Research Article

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Effect of beef long-storage under different temperatures and vacuum-packaging conditions on meat quality, oxidation processes and microbial growth

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Abstract

BACKGROUND: The global beef market demands the meat industry to ensure product quality and safety in markets that are often very distant. The present study aimed to evaluate the effects of chilled (CH, 120 d) and chilled-then-frozen (CHF, 28 d + 92 d) storage conditions of beef vacuum packaged (VP) and vacuum packaged with antimicrobial (VPAM) on meat quality, oxidative status and microbial loads. Treatments resulted from the combination of storage condition and packaging type: VP + CH, VP + CHF, VPAM + CH and VPAM + CHF.

RESULTS: Warner–Bratzler shear force values decreased in all treatments after 28 d of chilling. Except for VP + CH, *L values (lightness) of meat color did not differ in each treatment as the storage time increased. Meat from VP + CH had greater *a** values than CHF treatments on day 120 of storage. A consumer panel did not detect differences in tenderness, flavor and overall liking between VP and VPAM beef, but they preferred CHF steaks rather than CH beef. TBARS values did not differ between VP and VPAM and between CH and CHF at any time during the storage period. At the end of storage time, all treatments except VP + CHF presented a greater concentration of thiols than at 48 h post-mortem. On day 120 of storage, VP + CH had greater catalase enzyme activity than CHF treatments while VP + CH and VP + CHF showed a greater superoxide dismutase activity than VPAM + CHF. Storage condition (CH or CHF) had a greater impact on microbial counts than the type of packaging.**

CONCLUSION: Freezing meat after an ageing period represents a suitable strategy to extend beef storage life without a detrimental impact on its quality.

Keywords: beef; storage life; meat quality; oxidation; microbial growth

INTRODUCTION

The global beef market demands the meat industry to ensure product quality and safety in markets that are often very distant. The main strategy to fulfill this need has been based on controlling the temperature during meat preservation, such as chilled and frozen storage which have proven to be successful.[1] Under chilling conditions, the ageing process takes place which improves meat tenderness[2] and eating quality up to 20 weeks of storage.[3] On the other hand, frozen storage seems to be a key factor in maintaining meat quality and delaying microbial spoilage for export markets.[4] However, few studies have evaluated the combined effect of chilled and frozen storage (chilled-then-frozen) as a strategy to extend beef storage life.[1,5]

Meat packaging also plays a pivotal role in extending beef shelf-life under controlled temperature conditions and vacuum-

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packaging technology has been widely used in the industry to store and preserve meat.[6] Vacuum packaging creates a depleted oxygen environment that alters the product microbiota so that spoilage of fresh beef is usually caused by lactic acid-producing bacteria often resulting in a sour flavor.[7] However, the development of active packaging with antimicrobial properties has received increasing attention in the last few years and different compounds have been studied such as: silver, silver zeolite, glucose oxidase, ethanol vapor, triclosan, chlorine dioxide, natamycin, wasabi extract in cyclodextrin, allyl isothiocyanate, among others.[8] Fresh beef shelf-life is mainly determined by the activity of microorganisms, but it is also influenced by biochemical factors such as lipid and pigment oxidation.[9]

Conversion of muscle to meat includes changes in its antioxidant defense system[10] because of increased lipid and protein oxidation processes due to free radical formation.[11] Nutritional compounds such as amino acids which are highly abundant in meat undergo chemical modifications and formation of carbonyls and oxidized thiols and hydroxylation of aromatics[10] take place affecting flavor and promoting microorganism proliferation. As the post-mortem ageing time increases these chemical modifications become more pronounced.[12]

The study presented here aimed to evaluate the effects of chilled and chilled-then-frozen storage conditions of beef vacuum packaged and vacuum packaged with antimicrobial agent on meat quality, oxidative status and microbial loads.

MATERIALS AND METHODS

Carcass sampling and experimental treatments

Forty strip loins (longissimus lumborum muscle) were collected from left half-carcasses of steers fattened on a high-concentrate diet intended for the EU 481 quota. Slaughter took place in a commercial meat processing facility and carcasses were graded using the Uruguayan grading system as specified by the National Meat Institute.[13] Conformation, degree of finishing and dentition data were recorded. Different muscling grades were based on visual assessment of muscle mass development and were identified by the letters I – N – A – C – U – R, from very muscular development to thinly muscled. Degree of finishing was evaluated by observing the amount and distribution of subcutaneous fat where a lower number indicates lack of finishing (0: lack of fat cover to 4: excessive finishing) (Table 1).

Experimental treatments stem from a combination of two packaging types (vacuum packaging (VP) *versus* vacuum packaging with antimicrobial agent (VPAM)) and two storage conditions of meat (chilled for 120 days at 1.38 ± 0.21°C (CH) *versus* chilled for 28 days and then frozen at −20 °C for 92 days (CHF)) where 10 strip loins corresponded to each treatment ($n = 10$).

Two packaging types were evaluated: (1) VP (Supervac GK 842B; Supervac® GmbH, Mödling, Austria) with a barrier bag (50 µm thickness; maximum oxygen transmission rate (OTR) of 27 $cm^3\ m^{-2}\ (24\ h)^{-1}$ at 22–24 °C and 0% relative humidity (RH) and moisture vapor transmission rate (MVTR) of 5 $g\ m^{-2}\ (24\ h)^{-1}$ at 38 °C and 90% RH; Cryovac® Sealed Air Corp., BB 2620, Brazil); (2) VPAM (Multivac P605; Multivac Inc., São Paulo, Brazil) with a polyamide bag (50 µm thickness; OTR of 350 $cm^3\ (25\ \mu m)^{-1}\ m^{-2}\ (24\ h)^{-1}$ at 23 °C and 85% RH and MVTR of 58 $g\ (25\ \mu m)^{-1}\ m^{-2}\ (24\ h)^{-1}$ at 23 °C and 85% RH; M&Q Packaging®, BioPlastic 11, Limerick, Ireland) which was based on silver ion technology (Biomaster®, Addmaster Ltd, UK) and incorporated into the bag by extrusion.

Table 1. Hot carcass weight (± SEM) and carcass characteristics of steers ($N = 40$)

Variable	
Hot carcass weight (kg)	265.9 ± 3.2
Number of steers	
Dentition (teeth)	
2	26
4	14
Conformation[a]	
A	40
Degree of finishing[b]	
1	12
2	28

[a] Conformation according to the Uruguayan grading system (INAC, 1997), I – N – A – C – U – R: from large muscle development (I) to lack of muscle development (R).
[b] Degree of finishing according to the Uruguayan grading system (INAC, 1997), from 0: lack of fat cover to 4: excessive finishing.

Strip loins from the left 'pistola' cut (prepared from the hindquarter by the removal of the thin flank, lateral portion ribs and a portion of the navel end brisket) of each carcass were fabricated after 48 h of slaughter by cutting from the 10th rib to the lumbar–sacral junction and then were trimmed to approximately 1 cm of external fat thickness. Subsequently, each strip loin was cut in five pieces of 6 cm in thickness corresponding to one of the five time periods in which measurements were performed: 2 days post-mortem, and 28, 45, 90 and 120 days of storage. The cranial piece of each strip loin was used for the 2 days post-mortem evaluation and then each piece was randomly assigned to one of the four storage periods (28, 45, 90 and 120 days) within each strip loin. After the pieces were cut, an additional 2.5 cm thick steak was removed for a consumer panel assessment of meat stored for 120 days.

Once each storage period was completed, the 6 cm thick strip loin piece was cut into three steaks in a cranial to caudal direction for the following determinations: (a) microbial counts (1.5 cm), (b) color, cooking losses and Warner–Bratzler shear force (WBSF) (2.5 cm) and (c) lipid and protein oxidation (1.5 cm). Knives and saw blades were sanitized during sample processing to avoid cross-contamination.

Meat quality characteristics

Determinations of meat quality characteristics were performed at each time point (2 days post-mortem, and 28, 45, 90 and 120 days of storage). Instrumental lean color (CIE: L^*, lightness; a^*, redness; b^*, yellowness) was measured on a steak from the longissimus dorsi muscle in triplicate with a Minolta chromameter (CR-400, Konica Minolta Sensing Inc., Japan) using a C illuminant, a 2° standard observer angle and 8 mm aperture size and calibrated with a white tile before use. Next, steaks were weighed before cooking using an electronic scale (EP-41KA, A&D Company, Tokyo, Japan) and then cooked in a preheated clam shell style grill (GRP100 The Next Grilleration, Spectrum Brands, Inc., Miami, FL, USA) until the internal temperature monitored with a thermometer (Comark N9094, Norwich, Norfolk, UK) in the geometric center reached 71 °C.[14] After cooking, steaks were allowed to cool and then the excess liquid was removed with a paper towel. Cooking losses were

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determined as: ((raw weight - cooked weight)/raw weight) x 100. Subsequently, six cores (1.27 cm in diameter) from each steak were removed parallel to the longitudinal orientation of muscle fibers and shear force was assessed with a TA.XT Plus texturometer (Stable Micro Systems, Godalming, Surrey, UK) fitted with a Warner–Bratzler V-shaped blade. Individual shear force values were averaged to assign a mean peak WBSF value to each sample.[14]

Lipid and protein oxidation

Lipid oxidation in meat samples was determined following the TBARS (thiobarbituric acid reactive species) method of Lynch and Frei[15] as described by Terevinto *et al.*[16] Briefly, 5 g of frozen meat sample was homogenized in a Waring-Blender (Fisher Inc., USA) with 100 mL of an extraction buffer (0.15 mol L^{-1} KCl, 0.02 mol L^{-1} EDTA and 0.30 mol L^{-1} butylated hydroxytoluene (BHT)) at 12 000 rpm for 1 min and then centrifuged (Sorvall ST 16-R, Thermo Scientific Inc., USA) at 4 °C and 2000 × *g* for 10 min. The sample supernatant was incubated with a 2-thiobarbituric acid (35 mmol L^{-1})–trichloroacetic acid (10%) solution (in 125 mmol L^{-1} HCl) in a glass tube which was incubated in a boiling water bath (Fisher Inc., USA). After 30 min the tubes were removed, put in an ice bath to stop the reaction and then left at room temperature. *n*-Butanol was added to the tubes and then centrifuged at 3000 × *g* for 10 min (Sorvall ST 16-R, Thermo Scientific Inc., USA) to measure the absorbance of the supernatant at 535 nm in a spectrophotometer (T70+ UV/Vis, PG Instruments Ltd, UK). To express the results in mg of malondialdehyde (MDA) per kg of fresh meat, the MDA concentration was calculated using its molar extinction coefficient (156 000 L mol^{-1} cm^{-1}).

The total sulfhydryl content (T-SH) was determined spectrophotometrically after derivatization by Ellman's reagent, 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB).[17] One gram of meat sample was homogenized in 20 mL of urea–sodium dodecylsulfate (SDS)–phosphate buffer saline (PBS) solution (8 mol L^{-1} urea, 3% SDS, 0.1 mol L^{-1} PBS, pH 7.4) with an Ultra Turrax (IKA T18 Basic), 1 min, 8000 rpm. Then, it was vortexed (1 min) and incubated for 1 h (Roto Mix, Thermolyne, 140 rpm). Subsequently, samples were filtered with Whatman No. 1 to obtain 4 mL of filtrate. An aliquot of 40 µL of sample filtrate was added to 1960 µL of urea–SDS–PBS solution followed by 600 µL of DTNB (10 mmol L^{-1} in 0.1 mol L^{-1} PBS). A blank reactive was prepared with 2 mL of urea–SDS–PBS solution and 600 µL of DTNB and a blank of samples was prepared with urea–SDS–PBS without DTNB. All tubes are vortexed and incubated in darkness, at room temperature for 15 min. Absorbance was recorded at 412 nm (T70 UV/Vis spectrophotometer, PG Instruments Ltd). T-SH content was calculated based on sample absorbance using a molar extinction coefficient of 13 600 L mol^{-1} cm^{-1}. The protein concentration in the samples was determined spectrophotometrically at 280 nm using a BSA standard curve (0.5–1.5 mg L^{-1}).[18] Results of thiol concentration are given in nmol thiol (T-SH) per mg of protein.

Assay for *in vitro* oxidation

Beef homogenate lipid oxidation with Fe^{2+}/H_2O_2 was measured following Mercier *et al.*[19] with slight modifications. Briefly, 2 g of frozen meat was homogenized in 20 mL of 0.15 mol L^{-1} KCl (pH 7.2) with an Ultra-Turrax (IKA T18 Basic) at 12000 rpm for 1 min on chilled recipe, then 5 mL of homogenate was incubated at 37 °C in a dry bath, under agitation, with 5 mL of a mixture of ferrous sulfate (0.5 mmol L^{-1}) and hydrogen peroxide (1 mmol L^{-1}) for 0, 30 and 60 min. After each incubation time, oxidations were stopped by addition of BHT (to 0.02% final concentration) to aliquots of 2 mL of homogenate. At each assessment time, lipid oxidation was measured by the TBARS method as described above. Results were expressed as mg of MDA per kg of meat.

Antioxidant enzyme activities

For the determination of catalase (CAT) and superoxide dismutase (SOD) enzyme activities, 3.15 g of frozen meat sample was homogenized in an Ultra-Turrax (IKA T18 Basic, Germany) at 18 000 rpm for 30 s with 35 mL of an extraction buffer (0.15 mol L^{-1} KCl, 0.1 mmol L^{-1} EDTA, pH 7.4) and centrifuged (Sorvall ST 16-R, Thermo Scientific, USA) for 10 min at 9000 × *g* and 4 °C. CAT activity was measured following the method of Aebi[20] placing 2730 µL of the extraction buffer, 180 µL of the sample supernatant and 90 µL of 3.53 mol L^{-1} H_2O_2 in a quartz cuvette, and recording the decrease in absorbance (H_2O_2 disappearance) at 240 nm every 30 s during 3 min with a spectrophotometer (T70+ UV/Vis, PG Instruments Ltd, UK). For the expression of the results in nmol H_2O_2 min^{-1} mg^{-1} protein, the molar extinction coefficient of H_2O_2 (39.4 L mol^{-1} cm^{-1}) was used and protein concentration in each sample was determined at 280 nm following the method described by Stoscheck.[18] SOD activity was measured following the method of Marklund and Marklund[21] with modifications of Gatellier *et al.*[22] which is based on the inhibition of pyrogallol autoxidation. A volume 2950 µL of 50 mmol L^{-1} phosphate buffer (pH 8.2), 40 µL of the sample supernatant and 10 µL of 10 mmol L^{-1} pyrogallol were placed in a quartz cuvette, and the increase in absorbance at 340 nm was recorded every 10 s during 2 min with a spectrophotometer (T70+ UV/Vis, PG Instruments Ltd, UK). Results were expressed as UI g^{-1} fresh meat, where UI (unit of inhibition) was assumed as the activity that inhibits the reaction by 50%.

Microbial counts

Total viable counts (TVC) were performed in the strip loin samples intended for the microbiological assessment. Meat samples were homogenized in sterile PBS, pH 7.4 (Sigma) with a ratio of mass to volume of 5:1 using a Seward Stomacher® 400. Subsequently, a series of 10-fold dilutions were carried out and then diluted samples were cultured on duplicate plates of plate count agar (Oxoid) and incubated at 37 °C for 48 h. Results were expressed as

Table 2. Sociodemographic characteristics of consumers and meat consumption frequency

Variable	Frequency relative (%)
Gender	
Female	39
Male	61
Age	
<30 years	8
30–50 years	75
>50 years	17

	Frequency of consumption (%)			
	Less than once a month	Once a month	Every 2 weeks	Every week
Pork	34	40	16	10
Beef	—	1	7	92
Chicken	1	8	33	58
Sheep	38	44	11	7

log(CFU g^{-1}) of the sample. When bacteria were not detected, a log value of half of the detection limit was used for the calculation of the mean number.

Sensory panel

Consumer panel was conducted according to the guidelines of the Declaration of Helsinki (Code of Ethics of the World Medical Association) for experiments involving humans.

After 120 days of storage, samples from the longissimus dorsi muscle were assessed by consumers and those stored frozen were previously thawed at 2 °C for 24 h. Steak samples for sensory evaluation were wrapped with aluminium foil and then grilled in a preheated clam shell style grill (GRP100 The Next Grilleration, Spectrum Brands, Inc., Miami, FL, USA) until the internal temperature measured with a thermometer (Comark N9094, Norwich, Norfolk, UK) in the geometric center reached 71 °C.[14] After cooking, external fat and connective tissue were removed, and samples were cut into ten pieces which were wrapped with aluminium foil, coded and placed in a heater to avoid them cooling down.

Untrained panelists ($n = 100$) evaluated four samples (1.5 cm × 1.5 cm × 2.5 cm) representing the combination of the two packaging types and the two storage conditions (four treatments). Each panel session included 10 panelists and lasted approximately 20 min. Individual panelists were supplied with a ballot, plastic eating utensils, a napkin, a cup of water and unsalted crackers to serve as a palate cleanser between samples. Before each panel session, participants filled out a brief demographic questionnaire and then verbal instructions were given outlining procedures for the sensory evaluation. Procedures were followed to reduce the effects of sample order of presentation and first order carry-over effects.[23] Each strip loin (steak) was evaluated by 10 consumers who assessed the same four strip loins.

Each consumer was asked to assess tenderness, flavor and overall liking acceptability on 8-point category scales: (1) like extremely, (2) like very much, (3) like moderately, (4) like slightly, (5) dislike slightly, (6) dislike moderately, (7) dislike very much and (8) dislike extremely. Characteristics of the participants

Table 3. Meat quality characteristics of longissimus thoracis muscle by packaging type (VP or VPAM) and storage conditions (CH or CHF) at 48 h post-mortem, and 28, 45, 90 and 120 d of storage

	Variable	VP	VPAM	*P*	CH	CHF	*P*
48 h post-mortem	WBSF (kg)	$5.66^{z} \pm 0.33$	$5.77^{z} \pm 0.33$	0.8155	$5.50^{z} \pm 0.33$	$5.93^{z} \pm 0.33$	0.3737
28 d storage		$2.34^{y} \pm 0.10$	$2.48^{y} \pm 0.10$	0.2992	$2.40^{y} \pm 0.10$	$2.42^{y} \pm 0.10$	0.8970
45 d storage		$2.73^{y} \pm 0.10$	$2.58^{y} \pm 0.11$	0.3070	$2.55^{y} \pm 0.10$	$2.76^{y} \pm 0.11$	0.1732
90 d storage		$2.36^{y} \pm 0.11$	$2.69^{y} \pm 0.11$	0.0342	$2.29^{y} \pm 0.11$	$2.77^{y} \pm 0.11$	0.0034
120 d storage		$2.54^{y} \pm 0.10$	$2.40^{y} \pm 0.11$	0.3402	$2.25^{y} \pm 0.10$	$2.68^{y} \pm 0.11$	0.0057
P		<0.0001	<0.0001		<0.0001	<0.0001	
Meat color[a]							
48 h post-mortem	*L**	$38.8^{x} \pm 0.49$	$38.2^{y} \pm 0.49$	0.3400	$38.6^{y} \pm 0.49$	$38.4^{y} \pm 0.49$	0.8172
28 d storage		$40.9^{zy} \pm 0.54$	$39.5^{zy} \pm 0.54$	0.0794	$40.6^{zy} \pm 0.54$	$39.9^{zy} \pm 0.54$	0.3578
45 d storage		$40.5^{yx} \pm 0.45$	$40.2^{z} \pm 0.45$	0.6653	$41.0^{z} \pm 0.45$	$39.7^{zy} \pm 0.45$	0.0527
90 d storage		$42.5^{z} \pm 0.53$	$40.4^{z} \pm 0.53$	0.0078	$41.9^{z} \pm 0.53$	$41.0^{z} \pm 0.53$	0.2854
120 d storage		$41.0^{zy} \pm 0.39$	$40.1^{z} \pm 0.39$	0.0800	$41.5^{z} \pm 0.39$	$39.6^{zy} \pm 0.39$	0.0009
P		0.0001	0.0093		0.0002	0.0058	
48 h post-mortem	*a**	22.0 ± 0.28	22.4 ± 0.28	0.3503	$22.3^{yx} \pm 0.28$	$22.1^{z} \pm 0.28$	0.7158
28 d storage		22.4 ± 0.33	21.8 ± 0.33	0.1980	$22.1^{x} \pm 0.33$	$22.1^{z} \pm 0.33$	0.9463
45 d storage		23.1 ± 0.35	23.0 ± 0.35	0.8505	$23.5^{zy} \pm 0.35$	$22.7^{z} \pm 0.35$	0.0916
90 d storage		22.2 ± 0.34	21.8 ± 0.35	0.3289	$22.9^{zyx} \pm 0.34$	$20.9^{y} \pm 0.35$	0.0011
120 d storage		22.2 ± 0.41	22.3 ± 0.41	0.9678	$23.6^{z} \pm 0.41$	$21.1^{y} \pm 0.41$	0.0002
P		0.3541	0.2085		0.0023	0.0180	
48 h post-mortem	*b**	$10.6^{y} \pm 0.21$	$10.9^{y} \pm 0.21$	0.2910	$10.7^{x} \pm 0.21$	$10.8^{y} \pm 0.21$	0.7956
28 d storage		$11.6^{z} \pm 0.22$	$11.1^{zy} \pm 0.22$	0.0944	$11.3^{yx} \pm 0.22$	$11.4^{zy} \pm 0.22$	0.7875
45 d storage		$12.1^{z} \pm 0.23$	$11.9^{z} \pm 0.23$	0.5466	$12.3^{z} \pm 0.23$	$11.8^{z} \pm 0.23$	0.1169
90 d storage		$11.8^{z} \pm 0.18$	$11.2^{zy} \pm 0.18$	0.0143	$11.9^{zy} \pm 0.18$	$11.1^{zy} \pm 0.18$	0.0099
120 d storage		$11.7^{z} \pm 0.19$	$11.4^{zy} \pm 0.19$	0.2675	$12.2^{zy} \pm 0.19$	$11.0^{zy} \pm 0.19$	0.0006
P		<0.0001	0.0215		<0.0001	0.0088	
48 h post-mortem	Cooking losses (%)	$22.0^{y} \pm 0.81$	21.7 ± 0.81	0.8035	$22.1^{zy} \pm 0.81$	21.6 ± 0.81	0.6358
28 d storage		$21.9^{y} \pm 0.59$	21.3 ± 0.59	0.5166	$22.0^{zy} \pm 0.59$	21.2 ± 0.59	0.3289
45 d storage		$23.0^{zy} \pm 0.53$	22.7 ± 0.53	0.6266	$23.8^{z} \pm 0.53$	21.9 ± 0.53	0.0214
90 d storage		$22.1^{y} \pm 0.55$	20.7 ± 0.55	0.0741	$21.5^{y} \pm 0.55$	21.3 ± 0.55	0.8706
120 d storage		$24.8^{z} \pm 0.69$	22.4 ± 0.69	0.0203	$24.1^{z} \pm 0.69$	23.1 ± 0.69	0.3188
P		0.0096	0.1783		0.0301	0.1665	

Least squares means with different superscripts in the same column differ significantly ($P < 0.05$).
[a] CIELAB color space, L^* = lightness, a^* = redness, b^* = yellowness.
VP, vacuum packaging; VPAM, vacuum packaging with antimicrobial; CH, meat chilled for 120 d; CHF, meat chilled for 28 d and then frozen for 92 d; WBSF, Warner–Bratzler shear force.

(gender, age and frequency of meat consumption) are presented in Table 2.

Statistical analysis

Response variables were analyzed as a 2 × 2 factorial design with type of packaging (VP or VPAM) and storage conditions (CH or CHF) as fixed effects and the strip loin (animal) as a random effect using the PROC MIXED procedure of Statistical Analysis System software (SAS Institute, Cary, NC, version 9.4). For the consumer panel data, the participant was included as a random effect. Studentized residuals were calculated to evaluate outliers and normality for all data. The Kenward–Roger approximation was used to calculate denominator degrees of freedom for different covariance structures for adjustment of the *F*-statistic. After analysis of variance, least squares means were calculated for treatment comparisons with a significance level of $\alpha = 0.05$, using the PDIFF option of LSMEANS, when *F*-tests were significant ($P < 0.05$).

RESULTS

Meat quality characteristics and sensory panel

The values of WBSF at 48 h post-mortem did not differ ($P > 0.05$) between packaging type (VP *versus* VPAM), storage conditions (CH *versus* CHF) and treatments (Tables 3 and 4). However, greater values ($P < 0.05$) of WBSF were observed at 48 h post-mortem compared to the end of the storage period after 120 d. Indeed, after 28 d of chilling storage conditions a significant decrease ($P < 0.05$) of WBSF values was observed in all treatments (Table 4). Furthermore, from day 28 to 120 of storage no differences ($P > 0.05$) of WBSF values were found within the same packaging type, storage condition and each treatment, except for VP + CH (Tables 3 and 4). After 120 d of storage, WBSF values did not differ ($P > 0.05$) among treatments.

In terms of color, meat was lighter (*L** values; $P < 0.05$) on day 120 of storage than 48 h post-mortem within the same packaging type (VP or VPAM), and within CH storage condition. Nevertheless, no differences ($P > 0.05$) were observed in the initial and final *L**

Table 4. Meat quality characteristics of longissimus thoracis muscle by treatment at 48 h post-mortem, and 28, 45, 90 and 120 d of storage

Time	Variable	VP + CH	VP + CHF	VPAM + CH	VPAM + CHF	*P*
48 h post-mortem	WBSF (kg)	$5.26^{x} \pm 0.48$	$6.06^{y} \pm 0.47$	$5.75^{y} \pm 0.48$	$5.79^{y} \pm 0.47$	0.4446
28 d storage		$2.25^{zy} \pm 0.14$	$2.42^{z} \pm 0.14$	$2.56^{z} \pm 0.14$	$2.41^{z} \pm 0.14$	0.2707
45 d storage		$2.66^{y} \pm 0.15$	$2.81^{z} \pm 0.15$	$2.44^{z} \pm 0.15$	$2.72^{z} \pm 0.16$	0.6773
90 d storage		$2.15^{z} \pm 0.15$	$2.57^{z} \pm 0.15$	$2.43^{z} \pm 0.15$	$2.96^{z} \pm 0.15$	0.7122
120 d storage		$2.23^{zy} \pm 0.15$	$2.84^{z} \pm 0.14$	$2.27^{z} \pm 0.15$	$2.52^{z} \pm 0.15$	0.4008
P		<0.0001	<0.0001	<0.0001	<0.0001	
Meat color[†]						
48 h post-mortem	*L**	$38.9^{y} \pm 0.69$	38.8 ± 0.69	38.3 ± 0.69	38.1 ± 0.69	0.9321
28 d storage		$41.6^{z} \pm 0.77$	40.2 ± 0.77	39.5 ± 0.77	39.5 ± 0.77	0.3363
45 d storage		$41.4^{z} \pm 0.64$	39.5 ± 0.64	40.5 ± 0.64	39.9 ± 0.64	0.2974
90 d storage		$43.4^{z} \pm 0.76$	41.6 ± 0.76	40.3 ± 0.76	40.5 ± 0.76	0.1952
120 d storage		$42.1^{z} \pm 0.55$	39.9 ± 0.55	40.9 ± 0.55	39.2 ± 0.55	0.6656
P		0.0007	0.0819	0.0813	0.1223	
48 h post-mortem	*a**	$21.7^{bx} \pm 0.40$	$22.4^{abz} \pm 0.40$	$22.9^{a} \pm 0.40$	$21.9^{ab} \pm 0.40$	0.0389
28 d storage		$22.5^{yx} \pm 0.47$	$22.2^{zy} \pm 0.47$	21.6 ± 0.47	21.9 ± 0.47	0.4955
45 d storage		$23.8^{zy} \pm 0.52$	$22.5^{z} \pm 0.49$	23.3 ± 0.49	22.8 ± 0.52	0.4575
90 d storage		$23.4^{zy} \pm 0.48$	$21.1^{zy} \pm 0.48$	22.4 ± 0.48	21.2 ± 0.50	0.2992
120 d storage		$24.0^{az} \pm 0.58$	$20.4^{cy} \pm 0.58$	$22.8^{ab} \pm 0.58$	$21.7^{bc} \pm 0.58$	0.0384
P		<0.0001	0.0073	0.1741	0.4382	
48 h post-mortem	*b**	$10.2^{by} \pm 0.29$	$11.0^{a} \pm 0.29$	$11.2^{a} \pm 0.29$	$10.6^{ab} \pm 0.29$	0.0250
28 d storage		$11.8^{z} \pm 0.31$	11.5 ± 0.31	10.9 ± 0.31	11.3 ± 0.31	0.2147
45 d storage		$12.4^{z} \pm 0.32$	11.9 ± 0.32	12.2 ± 0.32	11.7 ± 0.32	0.8910
90 d storage		$12.3^{z} \pm 0.26$	11.4 ± 0.26	11.4 ± 0.26	10.9 ± 0.26	0.5432
120 d storage		$12.4^{z} \pm 0.28$	11.0 ± 0.28	11.7 ± 0.28	11.0 ± 0.28	0.1709
P		<0.0001	0.7347	0.0824	0.1278	
48 h post-mortem	Cooking losses (%)	$22.0^{y} \pm 1.15$	22.0 ± 1.15	22.2 ± 1.15	21.2 ± 1.15	0.6674
28 d storage		$21.8^{y} \pm 0.83$	21.9 ± 0.83	22.2 ± 0.83	20.4 ± 0.83	0.2713
45 d storage		$24.5^{zy} \pm 0.75$	21.6 ± 0.75	23.0 ± 0.75	22.3 ± 0.75	0.1762
90 d storage		$22.4^{y} \pm 0.78$	21.8 ± 0.78	20.5 ± 0.78	20.9 ± 0.78	0.4966
120 d storage		$26.0^{z} \pm 0.98$	23.7 ± 0.98	22.3 ± 0.98	22.6 ± 0.98	0.1971
P		0.0282	0.3133	0.3324	0.4591	

[a–c] Least squares means with different superscripts in the same row differ significantly ($P < 0.05$). [x–z] Least squares means with different superscripts in the same column differ significantly ($P < 0.05$).
[†] CIELAB color space, *L** = lightness, *a** = redness, *b** = yellowness.
VP + CH, vacuum packaging and chilled for 120d; VP + CHF, vacuum packaging and chilled for 28 d and then frozen for 92 d; VPAM + CH, vacuum packaging with antimicrobial and chilled for 120 d; VPAM + CHF, vacuum packaging with antimicrobial and chilled for 28 d and then frozen for 92 d; WBSF, Warner–Bratzler shear force.

Table 5. Consumer panel assessment of longissimus thoracis muscle by type of packaging (VP *versus* VPAM), storage conditions (CH *versus* CHF) and treatments at the end of the storage period (120 d)

Variable	VP	VPAM	*P*	CH	CHF	*P*
Tenderness	3.47 ± 0.10	3.64 ± 0.10	0.1953	3.73^{b} ± 0.10	3.38^{a} ± 0.10	0.0105
Flavor	3.68 ± 0.10	3.86 ± 0.10	0.1105	4.07^{b} ± 0.10	3.47^{a} ± 0.10	<0.0001
Overall liking	3.64 ± 0.09	3.84 ± 0.09	0.0806	3.99^{b} ± 0.09	3.49^{a} ± 0.09	<0.0001

Variable	VP + CH	VP + CHF	VPAM + CH	VPAM + CHF	*P*
Tenderness	3.69^{ab} ± 0.14	3.24^{b} ± 0.14	3.76^{a} ± 0.14	3.51^{ab} ± 0.14	0.0346
Flavor	3.94^{b} ± 0.12	3.43^{b} ± 0.12	4.21^{a} ± 0.12	3.51^{b} ± 0.12	<0.0001
Overall liking	3.88^{ab} ± 0.12	3.41^{c} ± 0.12	4.11^{a} ± 0.12	3.57^{bc} ± 0.12	0.0003

Sensory scale: (1) like extremely, (2) like very much, (3) like moderately, (4) like slightly, (5) dislike slightly, (6) dislike moderately, (7) dislike very much and (8) dislike extremely. Least squares means with different superscripts in the same row differ significantly ($P < 0.05$).
VP, vacuum packaging; VPAM, vacuum packaging with antimicrobial; CH, meat chilled for 120 d; CHF, meat chilled for 28 d and then frozen for 92 d.

values in the CHF storage condition (Table 3). Except for the VP + CH treatment, *L** values did not differ ($P > 0.05$) in each of the treatments as the storage time increased. No effect of packaging type ($P > 0.05$) was detected in *a** values during the storage time and these values did not differ ($P < 0.05$) within VP or VPAM from 48 h post-mortem to 120 d of storage (Table 3). Meat under CH storage condition presented greater ($P < 0.05$) *a** values than CHF on day 120 of storage. Meat from VP + CH treatment had greater ($P < 0.05$) *a** values than VP + CHF and VPAM + CHF at 120 d of storage (Table 4). Meat from VP, CH and VP + CH showed greater ($P < 0.05$) *b** values on day 120 of storage than 48 h post-mortem (Tables 3 and 4).

Cooking losses were greater ($P < 0.05$) in VP on day 120 of storage compared to 48 h post-mortem and higher ($P < 0.05$) than VPAM at the end of the storage time (Table 3). The VP + CH treatment exhibited greater ($P < 0.05$) cooking losses on day 120 than 48 h post-mortem (Table 4).

Table 6. Lipid and protein oxidation and antioxidant enzyme activities of longissimus thoracis muscle by packaging type (VP or VPAM) and storage conditions (CH or CHF) at 48 h post-mortem, and 28, 45, 90 and 120 d of storage

	Variable	VP	VPAM	*P*	CH	CHF	*P*
48 h post-mortem	TBAR (mg MDA kg^{-1})	0.69^{zy} ± 0.03	0.79 ± 0.04	0.0656	0.74 ± 0.04	0.74^{x} ± 0.03	0.9927
28 d storage		0.75^{y} ± 0.03	0.78 ± 0.04	0.4697	0.78 ± 0.04	0.74^{yx} ± 0.04	0.4445
45 d storage		0.74^{y} ± 0.04	0.82 ± 0.05	0.2442	0.79 ± 0.03	0.77^{yx} ± 0.04	0.8512
90 d storage		0.82^{xy} ± 0.04	0.85 ± 0.05	0.5777	0.87 ± 0.06	0.80^{yx} ± 0.04	0.1941
120 d storage		0.85^{x} ± 0.04	0.91 ± 0.05	0.1962	0.87 ± 0.05	0.89^{y} ± 0.05	0.6331
P-values		0.0080	0.1739		0.0731	0.0300	
48 h post-mortem	T-SH (nmol	50.0^{z} ± 4.9	57.5^{z} ± 4.7	0.1789	53.4^{z} ± 2.4	54.04^{z} ± 4.9	0.9161
28 d storage	thiol mg^{-1} protein)	121.4^{x} ± 4.4	136.3^{x} ± 4.7	0.0343	123.2^{x} ± 4.9	134.4^{x} ± 5.4	0.1142
45 d storage		89.4^{y} ± 5.1	107.0^{y} ± 5.0	0.0257	112.8^{xy} ± 4.5	83.6^{y} ± 4.1	0.0007
90 d storage		90.5^{y} ± 3.7	107.6^{y} ± 3.8	0.0001	105.5^{y} ± 3.9	92.9^{y} ± 3.8	0.0249
120 d storage		89.3^{y} ± 4.3	111.7^{y} ± 4.3	0.0012	109.7^{y} ± 4.8	91.3^{y} ± 4.2	0.0098
P		0.0010	0.0013		0.0020	0.0001	
48 h post-mortem	CAT (nmol	2311^{x} ± 192	2059^{x} ± 189	0.3617	2243^{x} ± 198	2128^{x} ± 188	0.6781
28 d storage	H_2O_2 min^{-1} mg^{-1}	1891^{x} ± 171	2102^{x} ± 170	0.3878	2090^{x} ± 173	1902^{x} ± 171	0.4414
45 d storage	protein)	1635^{y} ± 223	1800^{xy} ± 220	0.6038	2033^{x} ± 212	1402^{y} ± 198	0.0420
90 d storage		1306^{y} ± 115	1345^{y} ± 143	0.7806	1496^{y} ± 132	1155^{y} ± 122	0.0106
120 d storage		1380^{y} ± 125	1244^{y} ± 170	0.4148	1576^{y} ± 190	1047^{y} ± 60	0.0007
P		0.0001	0.0010		0.0023	0.0200	
48 h post-mortem	SOD (IU g^{-1})	870^{x} ± 21	851^{x} ± 28	0.5979	811^{x} ± 36	911^{x} ± 21	0.0035
28 d storage		726^{y} ± 31	778^{x} ± 34	0.2544	743^{x} ± 44	760^{y} ± 39	0.7215
45 d storage		955^{x} ± 33	888^{x} ± 43	0.2553	940^{x} ± 74	904^{x} ± 36	0.5495
90 d storage		787^{y} ± 55	856^{x} ± 50	0.2242	790^{x} ± 55	853^{xy} ± 50	0.2608
120 d storage		681^{y} ± 34	542^{y} ± 28	0.0001	619^{y} ± 30	605^{z} ± 33	0.7259
P		0.0001	0.0210		0.0001	0.0080	

Least squares means with different superscripts in the same column differ significantly ($P < 0.05$).
TBAR, thiobarbituric acid reactive species; T-SH, total sulfhydryl content; CAT, catalase enzyme activity; SOD, superoxide dismutase enzyme activity; VP, vacuum packaging; VPAM, vacuum packaging with antimicrobial; CH, meat chilled for 120 d; CHF, meat chilled for 28 d and then frozen for 92 d.

Table 7. Lipid and protein oxidation and antioxidant enzyme activities of longissimus thoracis muscle by treatment at 48 h post-mortem, and 28, 45, 90 and 120 d of storage

Time	Variable	VP + CH	VP + CHF	VPAM + CH	VPAM + CHF	*P*
48 h post-mortem	TBAR (mg MDA kg^{-1})	0.69 ± 0.05	0.70 ± 0.05	0.80 ± 0.04	0.78 ± 0.07	0.3414
28 d storage		0.74 ± 0.04	0.76 ± 0.04	0.83 ± 0.04	0.74 ± 0.06	0.5392
45 d storage		0.68 ± 0.09	0.80 ± 0.04	0.89 ± 0.05	0.75 ± 0.08	0.1526
90 d storage		0.82 ± 0.04	0.83 ± 0.05	0.93 ± 0.06	0.78 ± 0.05	0.2107
120 d storage		0.85 ± 0.03	0.85 ± 0.03	0.89 ± 0.06	0.93 ± 0.05	0.5622
P		0.1037	0.1063	0.3729	0.2029	
48 h post-mortem	T-SH (nmol	49.0^{y} ± 2.6	50.8^{y} ± 6.0	57.8^{z} ± 8.7	57.2^{z} ± 2.8	0.6111
28 d storage	thiol mg^{-1} protein)	111.0^{bx} ± 9.7	131.8^{abx} ± 4.8	135.5^{abx} ± 6.2	137.1^{ax} ± 3.6	0.0200
45 d storage		106.9^{bx} ± 11.2	71.8^{dy} ± 3.6	118.7^{axy} ± 1.2	95.3^{cy} ± 2.2	0.0100
90 d storage		99.4^{abx} ± 7.5	81.7^{by} ± 1.5	111.6^{ay} ± 2.0	104.1^{ay} ± 4.9	0.0100
120 d storage		102.8^{ax} ± 2.9	75.8^{by} ± 7.8	116.6^{axy} ± 3.4	106.8^{ay} ± 6.9	0.0100
P		0.001	0.001	0.001	0.001	
48 h post-mortem	CAT (nmol	2377^{x} ± 288	2245^{x} ± 238	2108^{x} ± 350	2010^{x} ± 225	0.8049
28 d storage	H_2O_2 min^{-1} mg^{-1}	1814^{x} ± 186	1969^{x} ± 244	2367^{x} ± 309	1836^{x} ± 196	0.3412
45 d storage	protein)	1722^{xy} ± 360	1548^{xy} ± 306	2343^{x} ± 327	1257^{xy} ± 148	0.0847
90 d storage		1443^{y} ± 110	1170^{xy} ± 120	1550^{y} ± 154	1140^{xy} ± 132	0.0831
120 d storage		1720^{axy} ± 201	1040^{by} ± 56	1433^{aby} ± 184	1055^{by} ± 64	0.0100
P		0.001	0.001	0.001	0.001	
48 h post-mortem	SOD (IU g^{-1})	828^{x} ± 38	913^{x} ± 21	794^{x} ± 32	909^{x} ± 37	0.0531
28 d storage		725^{xy} ± 65	726^{xy} ± 31	762^{x} ± 33	794^{x} ± 47	0.6808
45 d storage		983^{x} ± 87	928^{x} ± 27	896^{x} ± 61	881^{x} ± 46	0.6287
90 d storage		772^{xy} ± 64	803^{xy} ± 59	808^{x} ± 50	904^{x} ± 49	0.3823
120 d storage		662^{aby} ± 36	700^{ay} ± 34	576^{bcy} ± 25	509^{cy} ± 33	0.0010
P		0.0001	0.0001	0.0001	0.0001	

$^{a–c}$ Least squares means with different superscripts in the same row differ significantly ($P < 0.05$). $^{x–z}$ Least squares means with different superscripts in the same column differ significantly ($P < 0.05$).
TBAR, thiobarbituric acid reactive species; T-SH, total sulfhydryl content; CAT, catalase enzyme activity; SOD, superoxide dismutase enzyme activity; VP + CH, vacuum packaging and chilled for 120d; VP + CHF, vacuum packaging and chilled for 28 d and then frozen for 92 d; VPAM + CH, vacuum packaging with antimicrobial and chilled for 120 d; VPAM + CHF, vacuum packaging with antimicrobial and chilled for 28 d and then frozen for 92 d.

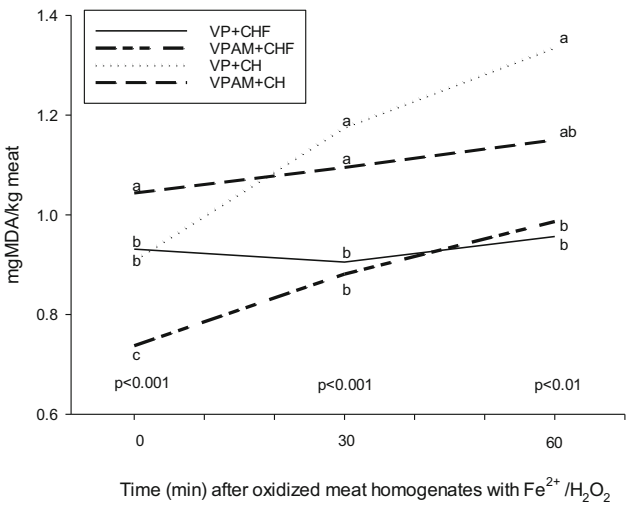

Figure 1. Resistance to oxidation during 60 min, expressed as TBARS level (mg MDA kg^{-1} meat) in beef homogenates oxidized by Fe^{2+}/H_2O_2 treated with different packaging and processes at 120 days of aging. VP + CH, vacuum packaging and chilled for 120 d; VP + CHF, vacuum packaging and chilled for 28 d and then frozen for 92 d; VPAM + CH, vacuum packaging with antimicrobial and chilled for 120 d; VPAM + CHF, vacuum packaging with antimicrobial and chilled for 28 d and then frozen for 92 d. Means with different letters at the each time point differ significantly ($P < 0.05$).

Consumer panel did not detect differences ($P > 0.05$) in tenderness, flavor and overall liking between VP and VPAM beef samples (Table 5). Nevertheless, consumers preferred CHF steaks ($P < 0.05$) rather than CH beef regarding the three characteristics evaluated. Steaks from VP + CHF treatment were preferred ($P < 0.05$) compared to VPAM + CH beef in terms of tenderness and overall liking. Meanwhile, the flavor of VPAM + CH steaks was the least preferred ($P< 0.05$) by consumers.

Lipid oxidation, total thiols and antioxidant activity

Lipid oxidation measured as TBARS values did not differ ($P > 0.05$) between VP and VPAM and between CH and CHF at any time during the storage period. However, greater ($P < 0.05$) TBARS values were detected on day 120 of storage than 48 h post-mortem in VP and CHF meat (Table 6). TBARS values were not affected ($P > 0.05$) by treatments at any time point of the storage period (Table 7). In addition, TBARS values did not change ($P > 0.05$) within each treatment over time (Table 7).

Total thiols content increased ($P < 0.05$) in VPAM compared to VP meat from day 28 of storage and in CH compared to CHF beef from day 45 of storage (Table 6). Regarding packaging type, greater ($P < 0.05$) content of total thiols was observed in VP and VPAM at the end of the storage period than 48 h post-mortem. Total thiols increased ($P < 0.05$) from 48 h post-mortem to 28 d of storage in all treatments. At the end of storage time, all

Table 8. TVC (log CFU g^{-1}) of longissimus thoracis muscle by type of packaging (VP *versus* VPAM), storage conditions (CH *versus* CHF) and treatments at 48 h post-mortem, and 28, 45, 90 and 120 d of storage

Time	VP	VPAM	*P*	CH	CHF	*P*	VP + CH	VP + CHF	VPAM + CH	VPAM + CHF	*P*
48 h post-mortem	$1.13^{az} \pm 0.09$	$1.10^{az} \pm 0.09$	0.8553	$1.06^{az} \pm 0.09$	$1.17^{az} \pm 0.09$	0.4343	$1.27^{bv} \pm 0.09$	$0.99^{abv} \pm 0.11$	$0.86^{av} \pm 0.09$	$1.35^{bv} \pm 0.15$	0.0265
28 d of storage	$3.23^{ay} \pm 0.18$	$4.54^{by} \pm 0.18$	<0.0001	$4.11^{ay} \pm 0.18$	$3.66^{ax} \pm 0.18$	0.0678	$3.46^{aw} \pm 0.27$	$3.00^{ax} \pm 0.31$	$4.75^{bw} \pm 0.27$	$4.32^{by} \pm 0.14$	0.0001
45 d of storage	$3.32^{ay} \pm 0.14$	$4.89^{bxy} \pm 0.14$	<0.0001	$5.10^{bx} \pm 0.14$	$3.04^{ay} \pm 0.14$	<0.0001	4.43^{bx} 0.30	$2.20^{aw} \pm 0.17$	$5.74^{cx} \pm 0.30$	$3.88^{bxy} \pm 0.12$	0.0001
90 d of storage	$4.23^{ax} \pm 0.20$	$5.34^{bwx} \pm 0.20$	0.0002	$6.28^{bw} \pm 0.20$	$3.30^{axy} \pm 0.20$	<0.0001	$5.34^{by} \pm 0.19$	$3.09^{ax} \pm 0.30$	$7.17^{cy} \pm 0.19$	$3.51^{awx} \pm 0.29$	<0.0001
120 d of storage	$4.54^{ax} \pm 0.17$	$5.53^{bw} \pm 0.18$	<0.0001	$7.03^{bv} \pm 0.17$	$3.05^{ay} \pm 0.18$	<0.0001	$6.47^{bz} \pm 0.26$	$2.62^{awx} \pm 0.32$	$7.58^{cy} \pm 0.26$	$3.46^{aw} \pm 0.23$	<0.0001
P	<0.0001	<0.0001		<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001	

$^{a–c}$ Least squares means with different superscripts in the same row differ significantly ($P < 0.05$). $^{v–z}$ Least squares means with different superscripts in the same column differ significantly ($P < 0.05$). VP, vacuum packaging; VPAM, vacuum packaging with antimicrobial; CH, meat chilled for 120 d; CHF, meat chilled for 28 d and then frozen for 92 d; VP + CH, vacuum packaging and chilled for 120d; VP + CHF, vacuum packaging and chilled for 28 d and then frozen for 92 d; VPAM + CH, vacuum packaging with antimicrobial and chilled for 120 d; VPAM + CHF, vacuum packaging with antimicrobial and chilled for 28 d and then frozen for 92 d.

treatments, except VP + CHF, presented a greater ($P < 0.05$) concentration of thiols than 48 h post-mortem (Table 7).

The activity of CAT enzyme did not differ ($P > 0.05$) between VP and VPAM at no time during the storage period, but a lower ($P < 0.05$) activity was observed in both packaging on day 120 of storage than 48 h post-mortem (Table 6). Meat frozen after 28 d of ageing (CHF) showed a lower ($P < 0.05$) CAT activity than CH meat from day 45 of storage. Meat from all treatments had a lower ($P < 0.05$) activity of the CAT enzyme at the end of the storage period compared to the beginning (Table 7). On day 120 of storage, meat from VP + CH treatment had greater ($P < 0.05$) activity of CAT enzyme than those treatments where meat was frozen.

The enzyme SOD had a greater ($P < 0.05$) activity in VP meat than VPAM at the end of the storage time while no differences ($P > 0.05$) were found between CH and CHF meat (Table 6). Meat from VP + CH and VP + CHF showed a greater ($P < 0.05$) SOD activity than VPAM + CHF treatment at the end of the storage period. In general, both antioxidant enzymes decreased their activities during the storage time. Meat that was frozen after 28 d ageing (CHF) exhibited a greater ($P < 0.05$) resistance to lipid oxidation than CH meat, expressed as mg MDA per kg of meat, during *in vitro* assay for 60 min, induced by Fe^{2+}/H_2O_2 (Fig. 1).

Total viable counts

TVC at 48 h post-mortem were relatively low and did not differ ($P > 0.05$) between packaging type (VP *versus* VPAM) or storage conditions (CH *versus* CHF) (Table 8). After 28 days of chilling storage conditions a significant increase ($P < 0.05$) of TVC values was observed in all treatments (Table 8). The increase of TVC in the VPAM steaks was significantly greater ($P < 0.05$) than in the VP beef (Table 8). Furthermore, in chilled loins (CH) from day 28 to 120 of storage, TVC increased over time and were always higher in the VPAM packaging condition. For loins frozen after day 28 of chilling storage (CHF), TVC remained almost constant or even decreased over time for both packaging conditions. TVC at the end of the storage period were lower ($P < 0.05$) in VP + CHF and VPAM + CHF than in those treatments under chilling conditions for 120 d (Table 8). Storage conditions (CH or CHF) had a greater ($P < 0.05$) impact on the microbial count than the type of packaging used (Table 8).

DISCUSSION

Meat quality and consumer panel

Previous research has shown that tenderness is the most important palatability attribute in meat affecting overall consumer acceptance.[24-26] Meat tenderness is mainly affected by the amount and solubility of connective tissue, the sarcomere length of muscle fibers and the extent of post-mortem proteolysis.[27]

In a review carried out by Lonergan *et al.*,[28] the effect of meat ageing on the increase of its tenderness is well documented. In the present study, WBSF values decreased significantly in all treatments from 48 h post-mortem to 28 d of storage when meat was under chilling conditions. In this sense, Gruber *et al.*[2] reported an improvement of meat tenderness of longissimus dorsi muscle up to 28 d under chilling conditions. It has been stated that a degradation process of proteins associated with the thick and thin filaments within myofibrils takes place in aged meat. Titin, nebulin and troponin-T have been identified as the main proteins whose disruption would be associated with loss of muscle cell integrity and thus to meat tenderization.[28] Calcium-dependent proteases,

known as calpains 1 and 2, have been extensively studied and considered mainly responsible for the post-mortem proteolysis. Once meat is frozen, proteolysis ceases due to suppression of calpain activity, but the enzymes remain functional and are re-activated after thawing.[29-31] In our study, after the first 28 d of storage, either chilling or frozen conditions did not affect WBSF values as the storage time increased.

It has been reported that during ageing meat tends to become lighter in color.[32] Protein degradation during ageing would trigger a myofibrillar spacing generating the opportunity for more light scattering and reflection.[33] In our study, L^* values of meat color were greater (lighter meat) on day 120 of storage in both packaging types (VP and VPAM) and in meat kept under CH conditions throughout the storage time. We did not observe lighter meat color on day 120 of storage for CHF, although a nonlinear increase has been reported in L^* values in frozen beef up to 8 months.[34] In the present study, redder color (greater a^* values) of steaks was found under CH condition than in CHF after 120 d of storage. Kim *et al.*[35] reported greater a^* values on days 1, 4 and 7 of retail display for meat aged for 4 weeks compared to steaks aged for 4 weeks and then frozen for 2 weeks. On the other hand, Farouk *et al.*[36] observed greater a^* values of beef with increase in ageing time prior to freezing. Those authors pointed out that the color of thawed beef improved by ageing the meat prior to freezing. Farouk and Wieliczko[34] found that the b^* values (yellowness) of meat color increased as the period of frozen storage extended but in the present study steaks frozen for 92 d after 28 d ageing (CHF) did not show greater b^* values on day 120 of storage.

Shanks *et al.*[37] observed no differences in cooking losses between beef steaks aged for 35 d compared to those aged for 35 d and then frozen for 2 months. However, Kim *et al.*[35] observed greater cooking losses in beef loins aged for 4 weeks than in those aged for 4 weeks and then frozen for 2 weeks. In addition, Farouk *et al.*[38] found that ageing time (0, 1, 3 and 9 weeks) prior to freezing did not affect the cooking losses. In our study, no differences were observed in cooking losses among treatments after 120 d of storage. However, VP beef presented greater cooking losses on day 120 of storage than 48 h post-mortem as was also reported by Colle *et al.*[39] in beef aged for 63 d compared to loins aged for 2 d.

A study conducted by Wiklund *et al.*[40] found that chilled beef longissimus dorsi muscle for 9 weeks was preferred by consumers in terms of tenderness compared to beef aged 3 weeks prior to frozen storage for 6 weeks. However, no differences were observed in overall liking between both treatments. A trained sensory panel did not detect differences in tenderness, flavor and general acceptability between beef aged for 10 d and steaks aged for 10 d and then frozen for 90 d.[41] In our study, consumers preferred the tenderness, flavor and overall liking of CHF steaks to CH beef. However, it is important to highlight that both CHF and CH meat were scored positively (at least 'like slightly'). It has been reported that ageing prior to freezing may be an effective procedure to improve beef tenderness and sensorial properties.[40,42] The flavor of VPAM + CH treatment was the least preferred by consumers. We hypothesized that meat stored for 120 d under CH condition and packaged with VPAM bags that had greater OTR and MVTR would promote the slight appearance of some off flavors having a small negative impact on consumers' preferences.

Lipid oxidation, total thiols and antioxidant enzyme activities

Storage at 28, 45, 90 and 120 d did not trigger a significant increase in lipid oxidation as reported by a previous study,[43] mainly due to long storage that promotes the release of iron from heme proteins which has a main role in the initiation and propagation phases of lipid oxidation. However, VP meat and frozen meat after 28 d ageing showed an increase of MDA at the end of the storage period contrary to what was expected as reported by Xiong.[44] Ageing is a complex process that involves proteolysis and lipolysis pathways with many molecules formed that enhance and change attributes such as flavor and texture, antioxidant capacity and loss of solubility by changing protein structure, depending on storage conditions.[45] MDA is one of the most important aldehydes produced during the secondary lipid oxidation of polyunsaturated fatty acids. This aldehyde is considered the major biomarker of lipid oxidation, largely used in routine analysis[46] because, at low amounts, it produces rancid aromas[47] and has a good association with sensory evaluation. Previous research pointed out values of 2–2.5 mg MDA kg^{-1} as the accepted threshold up to which rancidity would not occur in meat and meat products.[48,49] However, Domínguez *et al.*[45] stated that lipid oxidation measured as TBARS provides information about part of the oxidative process and therefore has some limitations. In our study, lipid oxidation values were aligned with the sensory assessment by the consumer panel. The low values of MDA obtained for each treatment on day 120 of storage would be associated with the positive (i.e. at least 'like slightly') overall liking scores assigned by consumers, indicating that probably rancidity was not perceived by consumers.

Thiols (SH) in meat are primarily located in muscle proteins[50] having cystine and cysteine as carriers. They have an important function in nutritional value, meat quality and processing. The SH group is one of most reactive groups in proteins and its loss during storage or processing has detrimental effects on protein solubility and affects quality attributes. In the present study, T-SH increased in all treatments on day 28 of storage reflecting the active metabolism of all compounds containing SH, such as sarcoplasmic and myofibrillar proteins, and no proteins such as glutathione and cysteine,[50] that occurs during ageing.[51] A review conducted by Hofmann and Hamm[50] has shown an increase of total SH during storage of frozen meat, but these findings are not consistent because factors such as temperature of freezing, packaging and time affect the oxidation of thiols released by the proteins. It is well documented that SH compounds have an antioxidant effect that delays the rancidity of lipids in meat,[52] and recent studies define SH as the first line of defense against lipids deterioration.[53] Indeed, residual thiols formed during meat storage coming from proteolysis of myofibrillar protein act as a protection, when in intact form, against free radicals formed.[53] When pro-oxidant conditions are present, thiols are rapidly oxidized, and this is one possible explanation for the decrease of T-SH observed in VP + CHF meat. These pro-oxidant conditions could be generated by lipid oxidation, since MDA values increased in VP meat and in CHF storage condition, as presented in Table 6. However, on day 120 of storage, VP + CHF meat showed the highest resistance to oxidation induced by Fe^{2+}/H_2O_2, and lower CAT and SOD enzyme activities than 48 h post-mortem.

Freezing affected the activity of CAT enzyme more than packaging type did, but SOD activity was affected by packaging type more than the storage condition. Then enzyme CAT is known for its ability to protect cells from the oxidizing action of hydrogen peroxide (H_2O_2) produced by SOD[54] and CAT would be stable during refrigerated and frozen storage of meat.[55] In our study, when analyzing the storage condition (CH and CHF) the CAT activity decreased from day 45 or 90 of storage. On day 120 of storage,

meat from VP + CHF and VPAM + CHF showed lower CAT activity than VP + CH treatment. These results would suggest that CAT enzyme activity diminishes after frozen storage of meat for 92 d.

The enzyme SOD is the first line of defense against injury in the antioxidant system, by transforming oxygen free radicals into H_2O_2.[56] Its activity indirectly reflects an important aspect of the endogenous antioxidant capacity of an organism.[57] It is produced continuously *in vivo* but in post-mortem conditions what remains must face and scavenge the increase in free radicals generated during meat storage.[57] Zheng *et al.*[58] reported that SOD activity decreases in meat during long cold storage depending on animal type, breed, muscle and storage conditions. The present study showed a decrease of SOD activity at the end of the storage time in all treatments, evidencing that antioxidant activity would be higher than the oxidation produced during long storage, which probably could be associated with a pasture-fed rearing phase prior to the fattening period with concentrate.[57] Finally, considering the response of different parameters of oxidation measured in the present study, it is possible that the active metabolism of proteins and redox-active muscle components during post-mortem period[59] could have a protective role against oxidation that would save up endogenous antioxidant enzymes for a long period. We hypothesized that although steers were fattened with concentrates, their previous backgrounding phase under pastoral systems would improve the antioxidant status of beef with a positive impact on meat shelf-life.

Microbial counts

Microbial growth has a significant impact on shelf-life and quality of vacuum-packaged beef stored under refrigerated conditions.[60,61] At the beginning of the study the low microbial load observed on meat samples would be related to the good hygienic practices of the abattoir. The levels of TVC on day 28 showed that microbial growth was favored under VPAM packaging conditions. The film used in VPAM had a greater OTR and MVTR, suggesting that the presence of oxygen within the package favored microbial growth that could not be prevented by the addition of the antimicrobial agent based on silver ions. Although we did not find an effect of the antimicrobial agent on TVC, Yusof *et al.*[62] reported the effectiveness of the same antimicrobial in reducing total plate counts in chicken breast meat. The onset of freezing on day 28 inhibited microbial growth preventing microbial proliferation which may reduce the rate of spoilage compared to chilled meat.[1,5] TVC after 90 d of storage was similar between VP + CHF and VPAM + CHF. Although similar in microbial counts, we have shown in a previous study[63] that the microbiome composition in VP + CHF and VPAM + CHF differed and was composed mainly of lactic acid bacteria and *Pseudomonas* spp., respectively. Lactic acid bacteria are recognized as causative agents of vacuum-packaged meat spoilage, but they also delay spoilage caused by other bacteria through the production of organic acids and bacteriocins.[64] TVC in CHF meat reached a stationary phase around 2–3 log CFU g^{-1} while in CH beef reached 5–7 log CFU g^{-1} at the end of the storage period. This would suggest that the end of shelf-life of CH meat would be closer to 120 d compared with meat that was kept frozen from day 28 of storage which presented lower bacterial counts at the end of storage.

CONCLUSIONS

Storage conditions had a greater impact on meat storage life than packaging type. CHF meat presented lower levels of microbial counts. The greater OTR and MVTR of the VPAM film promoted microbial growth that could not be mitigated by the antimicrobial agent. The oxidative deterioration did not reach levels that would determine negative scores (at least "dislike slightly") of the meat samples by the consumer panel. Freezing meat after an ageing period represents a suitable strategy to extend beef storage life without negatively affecting its quality.

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DATA AVAILABILITY STATEMENT

Research data are not shared.

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