,  $a^*$ , and  $b^*$  values. No interaction between physicochemical characteristics (color coordinates, pH, cooking losses, and shear force) and surface microbiological load was observed ( $P > 0.05$ ). Greater polyunsaturated fatty acids (PUFA), PUFA n-3, conjugated linoleic acid (c9, t11-18:2) ( $P < 0.01$ ), and PUFA/saturated fatty acid ratio ( $P < 0.05$ ) and lower n-6:n-3 ratio ( $P < 0.01$ ) were observed in pasture- than grain-fed steers. The consumer sensory panel showed acceptable scores for all treatments, although some differences between attributes were detected by cluster analysis. Different aging methods followed by a frozen storage period could be used to produce and export meat with the required quality attributes to meet consumer expectations.

**Key words:** beef, meat preservation, dry-aging bag, finishing diet, quality attributes

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## Introduction

The beef industry's success depends on adding value to meat products, to satisfy consumer's demands. The willingness to pay for this product is inspired by several attributes, among which tenderness, juiciness, and flavor are the most relevant. Postmortem aging improves tenderness (Campbell et al., 2001; Smith et al., 2008) through the action of the endogenous proteolytic system, influencing other attributes, such as flavor and overall palatability (Kim et al., 2020), affecting meat quality and its commercial value (Kim et al., 2018; Ha et al., 2019).

There are two fundamental ways to age beef: wet and dry. Wet aging involves vacuum packing meat into a highly moisture-impermeable bag and storing it under refrigerated conditions ( $-1$  to  $2^\circ\text{C}$ ) for a specified length of time. Traditional dry aging exposes unpackaged meat directly to cooler conditions with strict temperature ( $0$ – $4^\circ\text{C}$ ), relative humidity (RH; 80%–85%), and airflow control (0.5–2 m/s). This process implies higher costs associated with decreased yields and greater weight losses during aging and trimming (Parrish et al., 1991; Warren and Kastner, 1992), but it increases the intense beefy and roasted flavors (Iida et al., 2016; Ha et al., 2019;

Li et al., 2021). In recent decades, dry aging in a highly moisture-permeable bag has been widely used. Such technology decreases trim loss and microbial contamination, thus maximizing yield (Ahnström et al., 2006). The dry-aging bag acts as an oxygen barrier with the surrounding air, limiting oxidation and its associated consequences, which include oxidative deterioration and rancidity or off-flavor (DeGeer et al., 2009; Zhang et al., 2021).

Strategies to produce stable aged quality products (wet or dry) should be considered, especially if the products are meant to be traded globally where a long period of chilled/frozen storage is usually required. The advantages of frozen meat, rather than chilled, are the increased storage time and a greater flexibility in inventory for retailers (Wheeler et al., 1990). It is thought that freezing reduces meat quality, and although research findings are not conclusive (Farouk et al., 2003; Coombs et al., 2017; Bernardo et al., 2020; Luzardo et al., 2024), consumers tend to prefer meat that has not been frozen and thawed.

The beef cattle diet is known to play a pivotal role in influencing carcass composition and eating quality attributes of meat (Peripolli et al., 2018; Correa et al., 2020). Feeding beef cattle with grain before slaughter could improve beef flavor mainly due to an increase in the deposition of intramuscular fat (IMF) compared with meat from pasture-fed animals (Brito et al., 2014). Previous research reported that the greatest difference in the flavors of meat from cattle fed on pasture or grain is due to fatty acid concentration (Realini et al., 2004) and composition as they are the primary source of aromatic compounds such as carbonyls (Melton, 1983), which plays an important role in the interaction and generation of volatile flavor compounds (Ba et al., 2016).

Since consumer assessment is the golden standard for obtaining beef quality differentiation, it is important to understand the effect of aging and refrigeration on the sensory attributes of the product and its acceptability by consumers.

Few studies relate the type of finishing diet with the meat preservation methods: types of aging and freezing process. Therefore, there remains a need to develop and assess effective storage strategies for mitigating the inconsistency of meat quality associated with the preservation process. We hypothesized that aging methods (dry bag vs. wet aging) with or without subsequent freezing affect the meat quality of steers from pasture and grain finishing systems. The main goal of this study was to evaluate the effect of meat aging methods and then frozen storage conditions on the physicochemical,

microbiological, and sensory attributes of beef from steers finished on 2 different systems (pasture or grain).

## Materials and Methods

### Raw materials and aging process

A total of 30 steers (under 30 mo of age; British breed) finished (F) in the pasture ( $n = 15$ ) qualifying for UE Hilton quota (INAC, 2013) or grain ( $n = 15$ ) qualifying for UE 481 quota (MGAP, 2023) were slaughtered in a commercial meat processing plant (hot carcass weight: 266.5 kg and 253.2 kg, respectively). Hilton quota are selected cuts of beef from steers or heifers to produce superior quality beef that will have been raised exclusively on pasture, following the Uruguayan grading system. Meanwhile, the “481” quota is beef cuts obtained from carcasses of steers and heifers under 30 mo of age, which have been fed on a diet containing not less than 62% of concentrates, at least for 100 d previous to slaughter. The steer herd came from the same farm and was selected considering age, live weight, and fat cover to set up 2 similar groups. Sixty striploins (*Longissimus lumborum* [LL]) were obtained for analysis and assigned to an aging method (dry bag or wet) and then stored under frozen conditions. The striploin from the left side of each carcass was divided into 3 sections or pieces; a first section of 7.5 cm of length was used for fresh (initial or unaged) sample analysis, a second section of 16 cm of length was vacuum packaged in dry-aging bags (DAB; TUBLIN® 10 of 50  $\mu\text{m}$  thick, polyamide mix with a water vapor transmission rate of 2.5  $\text{kg}/50 \mu\text{m}^2/24 \text{ h}$  at 38°C, 50% RH, TUB-EX ApS, Denmark), and a third section of 14 cm of length was vacuum packaged for wet-aged (WA; vacuum packaged was a barrier bag of 50  $\mu\text{m}$  thickness; maximum oxygen transmission rate of 27  $\text{cm}^3/\text{m}^2/24 \text{ h}$  at 22–24°C and 0% RH and moisture vapor transmission rate of 5  $\text{g}/\text{m}^2/24 \text{ h}$  at 38°C and 90% RH; Cryovac® Sealed Air Corp., BB 2620, Brazil). The right striploin was processed in 2 sections following the same procedure for DAB (19 cm) and WA (17 cm), and after 40 d of aging they were immediately frozen (Fr) at  $-20^\circ\text{C}$  for 180 d to determine the effect of long-term frozen storage on the quality of dry bag and wet-aged beef (DAB+Fr and WA+Fr). The location of each meat portion from each striploin was alternated in cranial to caudal direction among carcasses. The striploin’s portions were laid out on wire racks inside the chamber during an aging period of 40 d.

During aging, the chamber was set up at  $2 \pm 0.5^\circ\text{C}$  and an RH of  $85\% \pm 5\%$ . Temperature and RH were recorded using 3 dataloggers (Electronic Temperature Instruments Ltd., UK), to obtain real-time information at different points in the chamber. The air velocity was recorded weekly in different chamber positions with a digital anemometer (HoldPeak 866A digi, China) averaging 0.5 m/s. The meat portions were relocated into the chamber every 8 d to prevent any potential confounding effects of location within the chamber. After 40 d of aging, the left striploin portions (DAb and WA) were divided into steaks (2.5 cm) for different analyses. Meanwhile, the right striploin portions (DAb+Fr and WA+Fr) were divided into steaks after the periods of frozen (40 d aging + 180 d frozen) for different analyses.

### **Instrumental color**

For the determination of instrumental color (King et al., 2023), one steak per loin section, from each treatment, was measured after blooming for 45 min under simulated retail display light at  $4^\circ\text{C}$ . Frozen samples were thawed at  $4^\circ\text{C}$  for 24 h before determinations were carried out. The surface color was measured using a colorimeter (Minolta Chroma Meter CR-400; Konica Minolta Sensing Inc., Japan) fitted with an illuminant C, a standard observer of 2 grade and 8 mm of opening size and previously calibrated using a standard white tile.  $CIE L^*a^*b^*$  (Commission Internationale de l'Eclairage, 1976) color space values,  $L^*$  (lightness),  $a^*$  (redness), and  $b^*$  (yellowness), were taken per triplicate on the lean surface of each steak. Values were averaged to obtain a mean for each sample.

### **Cooking losses and Warner-Bratzler shear force**

After color assessment steaks were weighed before and after cooking to determine the cooking losses (CL) according to the American Meat Science Association (AMSA, 2016). The percentage of CL was calculated according to the following equation:  $(\text{raw weight} - \text{cooked weight}/\text{raw weight}) \times 100$ . Steaks were cooked in a grill (GRP100 The Next Grilleration, Spectrum Brands, Inc., Miami, FL) until the internal core temperature of the steak reached  $71^\circ\text{C}$ . The internal temperature was monitored using thermocouples Type T and a recording thermometer (Comark N9094, Comark Instruments Inc., UK).

Warner-Bratzler shear force (WBSF; KgF) was evaluated on 6 cores from each steak. Each core was obtained parallel to the muscle fiber orientation using

a 1.27 cm diameter hand-held coring device and sheared using a TA-XT Plus texture analyzer (Stable Micro System Ltd., UK) set with a “V” Warner Bratzler slot blade. Shear force values resulted from the average of the 6 cores per steak.

### **Fatty acid composition and thiobarbituric acid-reactive substances**

Intramuscular fat (IMF) was determined using the chloroform-methanol lipid extraction procedure described by Bligh and Dyer (1959), and then the fatty acids profile was performed. The fatty acid profile was methylated with cold methanolic potash (IUPAC, 1987) and analyzed using a gas chromatograph (Shimadzu Nexis GC 2030 Tokyo, Japan). Fatty acids were identified using a 60 m SH-Rt-WAX capillary column (0.25 mm internal diameter and 0.25  $\mu\text{m}$  thick film, Shimadzu, Columbia, Maryland, USA) where nitrogen was used as a carrier gas at 1 ml/min flow.

The injection volume was 1  $\mu\text{l}$ , and a flame ionization detector (FID) was used. The detector was kept at  $260^\circ\text{C}$ , while the injector was at  $230^\circ\text{C}$ ; the temperature ramp was  $100^\circ\text{C}$  for 0.5 min, increasing  $10^\circ\text{C}/\text{min}$  until it reached  $120^\circ\text{C} \times 2$  min, increasing  $10^\circ\text{C}/\text{min}$  until  $220^\circ\text{C} \times 15$  min, totaling 29.5 min per sample. Fatty acids were identified by comparing retention times with those of a standard mixture of 37 FAME Supelco™ 37 compounds (Sigma, St. Louis, USA); meanwhile conjugated linoleic acid (CLA;  $c9, t11-18:2$ ) was identified using octadecadienoic acid, conjugated, methyl ester standard (No. O5632, Sigma, St. Louis, USA). Fatty acids were reported in mg/100 g of meat using methyl heneicosanoate (C21:0) as an internal standard. An internal standard, 1 ml of 1 mg methyl heneicosanoate (C21:0), was added before the addition of methylating reagents.

Lipid oxidation was determined by the thiobarbituric acid-reactive substances text. (TBARS) for the modified method of Ahn et al. (1998), at 0 d after the aging process (DAb and WA for 40 d) and after the frozen process (DAb+Fr and WA+Fr for 180 d). A 5 g meat sample was placed in a 50 ml test tube and homogenized with 15 ml of deionized distilled water (DDW) by using a tissue homogenizer (Wisd, HG-15A, Daihan Scientific) for 30 s. Meat homogenate (1 ml) was transferred to a disposable test tube (13  $\times$  100 mm), and butyrate 16 hydroxyanisole (50  $\mu\text{l}$ , 7.2%) and thiobarbituric acid/trichloroacetic acid (TBA/TCA) solution (2 ml) were added. The mixture was vortexed and then incubated in a boiling water bath for 15 min for color development. After that, the samples were cooled in cold

water for 10 min and then centrifuged for 15 min at 4000 rpm. The absorbance of the resulting supernatant solution was determined at 531 nm against a blank containing 1 ml DDW and 2 ml TBA/TCA solution. Malonaldehyde (MDA) standard curves were prepared by using 1,1,3,3-tetra-ethoxypropane. The TBARS concentrations were calculated from the standard curve and expressed as milligrams of MDA per kg of meat.

### Surface microbial counts

Microorganisms from the untrimmed surface of fresh beef samples were enumerated at 0 d, after the aging period (DAb and WA for 40 d), and after the frozen (DAb+Fr and WA+Fr for 180 d) storage period. Samples were analyzed for total bacterial count (TBC), *Psychrotrophic* microorganisms (PSY), and *Enterobacteriaceae* (ENT). At each sampling time, a 4 × 4 cm square was aseptically excised from the center of 10 steaks per treatment using disposable scalpels (Feather Sterile Scalpels 2975#21; Graham-Field Inc., Atlanta, GA) and placed into individual sterile Whirl-Pak bags (710 ml, 15 × 23 cm, 0.102 mm thick; Nasco Int., USA). The 4 × 4 cm squares for microbial analysis were homogenized with 90 ml of 0.1% peptone water (Oxoid, UK), using a stomacher (BagMixer®400 P, Interscience, Saint Nom, France) for 2 min. Tenfold serial dilutions were prepared in test tubes with 9 ml of 0.1% buffered peptone water (BPW; Oxoid, UK). Appropriate dilutions were surface plated in duplicate onto 2 sets of Petrifilms (3M; USA), one set for the enumeration of mesophilic microbial populations and the second set for the enumeration of psychrophilic microorganisms. Appropriate dilutions were also duplicated onto a set of Petrifilm surfaces (3M; USA) for the ENT microbial population. Petrifilm was enumerated before incubation at 37°C for 48 h for TBC or 7°C for 10 d for PSY and 37°C 24 h for ENT.

### Consumer sensory testing

Two analyses were carried out, one on fresh and the other on thawed samples. In each study, a total of 10 sessions of 10 consumers ( $n = 100$ , each one) were carried out. The consumer pool in both trials was homogeneous since they were recruited in the same region from a database of consumers (students, staff, and campus inter-institutional members: INIA, University, MGAP, etc.) that represent the Uruguayan population demographics in terms of gender and age (Table S1, Supplementary data). Moreover, consumers were selected since they eat meat as part of their diets. In the first sensory panel, consumers evaluated 4 samples, one of each combination of

finishing system (grain and pasture) and aging type (DAb and WA). In the second panel consumers assessed 4 samples stemming from the combination of finishing system (grain and pasture) and aging type followed by a frozen period (DAb+Fr and WA+Fr).

Each consumer was asked to taste the samples following the order in each ballot, which was designed to avoid the first sample and carry-over effect (MacFie and Bratchell, 1989). Water and unsalted crackers were provided as palate cleansers. Before tasting, consumers were asked to answer a questionnaire with demographics (gender, age range, education level) and frequency in the consumption of different types of meat, as well as to sign the consent form if they agreed to participate (Table S1, Supplementary data). After that, a ballot was provided to evaluate the steak samples' tenderness liking, flavor liking, and overall liking using a 9-point scale, where 1 represented "I like it extremely," 2 "I like very much," 3 "I quite like it," 4 "I like it," 5 "I neither like nor dislike," 6 "I dislike it," 7 "I quite dislike it," 8 "I dislike very much," and 9 "I dislike it extremely."

The day before the test, the steaks to be evaluated (4 per session, 1 for each treatment) were thawed at a 4°C chiller overnight. Before serving to consumers, samples were cooked in a grill (GRP100 The Next Grilleration, Spectrum Brands, Inc., Miami, FL) until the core (internal) temperature of steak reached 71°C (AMSA, 2016). Once cooked, steaks were trimmed of external fat and connective tissue and cut across the grain into a 1.3 × 1.3 × 2.0 cm piece, wrapped individually in coded aluminum foil, assigned to a cup, and kept warm in a heater/oven at 49°C for no more than 10 min until being tasted. The procedures used for consumer sensory evaluation in this study were approved by the Institute of Agrifood Research and Technology (IRTA) Ethics Committee, with the internal code CCSC 33/2023.

### Statistical analysis

Data analysis was carried out using the SAS software v. 9.4 (SAS Institute Inc., Cary, NC, USA). All data were screened for normality using the UNIVARIATE procedure and normalized as required using a  $\log_{10}$  transformation, but estimates have been back-transformed to the response scale.

The experimental design was a split-plot, where each finishing diet served as a main plot (F: pasture or grain), and carcass sides (pair of loins) served as sub-plot for the preservation methods (PM: DAb, WA, DAb+Fr and WA+Fr). The statistical analysis was performed with a model including the fixed effects of F and PM, their interactions, and the random effect

of the carcass using the MIXED procedure. The least-squares mean was calculated, and means separation was performed ( $P < 0.05$ ) using the PDIFF option. Peak cooking temperature was used as a covariable for WBSF and CL analysis.

Consumer tenderness liking, flavor liking, and overall liking scores were evaluated using the MIXED procedure of SAS. The 2 trials were considered together because, although there could be confusion between consumer population and treatment (fresh vs. frozen), the consumer population in both trials was homogeneous (Table S1, Supplementary data); consequently, it was assumed to be similar, allowing to take advantage of the full analysis. The model included the PM and F as fixed effects and their interaction. Consumers were considered as random effects in the model. A tasting session was included as a blocking factor. A segmentation by CLUSTER was carried out to find groups of consumers with similar preferences, since when considered as a pool, differences are diluted, and they are difficult to determine. Segmentation was performed by using the CLUSTER procedure applying Euclidian distance and the Ward method. The number of clusters to retain was based on the obtained dendrogram, considering the homogeneity within and among the segments and the principle of parsimony. An analysis of variance was carried out, considering PM and F as fixed effects and their interaction, for the pooled sample and by cluster. A Tukey test was applied to find differences between least-squares means. The significance level was set at  $P < 0.05$ .

## Results

### *Effects of preservation method and finishing system on instrumental color, pH, cooking losses, and shear force*

The interaction was not significant for any of these parameters; thus, results are presented independently

by effects. Lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) were affected by PM (Table 1). Lightness and  $a^*$  were significantly greater in WA followed by DAb, DAb+Fr, and WA+Fr samples ( $P < 0.01$ ). No difference in  $b^*$  values was detected between DAb and WA samples ( $P > 0.05$ ); lower values were observed in WA+Fr and DAb+Fr (the lowest values) ( $P < 0.01$ ). Meat color from grain-fed steers resulted in greater  $L^*$  values than pasture-fed animals ( $P < 0.01$ ). The pH was affected by the aging method; DAb and DAb+Fr samples presented greater pH values than WA and WA+Fr samples ( $P < 0.01$ ). However, it is worth mentioning that in all treatments, the pH was below 5.8. The higher percentage of CL was WA followed by WA+Fr, DAb, and DAb+Fr ( $P < 0.01$ ). Wet-aged and DAb samples presented lower WBSF values than WA+Fr and DAb+Fr samples ( $P < 0.021$ ). The finishing diet of the steers had no effect ( $P > 0.05$ ) on pH, CL, or WBSF (Table 1).

### *Effects of preservation method and finishing system on fatty acid profile and oxidation*

The initial (unaged) IMF content of LL from pasture and grain finishing diets was 3.48% and 3.63% (no tabulated data), respectively, which were not significantly different ( $P > 0.05$ ). However, after PM treatment, DAb+Fr showed a greater IMF than the other 3 treatments ( $P < 0.01$ ) (Table 2). Predominant fatty acids profile was analyzed and reported in Supplementary data (Table S2). Analyzing the fatty acid composition by effect (PM and F) did not affect any of the mentioned fatty acid groups and ratios ( $P > 0.05$ ) (Table 2). A greater concentration of polyunsaturated fatty acid (PUFA), PUFA n-3, CLA (c9, t11-18:2) ( $P < 0.01$ ), and PUFA/saturated fatty acid (SFA) ratio ( $P = 0.028$ ) was observed in pasture compared with grain-fed steers (Table 2). The *n-6:n-3* fatty acids ratio was greater in grain-fed compared to pasture-fed steers ( $P < 0.01$ ). A significant interaction effect (PM\*F)

**Table 1.** Effects (mean  $\pm$  SEM) of preservation method (PM) and finishing diet (F) on meat quality parameters

Traits	PM				<i>P</i> value	F		<i>P</i> value
	DAb	WA	DAb+Fr	WA+Fr		Pasture	Grain	
$L^*$	40.5 $\pm$ 0.4 <sup>b</sup>	41.8 $\pm$ 0.4 <sup>a</sup>	38.2 $\pm$ 0.4 <sup>d</sup>	39.7 $\pm$ 0.4 <sup>c</sup>	<0.001	38.8 $\pm$ 0.4	41.3 $\pm$ 0.4	<0.001
$a^*$	22.2 $\pm$ 0.4 <sup>b</sup>	24.0 $\pm$ 0.4 <sup>a</sup>	17.2 $\pm$ 0.4 <sup>d</sup>	19.5 $\pm$ 0.4 <sup>c</sup>	<0.001	20.7 $\pm$ 0.3	20.8 $\pm$ 0.3	0.783
$b^*$	11.8 $\pm$ 0.2 <sup>a</sup>	11.9 $\pm$ 0.2 <sup>a</sup>	10.0 $\pm$ 0.2 <sup>c</sup>	11.1 $\pm$ 0.2 <sup>b</sup>	<0.001	11.1 $\pm$ 0.1	11.3 $\pm$ 0.1	0.424
pH	5.76 $\pm$ 0.01 <sup>a</sup>	5.73 $\pm$ 0.01 <sup>b</sup>	5.77 $\pm$ 0.01 <sup>a</sup>	5.73 $\pm$ 0.01 <sup>b</sup>	<0.001	5.73 $\pm$ 0.02	5.76 $\pm$ 0.02	0.294
CL (%)	17.7 $\pm$ 0.4 <sup>c</sup>	23.0 $\pm$ 0.4 <sup>a</sup>	15.2 $\pm$ 0.4 <sup>d</sup>	21.4 $\pm$ 0.4 <sup>b</sup>	<0.001	19.5 $\pm$ 0.3	19.3 $\pm$ 0.3	0.602
WBSF (kgF)	2.6 $\pm$ 0.09 <sup>b</sup>	2.5 $\pm$ 0.09 <sup>b</sup>	2.8 $\pm$ 0.09 <sup>a</sup>	2.8 $\pm$ 0.09 <sup>a</sup>	0.021	2.8 $\pm$ 0.09	2.6 $\pm$ 0.09	0.093

DAb: Dry aging bag; WA: Wet aging; DAb+Fr; Dry aging bag + 180 d frozen; WA+Fr; Wet aging + 180 d frozen; CL: cooking losses; WBSF: Warner Brazler shear force. Different letters in the same row denote groups statistically different ( $P < 0.05$ ) among LSMeans.

**Table 2.** Effects (mean ± SEM) of preservation method (PM) and finishing diet (F) on intramuscular fat and content fatty acid composition

Traits	PM				P value	F		
	DAb	WA	DAb+Fr	WA+Fr		Pasture	Grain	P value
IMF (%)	3.9 ± 0.2 <sup>b</sup>	3.7 ± 0.2 <sup>b</sup>	4.5 ± 0.2 <sup>a</sup>	3.8 ± 0.2 <sup>b</sup>	<0.001	3.7 ± 0.2	4.3 ± 0.2	0.094
CLA (mg/100 g meat)	21.2 ± 1.9	23.0 ± 1.8	19.7 ± 1.9	20.6 ± 1.8	0.494	26.9 ± 1.9	15.3 ± 1.9	<0.001
MUFA (mg/100 g meat)	1826.5 ± 111.5	1774.9 ± 111.5	1633.5 ± 118.0	1702.4 ± 111.5	0.596	1632.1 ± 95.0	1836.5 ± 97.0	0.136
SFA (mg/100 g meat)	1902.7 ± 126.5	2003.0 ± 122.2	1938 ± 126.5	2024 ± 120.4	0.835	1925.0 ± 119.2	2009.4 ± 118.8	0.617
PUFA (mg/100 g meat)	253.8 ± 15.3	239.2 ± 14.4	216.2 ± 13.8	246.3 ± 14.9	0.304	268.9 ± 11.5	211.5 ± 9.3	<0.001
PUFA n-6 (mg/100 g meat)	183.4 ± 11.8	173.6 ± 10.9	160.3 ± 10.7	181.7 ± 11.5	0.419	179.2 ± 9.0	169.2 ± 8.7	0.460
PUFA n-3 (mg/100 g meat)	53.1 ± 3.8	58.3 ± 4.0	51.0 ± 3.6	58.4 ± 4.0	0.208	78.8 ± 5.7	38.6 ± 2.8	<0.001
n-6:n-3	3.1 ± 0.17	2.9 ± 0.16	3.1 ± 0.17	3.1 ± 0.17	0.049	2.1 ± 0.15	4.5 ± 0.34	<0.001
PUFA/SFA	0.12 ± 0.007	0.12 ± 0.007	0.12 ± 0.007	0.12 ± 0.007	0.570	0.14 ± 0.01	0.11 ± 0.008	0.028

DAb: Dry aging bag; WA: Wet aging; DAb+Fr; Dry aging bag + 180 d frozen; WA+Fr; Wet aging + 180 d frozen; CLA: conjugated linoleic fatty acid (c9, t11-18:2); PUFA: sum of polyunsaturated fatty acid (PUFA n-6 + PUFA n-3); MUFA: monounsaturated fatty acid (C14:1 + C16:1 + C18:1n9); SFA: saturated fatty acid (C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0); PUFA n-3 (C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3); PUFA n-6 (C18:2n-6 + C18:3n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6); IMF: intramuscular fat. Different letters in the same row denote groups statistically different ( $P < 0.05$ ) among LSMs.

was detected on C20:0 and C22:0 fatty acids and in lipid oxidation evaluated through TBARS. Greater concentrations of TBARS were observed in DAb and WA from grain-fed and DAb from pasture-fed than other treatments. The lowest concentration was in WA+Fr from pasture-fed ( $P < 0.01$ ) (Table 3).

### Effects of preservation method and finishing system on surface microbial counts

Meat samples analyzed before aging presented surface microbial count below the limit of detection ( $< 2 \log/\text{cm}^2$ ). The preservation method did not affect the TBC number, and the range was from 3.86 to 4.41 log CFU/cm<sup>2</sup> ( $P > 0.05$ ). However, PSY bacteria counts were greater in aged frozen meat than in samples only aged ( $P < 0.01$ ). Lower ENT numbers were observed in WA and WA+Fr compared to DAb and DAb+Fr ( $P < 0.01$ ). In addition, meat from grain-fed

steers presented a greater ENT count than that from pasture-fed steers ( $P < 0.05$ ) (Table 4).

### Effects of preservation method and finishing system on consumer sensory panel

Meat from grain-fed steers was preferred in terms of overall liking, flavor liking, and tenderness liking than pasture-finished animals when all the consumers were considered ( $P < 0.01$ ) (Table 5). There was an interaction effect between the preservation method and finishing system on tenderness liking, considering all the consumers ( $n = 200$ ), where grain-fed were found more tender than pasture-fed steers ( $P = 0.031$ ). All the consumers were segmented into 3 clusters depending on their acceptability scores. Cluster 1 ( $n = 77$ ) presented a PM\*F interaction for both overall liking ( $P < 0.01$ ) and flavor liking ( $P < 0.01$ ). The less preferred samples were DAb and DAb+Fr from

**Table 3.** Effects (mean ± SEM) of the interaction between the preservation method (PM) and the finishing diet (F) on the arachidic (C20:0), behenic (C22:0), and thiobarbituric acid-reactive substances (TBARS) concentrations

Traits	Pasture				Grain				Significance		
	DAb	WA	DAb+Fr	WA+Fr	DAb	WA	DAb+Fr	WA+Fr	PM	F	PM*F
C20:0 (mg/100 g)	11.9 ± 1.7 <sup>a</sup>	13.4 ± 1.9 <sup>a</sup>	6.2 ± 0.9 <sup>b</sup>	11.8 ± 1.7 <sup>a</sup>	3.9 ± 0.5 <sup>b</sup>	4.5 ± 0.6 <sup>b</sup>	4.3 ± 0.7 <sup>b</sup>	4.3 ± 0.6 <sup>b</sup>	0.013	<0.001	0.014
C22:0 (mg/100 g)	4.4 ± 0.5 <sup>a</sup>	5.1 ± 0.5 <sup>a</sup>	2.6 ± 0.3 <sup>b</sup>	2.9 ± 0.3 <sup>b</sup>	2.7 ± 2.3 <sup>b</sup>	3.0 ± 0.3 <sup>b</sup>	2.9 ± 0.3 <sup>b</sup>	3.0 ± 0.3 <sup>b</sup>	0.012	0.024	<0.001
TBARS (mg/kg)	0.391 ± 0.04 <sup>a</sup>	0.260 ± 0.02 <sup>b</sup>	0.293 ± 0.03 <sup>b</sup>	0.133 ± 0.01 <sup>c</sup>	0.379 ± 0.03 <sup>a</sup>	0.479 ± 0.04 <sup>a</sup>	0.254 ± 0.02 <sup>b</sup>	0.307 ± 0.03 <sup>b</sup>	<0.001	<0.001	0.001

DAb: Dry aging bag; WA: Wet aging; DAb+Fr; Dry aging bag + 180 d frozen; WA+Fr; Wet aging + 180 d frozen; TBARS: thiobarbituric acid-reactive substances (mg MDA/kg meat). Different letters in the same row denote groups statistically different ( $P < 0.05$ ) among LSMs.

**Table 4.** Effects (mean ± SEM) of preservation method (PM) and finishing diet (F) on microbiological growth

Traits	Initial	PM				P value	F		P value
		DAb	WA	DAb+Fr	WA+Fr		Pasture	Grain	
TBC (log10/cm <sup>2</sup> )	<1.0	4.35 ± 0.2	3.86 ± 0.2	4.19 ± 0.2	4.41 ± 0.2	0.137	4.22 ± 0.2	4.19 ± 0.2	0.890
PSY (log10/cm <sup>2</sup> )	<1.0	5.38 ± 0.13 <sup>b</sup>	4.96 ± 0.13 <sup>c</sup>	6.15 ± 0.14 <sup>a</sup>	5.52 ± 0.13 <sup>b</sup>	<0.001	5.55 ± 0.13	5.45 ± 0.13	0.573
ENT (log10/cm <sup>2</sup> )	<1.0	3.33 ± 0.2 <sup>a</sup>	2.72 ± 0.2 <sup>ab</sup>	3.40 ± 0.2 <sup>a</sup>	2.54 ± 0.2 <sup>b</sup>	0.002	2.86 ± 0.15	3.14 ± 0.15	0.020

DAb: Dry aging bag; WA: Wet aging; DAb+Fr: Dry aging bag + 180 d frozen; WA+Fr: Wet aging + 180 d frozen; TBC: total bacterial count; PSY: Psychotropic bacteria; ENT: Enterobacteriaceae bacteria. Different letters in the same row denote groups statistically different ( $P < 0.05$ ) among LSMs.

**Table 5.** Effect (mean ± SEM) of overall liking, tenderness, and flavor acceptability scores by consumers as a whole and segmented in clusters depending on the meat preservation method (PM) and the finishing diet (F) of steers

	Pasture				Grain				Significance		
	DAb	WA	DAb+Fr	WA+Fr	DAb	WA	DAb+Fr	WA+Fr	PM	F	PM*F
<b>Overall liking</b>											
All consumers	3.7 ± 0.14	3.7 ± 0.14	3.6 ± 0.14	3.7 ± 0.14	3.5 ± 0.14	3.1 ± 0.14	3.3 ± 0.14	3.3 ± 0.14	0.444	<0.001	0.196
Cluster 1	4.4 ± 0.17 <sup>a</sup>	3.6 ± 0.17 <sup>b</sup>	4.5 ± 0.15 <sup>a</sup>	3.5 ± 0.15 <sup>b</sup>	3.2 ± 0.17 <sup>c</sup>	3.3 ± 0.17 <sup>bc</sup>	3.2 ± 0.15 <sup>c</sup>	3.7 ± 0.15 <sup>b</sup>	0.048	<0.001	<0.001
Cluster 2	4.4 ± 0.26 <sup>c</sup>	5.5 ± 0.26 <sup>a</sup>	4.1 ± 0.31 <sup>c</sup>	5.4 ± 0.31 <sup>a</sup>	4.9 ± 0.26 <sup>b</sup>	4.1 ± 0.26 <sup>c</sup>	4.9 ± 0.31 <sup>b</sup>	4.7 ± 0.31 <sup>b</sup>	0.316	0.307	<0.001
Cluster 3	2.6 ± 0.17 <sup>b</sup>	2.8 ± 0.17 <sup>b</sup>	2.4 ± 0.18 <sup>c</sup>	3.2 ± 0.18 <sup>a</sup>	2.9 ± 0.17 <sup>b</sup>	2.3 ± 0.17 <sup>c</sup>	2.8 ± 0.18 <sup>b</sup>	2.4 ± 0.18 <sup>c</sup>	0.487	0.255	<0.001
<b>Tenderness</b>											
All consumers	3.3 ± 0.14 <sup>a</sup>	3.6 ± 0.14 <sup>a</sup>	3.2 ± 0.14 <sup>a</sup>	3.3 ± 0.14 <sup>a</sup>	2.7 ± 0.14 <sup>b</sup>	2.7 ± 0.14 <sup>b</sup>	2.9 ± 0.14 <sup>b</sup>	2.7 ± 0.14 <sup>b</sup>	0.740	<0.001	0.031
Cluster 1	3.9 ± 0.22	3.7 ± 0.22	3.9 ± 0.19	3.3 ± 0.19	2.7 ± 0.22	2.9 ± 0.22	2.8 ± 0.19	2.8 ± 0.19	0.470	<0.001	0.158
Cluster 2	3.8 ± 0.31 <sup>b</sup>	5.0 ± 0.31 <sup>a</sup>	3.7 ± 0.36 <sup>b</sup>	4.4 ± 0.36 <sup>a</sup>	3.3 ± 0.31 <sup>b</sup>	3.3 ± 0.31 <sup>b</sup>	3.7 ± 0.36 <sup>b</sup>	3.8 ± 0.36 <sup>b</sup>	0.062	0.001	0.023
Cluster 3	2.5 ± 0.18 <sup>a</sup>	2.7 ± 0.18 <sup>a</sup>	2.2 ± 0.19 <sup>b</sup>	2.8 ± 0.19 <sup>a</sup>	2.5 ± 0.18 <sup>a</sup>	2.0 ± 0.18 <sup>b</sup>	2.7 ± 0.19 <sup>a</sup>	2.1 ± 0.19 <sup>b</sup>	0.961	0.069	<0.001
<b>Flavor</b>											
All consumers	3.7 ± 0.14	3.7 ± 0.14	3.7 ± 0.14	3.7 ± 0.14	3.5 ± 0.14	3.3 ± 0.14	3.4 ± 0.14	3.4 ± 0.14	0.735	0.001	0.923
Cluster 1	4.4 ± 0.19 <sup>a</sup>	3.6 ± 0.19 <sup>b</sup>	4.6 ± 0.17 <sup>a</sup>	3.4 ± 0.17 <sup>b</sup>	3.2 ± 0.19 <sup>b</sup>	3.4 ± 0.19 <sup>b</sup>	3.4 ± 0.17 <sup>b</sup>	3.7 ± 0.17 <sup>b</sup>	0.028	<0.001	<0.001
Cluster 2	4.3 ± 0.30 <sup>b</sup>	5.2 ± 0.30 <sup>a</sup>	4.0 ± 0.35 <sup>b</sup>	5.1 ± 0.35 <sup>a</sup>	4.8 ± 0.30 <sup>a</sup>	4.4 ± 0.30 <sup>b</sup>	4.5 ± 0.35 <sup>b</sup>	4.4 ± 0.35 <sup>b</sup>	0.334	0.600	0.033
Cluster 3	2.8 ± 0.18 <sup>ba</sup>	2.8 ± 0.18 <sup>ba</sup>	2.6 ± 0.19 <sup>b</sup>	3.4 ± 0.19 <sup>a</sup>	3.0 ± 0.18 <sup>a</sup>	2.5 ± 0.18 <sup>b</sup>	2.9 ± 0.19 <sup>ab</sup>	2.6 ± 0.19 <sup>b</sup>	0.184	0.365	0.006

DAb: Dry aging bag; WA: Wet aging; DAb+Fr: Dry aging bag + 180 d frozen; WA+Fr: Wet aging + 180 d frozen. Scale 9 points: 1 “I like it extremely,” 2 “I like very much,” 3 “I quite like it,” 4 “I like it,” 5 “I neither like nor dislike,” 6 “I dislike it,” 7 “I quite dislike it,” 8 “I dislike very much,” and 9 “I dislike it extremely.” Different letters in the same row denote groups statistically different ( $P < 0.05$ ) among LSMs.

pasture-fed steers. Thus, Cluster 1 could be characterized by a higher preference for grain-fed steers independently of the preservation method, and within pasture-fed beef, consumers preferred meat from WA (either fresh or frozen) in terms of overall and flavor liking. They could be named “Grain-fed aged beef and pasture-fed WA beef likers.” Cluster 2 consumers ( $n = 43$ ) presented a significant PM\*F interaction for overall liking ( $P < 0.01$ ), tenderness liking ( $P = 0.023$ ), and flavor ( $P = 0.033$ ). Like Cluster 1, consumers’ preference for the 3 attributes was greater in grain-fed samples, but regarding pasture-fed beef, their preference was mainly for DAb. However, consumers from Cluster 2 have scores closer to “neither like nor dislike”; thus, they were more hesitant in making a decision. They could be named “Grain-fed aged beef and pasture-fed DAb beef likers.” On the other side, Cluster 3 ( $n = 80$ ) also presented PM\*F for all the attributes evaluated ( $P < 0.01$ ). Consumers preferred

( $P < 0.05$ ) WA and WA+Fr from grain-fed beef while DAb+Fr from pasture-fed animals were also preferred for overall liking. Regardless of these differences, consumers from Cluster 3 scored all the meat samples at good levels, between 2 to 3 on the scale; thus, they can be named “All types of beef likers.” Regarding sociodemographic characteristics, no important differences have been found among clusters (Table S1, Supplementary data).

## Discussion

### *Effects of preservation method and finishing system on instrumental color, pH, cooking losses, and shear force*

Meat color is widely used by consumers to determine the freshness and wholesomeness of meat

products (Lee et al., 2013). It is the single most important characteristic influencing consumers' purchase decisions even though preferences are variable among consumers (Realini et al., 2014; Altman et al., 2022). In line with Dikeman et al. (2013) and Gudjónsdóttir et al. (2015), WA meat was lighter (greater  $L^*$  values) and less red (lower  $a^*$  values) than DAb after 40 d aging and after aging and then frozen storage for 180 d. The lower lightness of DAb could be attributed to the moisture loss on the meat surface, resulting in more light absorption and darker color (Kim et al., 2011). However, Li et al. (2014) did not find any effect of the aging method (dry, dry bag, and wet) on instrumental color values. Bernardo et al. (2020) did not find differences between traditional dry aging (chilled condition) and meat aging and then frozen conditions in the first 5 d of retail display in  $L^*$  (32.7 vs. 30.8, respectively) values. However,  $a^*$  (20.8 vs. 16.6) and  $b^*$  values (17.4 vs. 14.4) were lower in meat that was frozen compared to chilled striploin samples.

Meat from cattle raised on pasture is reported to be darker than meat from grain-finished animals measured by both objective and subjective methods (Vestergaard et al., 2000; Priolo et al., 2001; Gatellier et al., 2005). In our study, lower  $L^*$  (lightness) values were observed in meat from pasture-fed steers. We hypothesize that a greater myoglobin concentration in pasture-fed animals would be responsible for less lightness of the meat since the final pH and IMF fat content did not differ between both finishing systems. Muscles from grass-fed cattle have more myoglobin (more muscle activity), perhaps making them darker in appearance, and have greater mitochondrial-based oxidative enzyme content, less glycolytic enzymes, and when subjected to an *in vitro* glycolysis system produce less lactate (Apaoblaza et al., 2020).

The increase of pH after 40 d aging compared to the initial value (data not shown) was more noticeable in Dab than WA, in both chilled and chilled and then frozen samples but, in any case, greater than 5.8 value. These results agreed with Zhang et al. (2019), who reported an increase in pH after 21 d of DAb beef. Other studies (Dikeman et al., 2013; Li et al., 2014; Obuz et al., 2014; Kim et al., 2017) indicated that pH increased in DAb and decreased in WA between 20 d and 40 d of aging. Later authors indicated that this increase of pH after DAb could be associated with the generation of nitrogenous compounds products from proteolysis; meanwhile, the lower pH in WA would be caused by the greater accumulation of lactic acid.

Cooking losses decreased in DAb compared to WA, and this effect was in greater magnitude after

frozen storage (DAb+Fr vs. WA+Fr), possibly due to the important amount of moisture loss by evaporation during the dry-aging process (Juárez et al., 2011; Zhang et al., 2019). In addition, freezing also reduces meat water-holding capacity due to muscle fiber disruption by ice crystal formation (Leygonie et al., 2012).

Several studies have indicated no differences in WBSF due to the aging methods (WA vs. DA and DAb) (Ahnström et al., 2006; Dikeman et al., 2013; Berger et al., 2018). All of them have an aging time ranging from 21 d to 28 d. However, Kahraman and Gürbüz (2019) reported greater WBSF values in DAb (3.77 kgF) than WA (3.27 kgF) in meat aged for 21 d and pointed out that striploin WBSF decreased as the aging time increased. Other studies observed a decrease in WBSF in dry aging (2.66 kgF) compared to stepwise aging (2.94 kgF) for 17 d (Kim et al., 2017). In our study, WA and DAb meat presented no difference between them in WBSF, and the values were lower than frozen samples; however, all of them were below 3 kgF, a tenderness threshold for consumer beef acceptability (Miller et al., 2001).

### ***Effects of preservation method and finishing system on fatty acid profile and oxidation***

Animal diet effect on fatty acid composition is demonstrated, e.g., animals fed in grass have higher omega 3 (n-3) fatty acids (Daley et al., 2010). The UK Department of Health (1994) recommended intakes of fatty acids with an  $n6:n3$  ratio  $\leq 4$ , which were reached in meat from pasture-fed steers and agreed with previous studies (Nuernberg et al., 2005; Brito et al., 2014). In the present study, the fatty acid profile (Supplementary Table S2) showed greater contents of myristic (C14:0), myristoleic (C14:1), palmitoleic (C16:1), oleic (C18:1), eicosadienoic (C20:2), and eicosatrienoic (C20:3) acids in the IMF of grain than pasture-fed steers. Pasture-fed beef cattle had greater contents of linolenic (C18:3), arachidic (C20:0), eicosapentaenoic (C20:5, EPA), and docosapentaenoic (C22:5, DPA) acids than grain-fed cattle. This information has been widely reported in previous studies (Realini et al., 2004; Ponnampalam et al., 2006; Jiang et al., 2010) where greater contents of stearic (C18:0), linolenic (18:3), and arachidonic (C20:4) acids were observed in pasture-fed than grain-fed animals.

Preservation methods increase IMF (%) in DAb+Fr, possibly due to water loss by evaporation during aging and frozen storage, which could also be associated with the lowest CL (%) (Zhang et al., 2019). However, the PM did not show an effect on most



fatty acids identified, except for C20:0 and C22:0, which presented the highest values in samples from pasture diet and during the aging process (DAb and WA). Berger (2017) reported no significant differences in fatty acid profile between aging methods (wet, dry, and dry-bag aging) except for DPA (22:5n-3), which showed a greater concentration in DAb than the other 2 aging methods. The author did not have a clear explanation of how and why only affected the greater content of DPA of beef samples compared to other aging methods in the current study. Jiang et al. (2010), working with ground beef, reported a greater concentration of C20:1n9 in un-aged samples than in dry-aged ones.

Lipid oxidation is a major cause of quality deterioration in meat and meat products. It leads to increase “rancidity” resulting in undesirable odors and flavors (Wang et al., 1997). In our study, a significant interaction between the preservation method and the finishing system was detected. Greater oxidative stability of lipids (lower TBARS values) was observed in frozen stored beef regardless of the diet, except for WA from pasture-fed steers that had similar values to the other treatments. The lowest value was in WA+Fr from pasture-fed steers. Similar results were reported in other studies on beef comparing aged and frozen meat: 0.33 vs. 0.23 mg MDA/kg meat (Zhang et al., 2021; dry-aging bag vs. stepwise [dry bag/wet] aging [21 d] and then 12 mo of frozen) and 0.24 vs. 0.25 mg MDA/kg meat (Bernardo et al., 2020; dry aged for 28 d vs. aged and then frozen for 1 mo), respectively. During storage, MDA may further degrade into organic alcohols and acids, or attach to free amino acids and proteins as MDA- amino-acids complex (Farouk et al., 2003), and these changes cannot be detected using the TBARS assay. Therefore, the variation during aging and frozen storage observed in the current studies could have resulted from different reaction rates between generation and degradation of MDA during storage. Differences observed in aging methods agree with previous findings that indicated less oxidation in dry-aged loin steaks in bags compared with traditional dry aging (DeGeer et al., 2009), which would suggest a protective effect of dry aging in bags from the oxidative deterioration (Zhang et al., 2021). It has been shown that animals fed on pasture have greater concentrations of vitamin E in muscle than those grain-fed (Realini et al., 2004; Nuernberg et al., 2005; Descalzo et al., 2007; Daley et al., 2010; Bernardo et al., 2020), which delays the lipid oxidation and metmyoglobin formation (Schwarz et al., 1998; Zerby et al., 1999; Descalzo and Sancho, 2008). The range of TBARS (0.133–0.479 mg

MDA/kg meat) found in this study was lower than the threshold (2 mg MDA/kg meat) for the detection of rancid flavor by trained panelists (Campo et al., 2006).

### ***Effects of preservation method and finishing system on superficial microbial counts***

Samples analyzed before aging (initial load) showed <1 log CFU/cm<sup>2</sup> for TBC and ENT. Dry-aging bag and DAb+Fr increased ENT compared with WA after the frozen storage period. Li et al. (2013) did not find an impact of aging treatment on ENT counts when comparing DAb and WA for 14 d, the same as in our study when the meat was aged for 40 d. Similar results were reported by Hulánková et al. (2018) and Ahnström et al. (2006) in dry-aging meat for 14 d. Counts of PSY are particularly relevant for products that are kept under chilling conditions since these microorganisms can still multiply (González-Gutiérrez et al., 2020). Psychotropic bacteria increased after the frozen period regardless of the aging method. This could be explained by the conditions for microbial growth that may occur during meat thawing, due to cell disruption and destruction of muscle fibers caused by freezing (Choe et al., 2016), and with the temperature increment, the exudate releases creating an ideal environment for microbial growth (Gill, 2014). Griffiths et al. (1981) indicated that levels of 6 to 8 log CFU/g of microorganisms are sufficient to produce off-odors and appearance defects in meat. In this sense, Borch et al. (1996) reported that the retail shelf-life of meat is estimated as the time required by the bacterial population to reach a level of 10<sup>7</sup> CFU/cm<sup>2</sup>. Stanbridge and Davies (1998) also state that levels of PSY over 7 to 8 log/cm<sup>2</sup> trigger strange smells and surface sliminess in meat. In our study, the PSY numbers were less than 6.5 log CFU/cm<sup>2</sup>, not affecting meat quality attributes. However, an off-flavor, which is a result of spoilage in meat, can be detected when the TBC is around 7 log CFU/cm<sup>2</sup> or g of meat product, although some negative changes can be observed much earlier with TBC numbers between 5 and 6 log CFU/cm<sup>2</sup> or g of meat product (Feiner, 2006). In this study, there was no effect of the aging method on TBC, suggesting that both methods are equally suitable for meat conditioning.

Some works have reported no significant differences in microbial counts between grain- and pasture-fed beef (Casas et al., 2021; Duarte et al., 2022), just like in our study for TBC and PSY bacteria counts. However, greater ENT counts were observed in meat from grain than in pasture-fed steers. It has been stated that high-grain diets can decrease ruminal pH, favoring the growth of acid-tolerant bacteria

(Diez-Gonzalez et al., 1998), e.g., *E. coli* O157:H7, a semi-acid-resistant pathogen that belongs to the ENT family. Nevertheless, Zhang et al. (2010) reported no differences in bacterial contamination between beef from grass- and grain-fed cattle. These authors also stated that other aspects than diet may play an important role in microbial contamination of meat such as how beef is processed.

### **Effects of preservation method and finishing system on consumer sensory panel**

Consumer preferences are very variable and dependent on an array of different factors (Font-i-Furnols and Guerrero, 2014). Thus, it is very common to find segments of consumers with different preferences, and it is important to identify them.

The sensory results showed that consumers from Cluster 1, i.e. “Grain-fed aged beef and pasture-fed WA beef likers,” preferred aged meat from grain-fed animals, especially those from DAb aging, as well as WA meat from pasture-fed animals for overall liking and flavor liking. The last combination (WA from pasture-fed) is the most common meat consumed in Uruguay and is possibly recognized and more accepted by this consumer group. Wet-aged beef from fresh samples presented lower aging odor and flavor, higher herb odor, lower hardness, and higher juiciness in mouth texture than DAb-aged beef (Panella-Riera et al., 2023), and this can be related to consumer preferences for beef meat. On the other hand, Realini et al. (2009) found that, for a segment of consumers, meat from beef fed on a combination of concentrate and pasture was preferred to those only from pasture-fed animals. Similar results were found in lamb by Font i Furnols et al. (2006).

Consumers from Cluster 2, i.e., “Grain-fed aged beef and pasture-fed DAb beef likers,” are similar to those of Cluster 1 in the sense that they liked pasture-fed beef, but are different from consumers of Cluster 1 in the sense that they preferred DAb (fresh or frozen) meat from pasture-fed steers instead of WA (fresh and frozen) samples from grain-fed steers, which had the highest scores (less like). Differences in preferences for the different sensory characteristics of meat (Panella-Riera et al., 2023) can explain these differences in acceptability.

In both cases, i.e. Cluster 1 and 2, results are surprising since Uruguayan consumers are used to eating beef from pasture-fed animals, and the habits greatly affect preferences (Font i Furnols et al., 2006; Font-i-Furnols and Guerrero, 2014). In fresh pasture-fed beef samples, both WA and DAb presented significantly

higher abnormal and herb odors (Panella-Riera et al., 2023) that might have influenced consumer acceptance. Moreover, in non-aged beef samples, those from pasture had a higher beef odor and flavor intensity and higher mouth tenderness than those from concentrate plus hay-fed animals (Resconi et al., 2010), which also can influence consumer acceptability. A cluster with similar preferences as this one was found by Realini et al. (2009). Studies have reported that grain-fed cattle produce greater IMF in meat (Schroeder et al., 1980; Hedrick et al., 1983), and the dry-aging process requires beef with a high content of IMF to help ensure products with consistent tenderness, flavor, and juiciness (Nishimura, 1998; Dashdorj et al., 2016). However, in this study, no differences in IMF were found for the F (grain vs. pasture) effect. The tenderness liking attribute tended to be best classified by this Cluster regarding the preservation methods in samples from grain-fed. This is in concordance with shear force values (<2.95 kgF) found (Table 1).

Cluster 3, i.e. “all types of beef likers,” is characterized by scoring all the samples close to “I like very much.” They did not discriminate between the different treatments; they all liked it equally. Debate about consumer preference and acceptability of dry, wet, and dry-bag aging is ongoing. In concordance with our study, Berger et al. (2018), in samples from 100% grass-fed heifers, indicated that overall liking scores were not different across aging treatments (wet, dry, and dry bag). However, the same consumer panel identified beef aged in dry-aging bags having higher tenderness and overall preference compared to the beef aged in a typical vacuum bag, indicating that dry aging in bags could be the preferable aging process in steak from pasture-fed, like our Cluster 1, in overall and flavor liking.

The information obtained in this study indicates that the consumer sensory panel showed levels of overall liking for all treatments, between 2 (“I like very much”) and 4 (“I like it”). Shear force values suggested high tenderness (<3 kgF), lipid oxidation was below the rancid flavor threshold (2 mg MDA/kg meat), and PSY microbial growth was under  $7 \log_{10}/\text{cm}^2$ . These findings suggest that any of the aging types and frozen conditions tested could produce meat quality within consumer satisfaction.

## **Conclusions**

Even though differences were found in physico-chemical characteristics among preservation methods, their magnitude would not have major implications for meat quality. The fatty acid profile of the IMF was

more affected by the finishing diet than the preservation method. Dry aging (and also with subsequent frozen storage) showed an increase in the *Psychrotrophic* and *Enterobacteriaceae* counts compared to wet aging, despite the consumers scoring positively (at least 4 [“I like it”]) the overall liking of all treatments. However, segmentation by clusters is necessary to better understand some preferences for treatments. Finally, frozen storage after aging beef would be a suitable strategy to supply high-quality meat for export markets, but further research is necessary.

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## Literature Cited

- Ahn, D. U., Olson, D. G., Lee, J. I., Jo, C., Wu, C., and Chen, X. 1998. Packaging and irradiation effects on lipid oxidation and volatiles in pork patties. *J. Food Sci.* 63:15–19.
- Ahnström, M. L., Seyfert, M., Hunt, M. C., and Johnson, D. E. 2006. Dry aging of beef in a bag highly permeable to water vapour. *Meat Sci.* 73:674–679. <https://doi.org/10.1016/j.meatsci.2006.03.006>
- Altmann, B. A., Anders, S., Risius, A., and Mörlein, D. 2022. Information effects on consumer preferences for alternative animal feedstuffs. *Food Policy* 106:102192. <https://doi.org/10.1016/j.foodpol.2021.10219>
- AMSA (American Meat Science Association). 2016. Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of meat. 2nd ed. Amer. Meat Sci. Assoc. Champaign, IL. pp. 106.
- Apaoblaza, A., Gerrard, S. D., Matarneh, S. K., Wicks, J. C., Kirkpatrick, L., England, E. M., Scheffler, T. L., Duckett, S. K., Shi, H., Silva, S. L., Grant, A. L., and Gerrard, D. E. 2020. Muscle from grass- and grain-fed cattle differs energetically. *Meat Sci.* 161:107996. <https://doi.org/10.1016/j.meatsci.2019.107996>
- Ba, H. V., Oliveros, C. M., Park, K., Dashdorj, D., and Hwang, I. 2016. Effect of marbling and chilled ageing on meat-quality traits, volatile compounds and sensory characteristics of beef *longissimus dorsi* muscle. *Anim. Prod. Sci.* 57:981–992. <https://doi.org/10.1071/AN15676>
- Berger, J. 2017. Effects of dry-aging on eating quality, physico-chemical, and microbiological attributes of grass-fed beef loins. Master’s thesis. Purdue University, West Lafayette, Indiana. ([https://scholar.google.com/scholar?hl=en&as\\_sdt=0%2C5&q=Jordy+Berger%2C+2017&oq=j#:~:text=%2D%202017%20%2D-,docs.lib.purdue.edu,-Abstract%20The%20United](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Jordy+Berger%2C+2017&oq=j#:~:text=%2D%202017%20%2D-,docs.lib.purdue.edu,-Abstract%20The%20United))
- Berger, J., Kim, Y. H. B., Legako, J. F., Martini, S., Lee, J., Ebner, P. E., and Zuelly, S. M. S. 2018. Dry aging improves meat quality attributes of pasture-fed beef loins. *Meat Sci.* 145:285–291. <https://doi.org/10.1016/j.meatsci.2018.07.004>
- Bernardo, A. P., Da Silva, A. C., Francisco, V. C., Ribeiro, F. A., Nassu, R., Calkins, C. R., Do Nascimento, M., and Pflanzer, S. 2020. Effects of freezing and thawing on microbiological and physical-chemical properties of dry-aged beef. *Meat Sci.* 161:108003. <https://doi.org/10.1016/j.meatsci.2019.108003>
- Bligh, E. G., and Dyer, W. J. 1959. A rapid method for total lipid extraction and purification. *Can. J. Biochem. Phys.* 37:911–917. <https://doi.org/10.1139/y59-099>
- Borch, E., Kant-Muermans, M.-L., and Blixt, Y. 1996. Bacterial spoilage of meat and cured meat products. *Int. J. Food Microbiol.* 33:103–120. [https://doi.org/10.1016/0168-1605\(96\)01135-x](https://doi.org/10.1016/0168-1605(96)01135-x)
- Brito, G., San Julián, R., La Manna, A., Del Campo, M., Montossi, F., Banhero, G., Chalkling, D., and Soares de Lima, J. M. 2014. Growth, carcass traits, and palatability: Can the influence of the feeding regimes explain the variability found on these attributes in different Uruguayan genotypes? *Meat Sci.* 98:533–538. <http://dx.doi.org/10.1016/j.meatsci.2014.07.003>
- Campbell, R. E., Hunt, M. C., Levis, P., and Chambers, E. 2001. Dry-aging effects on palatability of beef *longissimus* muscle. *J. Food Sci.* 66:196–199. <https://doi.org/10.1111/j.1365-2621.2001.tb11315.x>
- Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D., and Richardson, R. I. 2006. Flavour perception of oxidation in beef. *Meat Sci.* 72:303–311. <https://doi.org/10.1016/j.meatsci.2005.07.015>
- Casas, D. E., Manishimwe, R., Forgey, S. J., Hanlon, K. E., Miller, M. F., Brashears, M. M., and Sanchez-Plata, M. X. 2021. Biomapping of microbial indicators on beef subprimals subjected to spray or dry chilling over prolonged refrigerated storage. *Foods* 10:1403. <https://doi.org/10.3390/foods10061403>
- Choe, J.-H., Stuart, A., and Kim, Y. H. B. 2016. Effect of different aging temperatures prior to freezing on meat quality attributes of frozen/thawed lamb loins. *Meat Sci.* 116:158–164. <https://doi.org/10.1016/j.meatsci.2016.02.014>
- Coombs, C. E., Holman, B. W., Friend, M. A., and Hopkins, D. L. 2017. Long-term red meat preservation using chilled and frozen storage combinations: A review. *Meat Sci.* 125:84–94. <https://doi.org/10.1016/j.meatsci.2016.11.025>
- Correa, D., Lema, M., Ravagnolo, O., Clariget, J., Luzardo, S., and Brito, G. 2020. Effects of differences in level of post-weaning nutrition and in sire expected progeny differences for ribeye area on retail cuts yield in Hereford steers. *Anim. Prod. Sci.* 61:172–178. <https://doi.org/10.1071/AN19604>

- Daley, C. A., Abbot, A., Doyle, P. S., Nader, G. A., and Larson, S. 2010. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutr. J.* 9:10. <https://doi.org/10.1186/1475-2891-9-10>
- Dashdorj, D., Tripathi, V. K., Cho, S., Kim, Y., and Hwang I. 2016. Dry aging of beef; Review. *Journal of Animal Science and Technology* 58:20. <https://doi.org/10.1186/s40781-016-0101-9>
- DeGeer, S. L., Hunt, M. C., Bratcher, C. L., Crozier-Dodson, B. A., Johnson, D. E., and Stika, J. F. 2009. Effects of dry aging of bone-in and boneless strip loins using two aging processes for two aging times. *Meat Sci.* 83:768–774. <https://doi.org/10.1016/j.meatsci.2009.08.017>
- Descalzo, A. M., and Sancho, A. M. 2008. A review of natural antioxidants and their effects on oxidative status, odor and quality of fresh beef produced in Argentina. *Meat Sci.* 79:423–436.
- Descalzo, A. M., Rossetti, L., Grigioni, G., Irueta, M., Sancho, A. M., Carrete, J., and Pensel, N. A. 2007. Antioxidant status and odour profile in fresh beef from pasture or grain-fed cattle. *Meat Sci.* 75:299–307. <https://doi.org/10.1016/j.meatsci.2006.07.015>
- Diez-Gonzalez, F., Callaway, T. R., Kizoulis, M. G., and Russel, J. B. 1998. Grain feeding and the dissemination of acid-resistant *Escherichia coli* from cattle. *Science* 281:1666–1668.
- Dikeman, M. E., Obuz, E., Gök, V., Akkaya, L., and Stroda, S. 2013. Effects of dry, vacuum, and special bag aging; USDA quality grade; and end-point temperature on yields and eating quality of beef *Longissimus lumborum* steaks. *Meat Sci.* 94:228–233. <https://doi.org/10.1016/j.meatsci.2013.02.002>
- Duarte, T. L., Bolkenov, B., Klopatek, S. C., Oltjen, J. W., King, D. A., Shackelford, S. D., Wheeler, T. L., and Yang, X. 2022. Evaluating the shelf life and sensory properties of beef steaks from cattle raised on different grass feeding systems in the western United States. *Foods* 11:2141. <https://doi.org/10.3390/foods11142141>
- Farouk, M. M., Wieliczko, K. J., and Merts, I. 2003. Ultra-fast freezing and low temperatures are not necessary to maintain the functional properties of manufacturing beef. *Meat Sci.* 66:171–179. [https://doi.org/10.1016/S0309-1740\(03\)00081-0](https://doi.org/10.1016/S0309-1740(03)00081-0)
- Feiner, G. 2006. *Meat products handbook: Practical science and technology*. Elsevier, Amsterdam.
- Font-i-Furnols, M., and Guerrero, L. 2014. Consumer preference, behavior and perception about meat and meat products: An overview. *Meat Sci.* 98:361–371. <https://doi.org/10.1016/j.meatsci.2014.06.025>
- Font-i-Furnols, M., San Julián, R., Guerrero, L., Sañudo, C., C Campo, M. M., Olleta, J. L., Oliver, M. A., Cañeque, V., Álvarez, I., Díaz, M. T., Branscheid, W., Wicke, M., Nute, G. R. and Montossi, F. 2006. Acceptability of lamb meat from different producing systems and ageing time to German, Spanish and British consumers. *Meat Sci.* 72:545–554. <https://doi.org/10.1016/j.meatsci.2005.09.002>
- Gatellier, P., Mercier, Y., Juin., H., and Renner, M. 2005. Effect of finishing mode (pasture- or mixed-diet) on lipid composition, colour stability and lipid oxidation in meat from Charolais cattle. *Meat Sci.* 69:175–186. <https://doi.org/10.1016/j.meatsci.2004.06.022>
- Gill, C. O. 2014. Spoilage, factors affecting: Microbiological. *Encyclopedia of Meat Sciences*. 2nd ed. Elsevier, Amsterdam. pp. 388–393.
- González-Gutiérrez, M., García-Fernández, C., Alonso-Calleja, C. and Capita, R. 2020. Microbial load and antibiotic resistance in raw beef preparations from northwest Spain. *Food Science & Nutrition* 8:777–785. <https://doi.org/10.1002/fsn3.1319>
- Griffiths, M. W., Phillips, J. D., and Muir, D. D. 1981. Thermostability of proteases and lipases from a number of species of psychrotrophic bacteria of dairy origin. *J. Appl. Bacteriol.* 50:289–303. <https://doi.org/10.1111/j.1365-2672.1981.tb00894.x>
- Gudjónsdóttir, M., Gacutan Jr., M. D., Mendes, A. C., Chronakis, I. S., Jespersen, L., and Karlsson, A. H. 2015. Effects of electrospun chitosan wrapping for dry-ageing of beef, as studied by microbiological, physicochemical and low-field nuclear magnetic resonance analysis. *Food Chem.* 184:167–175. <https://doi.org/10.1016/j.foodchem.2015.03.088>
- Ha, M., McGilchrist, P., Polkinghorne, R., Huynh, L., Galletly, J., Kobayashi, K., Nishimura, T., Bonney, S., Kelman, K. R., and Warner, R. D. 2019. Effects of different ageing methods on colour, yield, oxidation and sensory qualities of Australian beef loins consumed in Australia and Japan. *Food Res. Int.* 125:108528. <https://doi.org/10.1016/j.foodres.2019.108528>
- Hedrick, H. B., Paterson, J. A., Matches, A. G., Thomas, J. D., Morrow, R. E., Stringer, W. G., and Lipsey, R. J. 1983. Carcass and palatability characteristics of beef produced on pasture, corn silage and corn grain. *J. Anim. Sci.* 57: 91–801. <https://doi.org/10.2527/jas1983.574791x>
- Hulánková, R., Kameník, J., Saláková, A., Závodský, D., and Borilova, G. 2018. The effect of dry aging on instrumental, chemical and microbiological parameters of organic beef loin muscle. *LWT-Food Sci. Technol.* 89:559–565. <https://doi.org/10.1016/j.lwt.2017.11.014>
- Iida, F., Miyazaki, Y., Tsuyuki, R., Kato, K., Egusa, A., Ogoshi, H., and Nishimura, T. 2016. Changes in taste compounds, breaking properties, and sensory attributes during dry aging of beef from Japanese black cattle. *Meat Sci.* 112:46–51. <https://doi.org/10.1016/j.meatsci.2015.10.015>
- INAC (Instituto Nacional de Carnes). 2013. Reglamento (UE) 593/2013. <https://www.inac.uy/innovaportal/v/9944/14/innova.front/hilton>. (Accessed 29 March 2024).
- IUPAC (International Union of Pure and Applied Chemistry). 1987. *Standard methods for the analysis of oils, fats and derivatives*. IUPAC Division Commission on Oils, Fats and Derivatives, 7th ed. Blackwell Jevent Publishers, Oxford.
- Jiang, T., Ssuboom, J. R., Nelson, M. L., O’Fallon, J., Ringkob, T. P., Rogers-Klette, K. R., Joos, D., and Piper, K. 2010. The influence of forage diets and aging on beef palatability. *Meat Sci.* 86:642–650. <https://doi.org/10.1016/j.meatsci.2010.05.016>
- Juárez, M., Caine, W. R., Dugan, M. E. R., Hidiroglou, N., Larsen, I. L., Uttaro, B., and Aalhus, J. L. 2011. Effects of dry-ageing on pork quality characteristics in different genotypes. *Meat Sci.* 88:117–121. <https://doi.org/10.1016/j.meatsci.2010.12.011>
- Kahraman, H. A., and Gürbüz, Ü. 2019. Effects of three aging methods on the *Longissimus lumborum* muscle from Holstein-Friesian steers. *Med. Weter.* 75:179–184. <https://dx.doi.org/10.21521/mw.6182>

- Kim, Y. H. B., Frandsen, M., and Rosenfold, K. 2011. Effect of ageing prior to freezing on colour stability of ovine longissimus muscle. *Meat Sci.* 88:332–337 <https://doi.org/10.1016/j.meatsci.2010.12.020>.
- Kim, J. H., Kim, D. H., Ji, D., Lee, H. J., Yoon, D. K., and Lee, C. H. 2017. Effect of aging process and time on physico-chemical and sensory evaluation of raw beef top round and shank muscles using an electronic tongue. *Korean J. Food Sci. Anim. Resour.*: 37(6):823–832. <https://doi.org/10.5851/kosfa.2017.37.6.823>
- Kim, J. H., Kim, T. K., Shin, D. M., Kim, H. W., Kim, Y. B., and Choi, Y. S. 2020. Comparative effects of dry-aging and wet-aging on physicochemical properties and digestibility of Hanwoo beef. *Asian Austral. J. Anim.* 33:501–505. <https://doi.org/10.5713/ajas.19.0031>
- Kim, Y. H. B., Ma, D., Setyabrata, D., Farouk, M. M., Lonergan, S. M., Huff-Lonergan, E., and Hunt, M. C. 2018. Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies (Review). *Meat Sci.* 144:74–90. <https://doi.org/10.1016/j.meatsci.2018.04.031>
- King, D. A., Hunt, M. C., Barbut, S., Claus, J. R., Cornforth, D. P., Joseph, P., Kim, Y. H. B., Lindahl, G., Mancini, R. A., Nair, M. A., Merok, K. J., Milkowski, A., Mohan, A., Pohlman, F., Ramanathan, R., Raines, C. R., Seyfert, M., Sørheim, O., Suman, S. P., and Weber, M. 2023. American Meat Science Association Guidelines for Meat Color Measurement. *Meat Muscle Biol.* 6:12473, 1–81. <https://doi.org/10.22175/mmb.12473>
- Lee, S. M., Lee, K. T., Lee, S. H., and Song, J. K. 2013. Origin of human colour preference for food. *J. Food Eng.* 119:508–515. <https://doi.org/10.1016/j.jfoodeng.2013.06.021>
- Leygonie, C., Britz, T. J., and Hoffman, L. C. 2012. Impact of freezing and thawing on the quality of meat: Review. *Meat Sci.* 91:93–98. <https://doi.org/10.1016/j.meatsci.2012.01.013>
- Li, X., Babol, J., Bredie, W. L. P., Nielsen, B., Tománková, J., and Lundstrom. 2014. A comparative study of beef quality after ageing longissimus muscle using a dry ageing bag, traditional dry ageing or vacuum package ageing. *Meat Sci.* 97:433–442. <https://doi.org/10.1016/j.meatsci.2014.03.014>
- Li, X., Babol, J., Wallby, A., and Lundström, K. 2013. Meat quality, microbiological status and consumer preference of beef gluteus medius aged in a dry ageing bag or vacuum. *Meat Sci.* 95:229–234. <https://doi.org/10.1016/j.meatsci.2013.05.009>
- Li, Z., Ha, M., Frank, D., McGilchrist, P., and Warner, R. D. 2021. Volatile profile of dry and wet aged beef loin and its relationship with consumer flavour liking. *Foods* 10:3113. <https://doi.org/10.3390/foods10123113>
- Luzardo, S., Saadoun, A., Cabrera, M. C., Terevinto, A., Brugnini, G., Rodriguez, J., de Souza, G., Rovira, P., and Rufo, C. 2024. Effect of beef long-storage under different temperatures and vacuum-packaging conditions on meat quality, oxidation processes and microbial growth. *J. Sci. Food Agr.* 104: 1143–1153. <https://doi.org/10.1002/jsfa.12999>
- MacFie, H. J., and Bratchell, N. K. 1989. Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests. *J. Sens. Stud.* <https://doi.org/10.1111/j.1745-459X.1989.tb00463.x>
- Melton, S. L. 1983. Effect of forage feeding on beef flavor. *Food Techn.* 37: 239–248.
- MGAP (Ministerio de Ganadería, Agricultura y Pesca). 2023. Available at [https://www.gub.uy/ministerio-ganaderia-agricultura-pesca/sites/ministerio-ganaderia-agricultura-pesca/files/2021-08/CarneAltaCalidad\\_8350\\_0621\\_UE\\_Ingles.pdf](https://www.gub.uy/ministerio-ganaderia-agricultura-pesca/sites/ministerio-ganaderia-agricultura-pesca/files/2021-08/CarneAltaCalidad_8350_0621_UE_Ingles.pdf). (Accessed 1 March 2024).
- Miller, M. F., Carr, M. A., Ramsey, C. B., Crockett, K. L., and Hoover, L. C. 2001. Consumer thresholds for establishing the value of beef tenderness. *J. Anim. Sci.* 79:3062–3068. <https://doi.org/10.2527/2001.79123062x>
- Nishimura, T. 1998. Mechanism involved in the improvement of meat taste during post-mortem aging. *Food Sci. Technol. Int.* 4:241–249. <https://doi.org/10.3136/fsti9596t9798.4.241>
- Nuernberg, K., Dannenberger, D., Nuernberg, G., Ender, K., Voigt, J. Scollan, N. D., Wood J. D., Nute, G. R., and Richardson, R. I. 2005. Effect of a grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of longissimus muscle in different cattle breeds. *Livest. Prod. Sci.* 94:137–147. <https://doi.org/10.1016/j.livprodsci.2004.11.036>
- Obuz, E., Akkaya, L., Gok, V., and Dikeman, N. E. 2014. Effects of blade tenderization, aging method and aging time on meat quality characteristics of Longissimus lumborum steaks from cull Holstein cows. *Meat Sci.* 96:1227–1232. <https://doi.org/10.1016/j.meatsci.2013.11.015>
- Panella-Riera, N., Correa, D., Brito, G., del Campo, M., Luzardo, S., and Font-i-Furnols, M. 2023. Sensory characteristics of wet and dry-bag aged beef from grain and pasture finished steers. *Proceedings of the 69th International Congress of Meat Science and Technology (ICoMST)*, 21–25th August, Padova, Italy. p. 402–403.
- Parrish, F. C., Boles, J. A., Rust, R. E., and Olson, D. G. 1991. Dry and wet aging effects on palatability attributes of beef loin and rib steaks from three quality grades. *J. Food Sci.* 56:601–603. <https://doi.org/10.1111/j.1365-2621.1991.tb05338.x>
- Peripolli, E., Banchemo, G., Pereira, A., Brito, G., La Manna, A., Fernandez, E., Montossi, F., and Baldi, F. 2018. Effect of growth path on the performance and carcass traits of Hereford steers finished on pasture or in feedlot. *Anim. Prod. Sci.* 58:1341–1348. <https://doi.org/10.1071/AN16061>
- Ponnampalam, E. N., Mann, N. J., and Sinclair, A. J. 2006. Effect of feeding systems on omega-3 fatty acids, conjugated linoleic acid and trans fatty acids in Australian beef cuts: Potential impact on human health. *Asia Pac. J. Clin. Nutr.* 15:21–29. [ajcn.nhri.org.tw/server/APJCN/15/1/21.pdf](http://ajcn.nhri.org.tw/server/APJCN/15/1/21.pdf)
- Priolo, A., Micol, D., and Agabriel, J. 2001. Effects of grass feeding systems on ruminant meat colour and flavour: A review. *Animal Resources* 50:185–200. <https://doi.org/10.1051/animres:2001125>
- Realini, C. E., Duckett, S. K., Brito, G. W., Dalla Rizza, M., and De Mattos, D. 2004. Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. *Meat Sci.* 66:567–577. [https://doi.org/10.1016/S0309-1740\(03\)00160-8](https://doi.org/10.1016/S0309-1740(03)00160-8)
- Realini, C. E., Font i Furnols, M., Guerrero, L., Montossi, F., Campo, M. M., Sañudo, C., Nute, G. R., Alvarez, I., Cañeque, V., Brito, G., and Oliver, M. A. 2009. Effect of

- finishing diet on consumer acceptability of Uruguayan beef in the European market. *Meat Sci.* 81:499–506. <https://doi.org/10.1016/j.meatsci.2008.10.005>
- Realini, C. E., Kallas, Z., Pérez-Juan, M., Gómez, I., Olleta, J. L., Beriain, M. J., Albertí, P., and Sañudo, C. 2014. Relative importance of cues underlying Spanish consumers' beef choice and segmentation, and consumer liking of beef enriched with n-3 and CLA fatty acids. *Food Qual. Prefer.* 33:74–85. <https://doi.org/10.1016/j.foodqual.2013.11.007>
- Resconi, V. C., Campo, M. M., Font i Furnols, M., Montossi, F., and Sañudo, C. 2010. Sensory quality of beef from different finishing diets. *Meat Sci.* 86:865–869. <https://doi.org/10.1016/j.meatsci.2010.07.012>
- Schroeder, J. W., Cramer, D. A., Bowling R. A., and Cook, C. W. 1980. Palatability, shelf life and chemical differences between forage- and grain-finished Beef. *J. Anim. Sci.* 50:852–859. <https://doi.org/10.2527/jas1980.505852x>
- Schwarz, F. J., Augustini, C., Timm, M., Kirchgessner, M., and Steinhart, H. 1998. Effect of vitamin E on alpha-tocopherol concentration in different tissues and oxidative stability of bull beef. *Livest. Prod. Sci.* 56:165–171. [https://doi.org/10.1016/S0301-6226\(98\)00189-4](https://doi.org/10.1016/S0301-6226(98)00189-4)
- Smith, R., Nicholson, K., Nicholson, J., Harris, K., Miller, R., Griffin, D., and Savell, J. 2008. Dry versus wet aging of beef: Retail cutting yields and consumer palatability evaluations of steaks from US Choice and US Select short loins. *Meat Sci.* 79:631–639. <https://doi.org/10.1016/j.meatsci.2007.10.028>
- Stanbridge, L. H., and Davies, A. R. 1998. The microbiology of chill-stored meat. In: A. R. Davies, R. J. Board, and R. G. Board, editors, *The microbiology of meat and poultry*. Springer. pp. 174–206.
- UK Department of Health. 1994. Report on health and social subjects. No. 46. Nutritional aspects of cardiovascular disease. London: HMSO.
- Vestergaard, M., N. Oksbjerg, and P. Henckel. 2000. Influence of feeding intensity, grazing and finishing feeding on muscle fibre characteristics and meat colour of *semitendinosus*, *longissimus dorsi* and *supraspinatus* muscles of young bulls. *Meat Sci.* 54:177–185. [https://doi.org/10.1016/S0309-1740\(99\)00097-2](https://doi.org/10.1016/S0309-1740(99)00097-2)
- Wang, C., Zhu, L., and Brewer, M. S. 1997. Comparison of 2-Thiobarbituric acid reactive substances determination methods in various types of frozen, fresh meat. *J. Food Lipids* 4:87–96. <https://doi.org/10.1111/j.1745-4522.1997.tb00083.x>
- Warren, K. E., and Kastner, C. L. 1992. A comparison of dry-aged and vacuum-aged beef strip loins. *J. Muscle Foods* 3:151–157. <https://doi.org/10.1111/j.1745-4573.1992.tb00471.x>
- Wheeler, T. L., Savell, J. W., Cross, H. R., Lunt, D. K., and Smith, S. B. 1990. Mechanisms associated with the variation in tenderness of meat from Brahman and Hereford cattle. *J. Anim. Sci.* 68:4206–4220. <https://doi.org/10.2527/1990.68124206x>
- Zerby, H. N., Belk, K. E., Sofos, J. N., McDowell L. R., and Smith, G. C. 1999. Case life of seven retail products from beef cattle supplemented with alpha-tocopheryl acetate. *J. Anim. Sci.* 77:2458–2463. <https://doi.org/10.2527/1999.7792458x>
- Zhang, J., Wall, S. K., Xu, L., and Ebner, P. D. 2010. Contamination rates and antimicrobial resistance in bacteria isolated from “grass-fed” labeled beef products. *Foodborne Pathog. Dis.* 7:1331–1336. <https://doi.org/10.1089/fpd.2010.0562>
- Zhang, R., Yoo, M. J.Y., and Farouk, M. 2021. Oxidative stability, proteolysis, and in vitro digestibility of fresh and long-term frozen stored in-bag dry-aged lean beef. *Food Chem.* <https://doi.org/10.1016/j.foodchem.2020.128601>
- Zhang, R., Yoo, M. J. Y., and Farouk, M. M. 2019. Quality and acceptability of fresh and long-term frozen in-bag dry-aged lean bull beef. *J. Food Quality* 2019:1975264.

**Table S1.** Socio-demographic characteristics of the consumers (%)

Characteristics		All Consumers (n = 100); aged	All Consumers (n = 100); aged+frozen	All Consumers (n = 200)	Cluster 1 (n = 77)	Cluster 2 (n = 43)	Cluster 3 (n = 80)	
Sex	Male	45.0	56.0	55.5	53.0	53.5	58.8	
	Female	55.0	44.0	44.5	47.0	46.5	41.2	
Age	<30 years	35.0	27.0	31.0	26.0	25.6	38.7	
	30–50 years	53.0	60.0	56.5	63.6	62.8	46.3	
	>50 years	12.0	13.0	12.5	10.4	11.6	15.0	
Educational level	Primary school	5.0	2.0	3.5	3.9	2.3	3.7	
	Secondary school	25.0	27.0	26.0	28.6	25.6	23.8	
	University	47.0	46.0	46.5	44.1	44.2	50.0	
	Post-graduate	23.0	25.0	24.0	23.4	27.9	22.5	
Frequency of fresh meat consumption	Pork	Never	24.3	19.0	21.5	16.9	30.2	21.2
		Once a month	39.4	52.0	45.5	44.1	48.8	45.0
		Every two weeks	22.2	13.0	17.5	19.5	9.4	20.0
	Beef	Every week	14.1	16.0	15.5	19.5	11.6	13.8
		Never	-	-	-	-	-	-
		Once a month	3.0	4.0	4.0	5.1	-	5.0
	Chicken	Every two weeks	9.1	10.0	9.5	7.8	9.3	11.2
		Every week	87.9	86.0	86.5	87.1	90.7	83.8
		Never	4.1	3.0	3.5	-	4.6	6.2
	Lamb	Once a month	7.2	4.0	6.0	5.2	7.0	6.2
		Every two weeks	24.7	25.0	25.5	23.4	27.9	25.0
		Every week	63.9	68.0	65.5	71.4	60.5	62.6
Lamb	Never	15.5	16.0	16.0	15.6	9.3	20.0	
	Once a month	54.6	42.0	48.0	41.6	58.1	48.7	
	Every two weeks	19.6	25.0	22.5	25.9	20.9	20.0	
	Every week	10.3	17.0	13.5	16.9	11.7	11.3	

**Table S2.** Effects (LSM and P-values) of preservation methods (PM) and finishing diet (F) PUFA (mg/100g meat) (Omega 6 and Omega 3), MUFA (mg/100g meat), and SFA (mg/100g meat).

Traits	DAb	PM				P-value	F		P-value	PM*F P-value
		WA	DAb+Fr	WA+Fr	Pasture		Grain			
<b>PUFA</b>	<b>253.8 ± 15.3</b>	<b>239.2 ± 14.4</b>	<b>216.2 ± 13.8</b>	<b>246.3 ± 14.9</b>	<b>0.304</b>	<b>268.9 ± 11.5</b>	<b>211.5 ± 9.3</b>	<b>&lt;0.001</b>	<b>0.081</b>	
<b>PUFA n6</b>	<b>183.4 ± 11.8</b>	<b>173.6 ± 10.9</b>	<b>160.3 ± 10.7</b>	<b>181.7 ± 11.5</b>	<b>0.419</b>	<b>179.2 ± 9.0</b>	<b>169.2 ± 8.7</b>	<b>0.460</b>	<b>0.138</b>	
C18:2n6	123.0 ± 7.9	117.3 ± 7.4	111.1 ± 7.5	124.5 ± 7.9	0.556	122.9 ± 6.5	114.9 ± 6.2	0.377	0.149	
C18:3n6	3.3 ± 0.22	3.5 ± 0.24	3.1 ± 0.22	3.3 ± 0.23	0.501	4.2 ± 0.27	2.6 ± 0.17	<0.001	0.180	
C20:2n6	4.4 ± 0.33	4.4 ± 0.32	4.1 ± 0.32	4.1 ± 0.30	0.770	3.2 ± 0.22	5.8 ± 0.4	<0.001	0.067	
C20:3n6	11.7 ± 0.8	11.5 ± 0.7	9.9 ± 0.7	11.5 ± 0.8	0.241	10.6 ± 0.6	11.4 ± 0.7	0.390	0.091	
C20:4n6	39.1 ± 2.9	36.1 ± 2.6	30.7 ± 2.4	36.6 ± 2.7	0.088	36.9 ± 2.4	34.1 ± 2.3	0.404	0.119	
<b>PUFA n3</b>	<b>53.1 ± 3.8</b>	<b>58.3 ± 4.0</b>	<b>51.0 ± 3.6</b>	<b>58.4 ± 4.0</b>	<b>0.208</b>	<b>78.8 ± 5.7</b>	<b>38.6 ± 2.8</b>	<b>&lt;0.001</b>	<b>0.767</b>	
C18:3n3	22.8 ± 1.8	25.4 ± 1.9	20.8 ± 1.7	23.2 ± 1.8	0.286	47.1 ± 3.1	11.2 ± 0.8	<0.001	0.193	
C20:3n3	2.1 ± 0.19	2.3 ± 0.9	1.8 ± 0.16	2.2 ± 0.18	0.172	2.1 ± 0.17	2.1 ± 0.17	0.800	0.089	
C20:5n3	10.0 ± 0.9	10.0 ± 0.9	8.0 ± 0.8	9.4 ± 0.9	0.110	11.8 ± 1.2	7.3 ± 0.8	0.002	0.110	
C22:5n3	19.7 ± 1.4	18.2 ± 1.4	15.3 ± 1.5	17.6 ± 1.4	0.164	20.2 ± 1.1	15.2 ± 1.1	0.002	0.095	
C22:6n3	3.4 ± 0.3	3.2 ± 0.3	3.6 ± 0.3	4.0 ± 0.3	0.167	3.3 ± 0.2	3.7 ± 0.2	0.143	0.342	
<b>n6:n3</b>	<b>3.1 ± 0.17</b>	<b>2.9 ± 0.16</b>	<b>3.1 ± 0.17</b>	<b>3.1 ± 0.17</b>	<b>0.049</b>	<b>2.1 ± 0.15</b>	<b>4.5 ± 0.34</b>	<b>&lt;0.001</b>	<b>0.767</b>	
<b>CLA</b>	<b>21.2 ± 1.9</b>	<b>23.0 ± 1.8</b>	<b>19.7 ± 1.9</b>	<b>20.6 ± 1.8</b>	<b>0.494</b>	<b>26.9 ± 1.9</b>	<b>15.3 ± 1.9</b>	<b>&lt;0.001</b>	<b>0.218</b>	
<b>MUFA</b>	<b>1826.5 ± 111.5</b>	<b>1774.9 ± 111.5</b>	<b>1633.5 ± 118.0</b>	<b>1702.4 ± 111.5</b>	<b>0.596</b>	<b>1632.1 ± 95.0</b>	<b>1836.5 ± 97.0</b>	<b>0.136</b>	<b>0.200</b>	
C14:1	19.1 ± 1.7	18.9 ± 1.6	18.1 ± 1.6	19.3 ± 1.7	0.858	15.0 ± 1.5	23.7 ± 2.8	0.002	0.283	
C16:1	30.2 ± 2.1	31.1 ± 2.1	29.5 ± 2.1	30.9 ± 2.1	0.905	26.5 ± 1.8	34.9 ± 2.4	0.005	0.296	
C18:1n9	1685.0 ± 100.7	1722.0 ± 99.0	1583.0 ± 104.7	1648.7 ± 99.0	0.756	1543.0 ± 86.0	1776.3 ± 87.2	0.060	0.291	
<b>SFA</b>	<b>1902.7 ± 126.5</b>	<b>2003.0 ± 122.2.0</b>	<b>1938 ± 126.5</b>	<b>2024 ± 120.4</b>	<b>0.835</b>	<b>1925.0 ± 119.2</b>	<b>2009.4 ± 118.8</b>	<b>0.617</b>	<b>0.447</b>	
C10:0	1.8 ± 0.13	1.9 ± 0.13	1.9 ± 0.13	2.1 ± 0.13	0.397	2.0 ± 0.13	1.9 ± 0.12	0.714	0.206	
C12:0	2.2 ± 0.16	2.2 ± 0.16	2.2 ± 0.17	2.3 ± 0.16	0.870	2.2 ± 0.15	2.2 ± 0.15	0.927	0.149	
C14:0	95.2 ± 7.1	98.8 ± 7.3	94.3 ± 7.3	100.3 ± 7.4	0.860	89.9 ± 7.0	105.0 ± 8.2	0.161	0.222	
C15:0	13.7 ± 1.0	14.7 ± 1.0	13.7 ± 1.0	14.8 ± 1.0	0.684	15.8 ± 1.1	12.8 ± 0.9	0.040	0.169	
C16:0	1106.4 ± 70.0	1150.1 ± 68.9	1081.1 ± 72.7	1136.1 ± 68.9	0.870	1097.0 ± 62.7	1139.8 ± 63.5	0.633	0.225	
C17:0	130.5 ± 9.5	124.8 ± 9.1	115.5 ± 8.8	122.7 ± 9.0	0.527	105.1 ± 8.1	144.6 ± 11.2	0.005	0.215	
C18:0	594.8 ± 41.6	636.8 ± 43.8	584.2 ± 42.4	609.0 ± 41.9	0.777	643.9 ± 41.2	570.1 ± 36.9	0.185	0.213	
C20:0	6.8 ± 0.7	7.7 ± 0.8	5.2 ± 0.5	7.1 ± 0.7	0.017	10.4 ± 1.0	4.2 ± 0.4	<0.001	<b>0.014</b>	
C22:0	3.4 ± 0.26	4.0 ± 0.29	2.8 ± 0.22	2.9 ± 0.22	0.001	3.6 ± 0.24	2.9 ± 0.20	0.024	<b>&lt;0.001</b>	
C24:0	4.4 ± 0.31	4.1 ± 0.28	3.7 ± 0.27	4.5 ± 0.31	0.168	3.7 ± 0.21	4.7 ± 0.27	0.008	0.226	
<b>PUFA/SFA</b>	<b>0.12 ± 0.007</b>	<b>0.12 ± 0.007</b>	<b>0.12 ± 0.007</b>	<b>0.12 ± 0.007</b>	<b>0.570</b>	<b>0.14 ± 0.01</b>	<b>0.11 ± 0.008</b>	<b>0.028</b>	<b>0.075</b>	

DAb: Dry aging bag; WA: Wet aging; DAb+Fr: Dry aging bag + 180 days frozen; WA+Fr: Wet aging + 180 days frozen. CLA: conjugated linoleic fatty acid (c9, t11–18:2); PUFA: sum of polyunsaturated fatty acid (PUFA n-6 + PUFA n-3); MUFA: monounsaturated fatty acid (C14:1 + C16:1 + C18:1n9); SFA: saturated fatty acid (C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0); PUFA n-3 (C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3); PUFA n-6 (C18:2n-6 + C18:3n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6); PUFA/SFA: Polyunsaturated/Saturated fatty acids; IMF: intramuscular fat. Different letter in the same row denotes groups statistically different ( $P < 0.05$ ) among LSM means.