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Yield and Quality Attributes of Aged Beef through Lipid Coatings: A Comparative Study of Milk Butter and Pork Lard

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Abstract: A new type of aging has been employed in which the meat receives a layer of fat prior to aging, known as being butter-aged. This study investigated the effects of using milk butter and pork lard as coating materials for loin during 28-d aging, focusing on yield and quality indicators. Samples were weighed ($n = 12$ per treatment; wet-aged, butter-aged, and lard-aged) throughout the process to determine yield indicators. After aging, samples were evaluated for physicochemical (pH, moisture and total lipids, color stability, cooking loss, instrumental tenderness, and protein oxidation) and microbiological counts. Data were analyzed as repeated measures in time (color) or factorial analysis of variance (yield and physicochemical traits) (Software Statistica 10.0). The samples aged with lipid coatings had lower final yield and surface water activity compared with wet-aged ($P < 0.01$). The type of aging did not influence inner water activity, moisture content, lipid content, cooking loss, instrumental tenderness, and carbonyl content ($P > 0.05$). During display, samples aged with fat coatings showed more intense discoloration (redness) compared with wet-aged, whereas aging with butter exhibited higher lipid oxidation compared with the other treatments ($P < 0.001$). Wet-aged showed the lowest microbial counts. Samples with fat coatings had higher microbial counts, particularly for psychrotrophic, mesophilic, and lactic acid bacteria groups ($P < 0.05$), with signs of deterioration. Using the same amount of fat in the meat coating, the sample with butter had a higher final yield, but the sample with lard had better color and microbiological quality. However, the lipid-coated aging process clearly has no advantages over wet aging under the conditions applied to the study because it results in exposure with color problems and represents a potential health risk. Therefore, we suggest further studies in which a shorter aging time is applied for the commercial viability of these products.

Introduction

The primary attribute assessed by consumers when consuming beef is tenderness (Aboah and Lees, 2020). The advancement of understanding of the mechanisms influencing tenderness and the implementation of technologies to enhance it should be actively encouraged to ensure consumer satisfaction, particularly among the most discerning clientele. Among the key methods to improve meat tenderness, the aging stands out, which relies on the action of endogenous enzymes present in the muscle tissue on the meat proteins (Bhat et al., 2018). It results in a structural disruption of the myofibrils, which enhances the tenderness of the

meat. Wet and dry aging techniques are employed for this purpose.

For wet aging, the most common type of aging, meats are packed in plastic film and kept under vacuum, which, due to low oxygen pressure, can alter the flavor and color, potentially influencing the consumer at the time of purchase. Dry aging, on the other hand, occurs without any packaging, leading to the evaporation of water from the meat, concentrating flavor and aroma compounds. However, due to potential losses of up to 50%, the cost and selling price are higher (Dashdorj et al., 2016).

To address some of the issues associated with different aging methods (off-flavor in wet-aged and lower yield in dry-aged meat), the meat market has introduced a new form of aging, known as butter-aged, which involves the use of a lipid coating (Rezende-de-Souza et al., 2021). In this process, a layer of butter is placed over the meat, increasing the process yield compared with dry-aged (Rezende-de-Souza et al., 2022). Furthermore, it is empirically reported in digital media that butter-aged meat exhibits enhanced flavor compared with those wet- and dry-aged. Moreover, no studies focused on the utilization of alternative fat sources to milk butter for cost reduction. Also, no studies evaluate the final quality of fat-coating–aged beef. Therefore, this pioneering study aimed to investigate the effects of dairy butter and pork lard as coating materials for meat during 28 d of aging, focusing on process yield and quality indicators.

Materials and Methods

Obtaining and processing samples

Vacuum-packaged bone less loins (*longissimus thoracis et lumborum* from the eighth thoracic vertebra to the 40 lumbar vertebra/∼60 cm) were obtained from Zebu cattle at a federally inspected commercial slaughterhouse regulated by the Federal Inspection Service 6 d post-slaughter. A total of 12 loins were used. After unpackaging, all apparent subcutaneous fat was removed from loins, though the connective tissue seam was kept intact. A 1.5-cm steak (cranial) was taken for volatile compound evaluation of nonaged samples. Subsequently, each loin was divided into 3 equal sections (∼20 cm each), totaling 36 sections. These sections were distributed in blocks, respecting the cranial, central, and caudal positions, in 3 different aging treatments: wet-aged—vacuum-aged; butter-aged—using unsalted milk butter (Milkpar, Dois

Vizinhos, Brazil); and lard-aged—using refined pork lard (Seara, São Paulo, Brazil).

Initially, all the samples were weighed to calculate the yield. The sections for the wet-aged treatment were vacuum-sealed (Cryovac BB 2620; Cryovac Brasil Ltd., São Paulo, Brazil; 50 μm thick, oxygen permeability of 20 cm^3 m^{-2}, 24 h, at 23°C, and 75% relative humidity [RH]; and maximum carbon dioxide permeability of 100 cm^3 m^{-2}, 24 h, at 23°C, and 75% RH) and placed in the aging chamber. For samples designated for lipid coating, sections were initially hung in the aging chamber (using food-grade twine and butcher hooks) for 48 h at 2°C, with RH of 80% ± 10% and an air flow of 2.5 ± 0.5 m/s, to allow an initial surface drying before applying the fats. This was done to ensure better adhesion of the fat to the meat surface, as determined by preliminary studies conducted in our laboratory. All the samples were aged in the same aging chamber.

After the 48-h drying period, the samples were weighed again, and the lipid coating process was initiated. For this, a single 10 kg batch of each lipid source (butter or lard) fat type was melted at 50°C, then cooled to a higher viscosity consistency, i.e., resistant to falling when lifted with a spatula. The initial temperatures of the fats during the coating process were 18°C for butter and 17°C for lard, determined by preliminary studies (Rezende-de-Souza et al., 2022).

For the coating, the entire loin section was immersed once in the fat to form a coating of approximately 1.5-cm thickness, as determined by preliminary studies. Some variation in thickness was observed on "corners," but most area was close to 1.5 cm thick. Subsequently, the loin sections were hung in the aging chamber for at least 2 h to allow the fat to dry before being weighed again. After weighing, the loin sections remained in the chamber for more 26 d, until the aging process was completed.

Aging conditions

Throughout the process, the aging chamber temperature was kept at 2°C ± 1°C, with RH of 80% ± 10% and an air flow of 2.5 ± 0.5 m/s. Samples from the wet-aged treatment were aged for 28 d, whereas samples from the lipid treatments (butter- and lard-aged) were dried for 2 d without coating, followed by an additional 26 d with the lipid coating, totaling 28 d of aging.

Characterization of coating fats

Before coating the meat, portions of samples of milk butter and pork lard were collected for characterization by determining the fatty acid profile and

crystallization and melting temperatures. The fatty acid profile was determined using chromatography (AOCS, 2009) on an Agilent 6850 Series GC System capillary gas chromatograph (Agilent, Santa Clara, CA) using an Agilent DB-23 capillary column (50% cyanopropyl-methylpolysiloxane), with dimensions of 60 m, an internal diameter of 0.25 mm, and a film size of 0.25 μm. The chromatograph's operating conditions were column flow rate of 1.00 mL/min, linear speed of 24 cm/s, detector temperature at 280°C, injector temperature at 250°C, oven temperature at 110°C for 5 min, followed by 110°C to 215°C (5°C/min) and 215°C for 24 min, using helium as the carrier gas, with an injected sample volume of 1.0 μL.

Crystallization and melting temperatures were determined using the AOCS Cj 1-94 method (AOCS, 2009). For this, approximately 10 mg of liquid sample was analyzed in a TA Q2000 differential scanning calorimeter, coupled to the RCS90 Refrigerated Cooling System (TA Instruments, Waters LLC, New Castle, DE). The conditions used to determine crystallization events were 80°C for 10 min, 80°C to −40°C at a rate of 10°C per minute, whereas for melting events, the conditions were −40°C for 30 min, −40°C to 80°C at a rate of 5°C per minute.

Process yield

At the end of the aging period, weighing was conducted to determine the process yield. In total, loin sections were weighed at 6 different time points: before the 48-h drying period, after the 48-h drying period, before vacuum packaging or lipid coating, immediately after the coating process, after the aging process with the coatings, and after the removal of packaging and fat. The calculations were based on the ratio of the initial weight to the weight at each stage of the process, according to Equations 1 to 5:

$$FA = (F \times 100)/M \tag{1}$$

$$DL_{2d} = ((Wi - Wf)/Wi) \times 100 \tag{2}$$

$$AL_{26d} = 100 - ((Wf \times 100)/Wi) \tag{3}$$

$$TL = DL + DL_{2d} + E \tag{4}$$

$$TY = 100 - TL \tag{5}$$

For the equations, the following definitions must be considered: FA (%) = fat adhesion; DL_{2d} (%) = loss due to dehydration in the first 2 d; AL_{26d} (%) = loss due to dehydration in the 26 d of aging; TL (%) = total loss during aging in the 28 d; TY (%) = total process yield;

Wi (g) = initial weight (day 0); Wf (g) = final weight (day 28); F (g) = weight of adhered fat; M (g) = weight of meat without fat; and E (g) = exudate.

Sample preparation for meat quality assessments

After aging and removal of the packaging or lipid coating, approximately 100 g of surface samples were taken for microbiological evaluation. These processes were carried out aseptically, cleaning and sanitizing the table, knives, and all accessories used for collection.

The sections were then cut into 2.5-cm-thick steaks for further analyses. One steak was designated for water activity (not frozen). The steaks designated for color stability and lipid oxidation were individually identified, placed in expanded polystyrene trays, wrapped in polyvinyl chloride (PVC) film, and refrigerated at 2°C. The samples used for other analyses (pH, total moisture and total lipids, cooking loss, instrumental tenderness, protein oxidation [carbonyl], and volatile compounds) were identified, vacuum-sealed, and frozen at −18°C until the time of analysis. Prior to analysis, the samples were thawed at 4°C for 24 h.

Microbiological analyses

The microbiological analyses were conducted using serial dilutions according to the official methodologies described by the American Public Health Association (Salfinger and Tortorello, 2015) and ISO 15214:1998 (International Organization for Standardization, 1998). The following microbial counts were performed: psychrotrophic and total mesophilic bacteria, lactic acid bacteria, molds and yeasts, and enterobacteria. The initial sample for dilution 10^1 was 25 g of sample homogenized in 225 mL of 0.1% peptone solution. In total, dilutions of up to 10^8 were used with 2 plates per dilution used.

The surface technique was used to count molds and yeasts, with Rose Bengal Chloramphenicol Dichloran Agar, under incubation conditions of 25°C for 5 d, and also for the analysis of total psychrotrophs, using the standard counting agar culture medium and an incubation temperature of 7°C for 10 d. Quantification of total mesophiles and enterobacteria, in turn, took place using a standard counting system on deep plates, using standard counting agar and red violet bile agar with glucose, respectively; both were incubated at 35°C for 48 h. Total lactic acid bacteria were also cultured in a depth-plating system using de Man–Rogosa–Sharpe agar, in which the plates were incubated at 30°C for 72 h.

Water activity and pH

Water activity was determined in both the surface and inner region of steaks. They were knife-cut into 2 horizontal strips of 0.5 cm each, starting from the dorsal region of the steak (in contact with fat coating) to the ventral region. The strip just below the fat was identified as "surface," and the bottom strip was identified as "inner." The 4TE instrument from Decagon Devices Inc. (Aqualab, Pullman, WA) was used for water activity measurement (Bernardo et al., 2020c).

The pH measurement procedure involved the initial calibration of the equipment with pH 7 and 4 buffer solutions, as previously described by Zenebon et al. (2008). The pH values were determined by inserting the probe in 3 random locations in the lean surface on 2.5-cm-thick steak samples employing the MP125 pH meter (Mettler-Toledo, Columbus, OH). The electrode was meticulously cleaned with distilled water between each replicate measurement.

Total moisture and lipids

The moisture and lipid content were determined using near-infrared spectroscopy with the assistance of the FoodScan 2 PRO equipment (FOSS, Hillerød, Denmark) (AOAC 2007.04; AOAC, 2007). The lean part of the steak was coarsely ground for 10 s using a conventional food grinder, and 180 g of the sample was spread evenly on a plate for subsequent reading. Each plate was read by the equipment 16 times to represent a single final reading per replicate. Three replicates were carried out on each sample.

Protein oxidation

Protein oxidation was determined using the colorimetric method for quantifying carbonyl content (Colombo et al., 2016). Myofibrillar proteins were extracted by homogenizing 3 g of meat in 20 mL of isolation buffer pH 7.0 (10 mM monosodium phosphate [NaH_2PO_4], 100 mM sodium chloride [NaCl], 2 mM magnesium chloride [$MgCl_2$], 1 mM ethylenediaminetetraacetic acid [EDTA]), followed by a series of 4 centrifugations ($3,000 \times g$ at 4°C for 15 min). Before the final centrifugation, the pellet was resuspended in 10 mL of 100 mM NaCl, homogenized, and filtered through voile fabric. The obtained pellets were diluted in 5 mL of deionized water. The protein carbonyl content was calculated by using an absorption coefficient of 22,000 M^{-1}/cm^{-1} and expressed in nanomoles of carbonyls per milligram of protein.

Color stability

The steaks prepared after aging were placed on a polystyrene tray covered with PVC film (as mentioned previously), put in a refrigerated (2°C) nonlight cooler, and were subjected to color measurement using a portable colorimeter MiniScan MSEZ 1314, v. 2.0 (HunterLab, Reston, VA), using the parameters of lightness ($L*$) and red/green ($a*$) and yellow/blue ($b*$) coordinates. The measurements were performed with illuminant D65, an 8° viewing angle, and a standard 10° observer (King et al., 2023), with prior calibration using black and white calibration plates, following the calibration steps provided by the instrument manufacturer. Colorimetric readings were taken on days 1 (24 h after exposure) and 3, and day 6 of storage on display, in triplicate for each sample.

Lipid oxidation

To determine lipid oxidation, the method used was the thiobarbituric acid reactive substances (TBARS) colorimetric assay, adapted from Bruna et al. (2001). For this, the samples were prepared prior to display storage: each sample was divided into 3 pieces and placed together in the same expanded polystyrene tray, wrapped with PVC film, and stored in a chamber at 2°C. On days 1, 3, and 6 of display, a portion of each steak was taken for analysis.

For this, 5 g of sample previously ground in a Grindomix GM 200 knife mill (Retsch, Haan, Germany) was homogenized for 20 s using an Ultra-Turrax T 25 (IKA, Staufen im Breisgau, Germany) with 10 mg of butylated hydroxytoluene (BHT) and 20 mL of 5% trichloroacetic acid. The mixture was centrifuged in an H2050R-1 centrifuge (Cence, Hunan, China) at 3,500 RPM for 10 min at 7°C. The solution was filtered using qualitative filter paper, and 3 mL of the filtrate was mixed with 3 mL of 0.02 M 2-thiobarbituric acid. The mixture was heated at 100°C for 1 h, and after cooling to room temperature, it was read in duplicate by using a UV-VIS AquaMate spectrophotometer (Thermo Fisher, China) at 532 nm.

Cooking loss and Warner-Bratzler shear force

The steps were based on a methodology described by the American Meat Science Association (2016). After thawing, the 2.56-cm steak, still raw, was weighed and placed on a baking tray with a rack. A thermocouple was inserted into the central region of the steak, and it was cooked in a conventional oven

(90 × 90; Fritomaq, São Paulo, Brazil), at 165°C. Cooking was completed when the internal temperature of the sample reached 71°C. Immediately after being removed from the oven, the steak was weighed to determine the cooking loss. Then, the cooked steak was cooled in a chamber at 4°C for 24 h. After cooling, 6 cores with a diameter of 1.27 cm were removed from the same steak, parallel to the longitudinal direction of the muscle fiber, using a coring cutter sampler. The cylinders were sheared using a TA.XT texture analyzer (Stable Micro Systems, Surrey, UK), with the assistance of a 1-mm-thick Warner-Bratzler blade, at a speed of 3.7 mm/s.

Volatile compounds

In addition to the aged samples from the 3 treatments (wet-aged, butter-aged, and lard-aged), the volatile compounds of fresh samples (unaged meat, day 0) were also evaluated.

The steaks were placed on a grill and cooked in a Ford 9 electric oven (NKS, São Paulo, Brazil) preheated to 180°C for 10 min until reaching 75°C at the center of the steak. The internal temperature was monitored using a thermometer. After cooking, the samples were placed in a plastic bag and kept in an ice bath for 10 min to halt the cooking process. Subsequently, the sample was ground using a Viva RI1364/06 food processor (Walita, São Paulo, Brazil).

For volatile compounds extraction, 1 g of the cooked and ground sample was mixed with 5 mL of a saline solution saturated with 0.01% BHT. The vial was sealed with a polytetrafluoroethylene (PTFE)/silicone septum, and the mixture was agitated at 30°C. A solid-phase microextraction fiber carboxen/polydimethylsiloxane was inserted into the sample and allowed to reach equilibrium for 5 min, followed by a 65-min exposure for volatile compound extraction.

Analysis was performed with a QP2010 gas chromatograph (Shimadzu, Kyoto, Japan) coupled with a quadrupole mass spectrometer. Thermal desorption of the analytes from the fiber was carried out at 250°C using a split/splitless injector in splitless mode for 1 min. The fiber remained in the injector for 12 min to ensure complete desorption of the volatile compounds. A fiber blank was performed between each extraction and injection procedure to confirm the absence of carryover effects.

The volatile compounds were separated on a DB-5ms column (5% phenyl, 95% dimethylpolysiloxane) with dimensions of 60 m × 0.25 mm internal diameter and 1 μm of stationary phase thickness (J&W Scientific, Folsom, CA). The oven temperature started at 40°C, increased at a rate of 4°C/min to 180°C, and then increased at a rate of 10°C/min to 280°C, remaining at this temperature for 5.3 min.

Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The quadrupole mass detector was operated under the following conditions: ionization energy of 70 eV, interface temperature of 300°C, ion source temperature of 250°C, and scanning mode, monitoring the mass-to-charge ratio (m/z) range of 35 to 350. Compounds were identified by their mass spectra, compared with the library database of gas chromatography/mass spectrometry (Thomas, 2002). To confirm identification, an n-alkane (C7-C30) (Supelco, Bellefonte, PA) solution was injected into the equipment under the same conditions as the samples to obtain the linear temperature programmed retention index (LTPRI) of the volatile compounds. The experimental identification was performed by comparing the LTPRI and the mass spectra to the reports from the literature, with a similarity of a minimum of 85% and maximum variation of ±10 (Thomas, 2002).

Data analyses

The results were evaluated using analysis of variance (ANOVA), and the means were compared with the Tukey test at a significance level of 5% in Statistica 10.0 software. For the parameters of yield, pH, surface and inner water activity (A_w), total moisture and total lipids, cooking loss, and instrumental tenderness, one-way ANOVA was used, with the type of aging as the source of variation. For color stability ($L*$, $a*$, and $b*$), variables were analyzed as repeated measures in time (days of display), whereas for lipid oxidation (TBARS), a 2-way factorial ANOVA was applied.

The qualitative analysis of volatile compounds was conducted based on their retention time and peak areas, with the reported volatile compounds being unique representatives of each respective aged treatment.

Results and Discussion

Characterization of coating fats

Butter, a dairy-derived product, primarily comprises saturated fatty acids in its composition, with a notable presence of palmitic acid (C16:0), whereas pork lard predominantly features unsaturation in its

total fatty acid composition, particularly oleic acid (C18:1) (Table 1).

An increase in saturated fatty acids within a lipid matrix raises its melting point, resulting in a firmer texture even at room temperature (Panchal and Bhandari, 2020). As demonstrated in Table 2, butter exhibits a higher melting point compared with pork lard, leading to its firm texture during meat aging, whereas pork lard exhibits greater plasticity when refrigerated during sample aging.

Process yield

No significant difference was found in the initial dehydration weight loss, calculated within the first 48 h of aging without lipid coating ($P = 0.65$), nor in the fat adhesion rate ($P = 0.06$) between all treatments. However, the treatment with pork lard had a higher aging loss compared with the samples coated with butter ($P < 0.01$) (Table 3). The texture of the fats may have influenced this loss. This is due to milk butter solidifying at refrigeration temperatures, whereas pork

Table 1. Fatty acid profile (m/m) of butter and lard used on loins for aging lipid-coated meats

Fatty acids	Butter	Lard
Total saturates	**69.91**	**36.44**
C4:0 butyric	1.59	0.00
C 6:0 caproic	1.89	0.00
C 8:0 caprylic	1.26	0.00
C10:0 capric	2.91	0.09
C12:0 lauric	3.49	0.11
C14:0 myristic	11.84	1.24
C15:0 pentadecanoic	1.15	0.06
C16:0 palmitic	33.08	22.42
C17:0 margaric	0.73	0.31
C18:0 stearic	11.64	11.70
C20:0 arachidic	0.21	0.20
C22:0 behenic	0.07	0.31
C24:0 lignoceric	0.05	0.00
Total unsaturated	**26.31**	**42.98**
C16:1 palmitoleic	1.87	2.70
C17:1 cis-10-heptadecenoic	0.25	0.18
C18:1 trans elaidic	2.53	0.11
C18:1 oleic	21.51	39.36
C20:1 eicosenoic	0.15	0.63
Total polyunsaturates	**3.84**	**19.82**
C18:2 linoleic	2.14	18.82
C18:2 trans t-linoleic	0.52	0.00
C18:3 linolenic	0.29	1.00
C18:4 octadecatetraenoic	0.75	0.00
C20:4 arachidonic	0.14	0.00
Not identified	**0.00**	**0.79**

Table 2. Crystallization and melting temperatures of butter and lard used in loins for aging lipid-coated meats

Parameter	Butter	Lard
Initial crystallization (°C)	18.67	19.52
Final crystallization (°C)	−0.52	−2.79
Initial merger (°C)	−41.01	−22.68
Final merger (°C)	37.42	14.12

lard remains semisolid within this temperature, resulting in greater spreadability compared with butter and consequently promoting higher permeability of water from the meat to the exterior. However, the samples from the butter- and lard-aged treatments exhibited less weight loss during the aging process compared with the losses reported in the literature for dry-aged. For those, which undergo an aging process of 21 to 30 d without packaging, an average weight loss of 10% to 15% is commonly observed due to dehydration (Dashdorj et al., 2016).

The total loss, characterized by evaporation and drip losses during aging, was higher for the samples with pork lard, followed by samples with butter, and lowest for wet-aged samples ($P < 0.001$). This influence was due to the sum of all losses during the process, meaning wet-aged meats only lost exudate from the packaging, whereas meats coated with butter lost weight due to initial drying and exudate during aging. The lard-aged samples showed an influence from the total loss because after a loss during the initial drying period (48 h), the samples continued drying during the remaining aging period, which lead to higher losses in these samples. Pork lard is a fat with intermediate

Table 3. Processing yields of beef loins aged under vacuum, coated with butter, or with lard for 28 d

Parameter	Wet-aged (n = 12)	Butter-aged (n = 12)	Lard-aged (n = 12)	P value
Dehydration loss (%)	NA	6.98 ± 0.26[a]	7.18 ± 0.35[a]	0.65
Fat adhesion (%)	NA	25.39 ± 0.98[a]	23.15 ± 0.56[a]	0.06
Aging loss (%)	NA	1.53 ± 0.09[b]	3.52 ± 0.64[a]	<0.01
Total loss (%)	3.21 ± 0.40[c]	8.51 ± 0.28[b]	10.70 ± 0.48[a]	<0.001
Total yield (%)	96.79 ± 0.40[a]	91.49 ± 0.28[b]	89.30 ± 0.48[c]	<0.001

[a–c]Means (± standard error of mean) with different letters in the same row differ from each other by analysis of variance ($P < 0.05$).

NA = not applicable. Fat adhesion = ((fat × 100)/meat); dehydration loss in the first 2 d = ((initial weight − final weight)/(initial weight)) × 100; aging loss in 26 d = 100 − ((final weight × 100)/(initial weight)); total loss = (loss to dehydration in 2 d + loss to dehydration in 2 d + exudate); total yield = 100 − total loss.

viscosity, meaning it has high spreadability even at low temperatures, resulting in greater permeability of water from the meat to the environment.

Due to the higher losses before and during the aging process, the samples aged with lipid coatings showed lower yields ($P < 0.001$) compared with wet-aged samples (Table 3). However, the total yields of the samples in this study were higher compared with the yields reported in the literature for dry-aged meats (Ha et al., 2019; Vilella et al., 2019; Bernardo et al., 2020a, 2020b) because superficial trimming was unnecessary.

pH and water activity

The samples with lipid coatings presented higher pH compared with the wet-aged loin ($P < 0.001$; Table 4). During wet aging, the pH of the meat remains stable and close to 5.4 to 5.5, a condition naturally promoted by anaerobic bacteria present in the meat prior to the start of aging (Terjung et al., 2021). This pH range was observed in the wet-aged samples in this study. Other reasons for the high pH in coated samples may be due to the high counts of bacteria because these samples, in general, had microbial counts that were 1 logarithmic unit higher than the wet-aged samples (Table 5). Some microorganisms, such as enterobacteria, can metabolize amino acids into volatile compounds associated with deteriorated meat, which consequently increases the pH. Different volatile

compounds were observed between the aged treatments in this study, which will be presented later (Table 5).

Aging type showed no effect on Aw values when evaluating the inner portion of the steak ($P = 0.090$). However, wet-aged samples had higher Aw values on the surface of the meat compared with lard-aged samples, and both did not differ from butter-aged samples ($P < 0.01$; Table 4). The Aw tends to decrease with increasing exposure times of unpackaged aged meats, as demonstrated in studies on dry-aged meats (Dashdorj et al., 2016). This is due to the movement of free water from regions of high concentration to regions with low concentration by diffusion and capillary flow, followed by the evaporation of water as vapor into the surrounding air (Ribeiro et al., 2021), which explains the lower Aw in unpackaged aged samples.

Total moisture, total lipids, and protein oxidation

The total moisture and lipid contents were not affected by the aging process ($P = 0.34$; Table 4). Note that during the removal of the lipid coatings, a thin layer of pork lard remained on the surface of the samples. However, even with this residual layer of pork lard, the overall lipid content of the meat was not altered.

Table 4. Physicochemical traits of beef loins aged under vacuum, coated with butter, or with lard for 28 d

Parameter	Wet-aged ($n = 12$)	Butter-aged ($n = 12$)	Lard-aged ($n = 12$)	P value
pH	5.42 ± 0.02^b	5.60 ± 0.02^a	5.59 ± 0.01^a	<0.001
Aw (inner region)	0.9919 ± 0.0005^a	0.9925 ± 0.0004^a	0.9906 ± 0.0007^a	0.09
Aw (surface region)	0.9917 ± 0.0020^a	0.9907 ± 0.0009^{ab}	0.9882 ± 0.0008^b	<0.01
Total moisture (%)	72.66 ± 0.25^a	72.32 ± 0.30^a	72.10 ± 0.25^a	0.34
Total lipids (%)	2.91 ± 0.20^a	2.89 ± 0.32^a	3.32 ± 0.36^a	0.52
Carbonyl (nmol/mg)	1.18 ± 0.10^a	1.29 ± 0.06^a	1.26 ± 0.12^a	0.75
Cooking loss (%)	20.51 ± 0.67^a	18.42 ± 0.43^b	18.57 ± 0.48^b	<0.05
WBSF (kg)	2.49 ± 0.14^a	2.85 ± 0.18^a	2.86 ± 0.17^a	0.20

[a,b]Means (± standard error of mean) with different letters in the same row differ from each other by analysis of variance ($P < 0.05$).

Aw = water activity; WBSF = Warner-Bratzler shear force.

Table 5. Volatile compounds identified exclusively in of beef loins aged under vacuum and coated with butter for 28 d

RT (min)	Exclusive compound in wet-aged	Class	LTPRI	Area
20.811	Hexyl acetate	Ester	809	3.277.174
38.159	2-decanone	Ketones	1.192	222.137
28.720	3-methyl nonane	Hydrocarbon	971	85.552
23.249	2,3-dimethyl, heptane	Hydrocarbon	858	125.693
20.772	3-methyl, 2-heptene	Hydrocarbon	808	185.296
22.136	2,5-dimethyl heptane	Hydrocarbon	836	223.354
29.991	Decane	Hydrocarbon	999	342.791
RT (min)	**Exclusive compound in butter-aged**	**Class**	**LTPRI**	**Area**
25.210	2-heptanol	Alcohol	898	201.066
19.648	2-methylpropyl 2-hydroxypropanoate	Ester	786	370.155
38.134	Ethyl octanoate	Ester	1,193	367.160
12.223	Methyl propionate	Ester	606	105.156
21.781	Methylpyrazine	Pyrazine	830	61.719

LTPRI = linear temperature programmed retention index; RT = retention time.

Meat proteins are susceptible to oxidation reactions, resulting in the irreversible generation of carbonyl compounds. The initiation of this process is attributed to inherent components of muscle tissue, including lipid oxidation compounds, heme pigments, transition metals, and oxidative enzymes (Xiong, 2000). Various external factors, such as pH, temperature, water activity, and light, further influence protein oxidation, with these conditions accentuating carbonyl formation in aged meats (Estévez, 2011). Notably, the type of aging did not exert a significant effect on the total carbonyl content ($P = 0.75$; Table 4). This suggests that the aging process of meats, whether coated with butter or lard, does not significantly amplify the production of carbonyl compounds when compared with wet aging.

Color stability

The aging type did not affect the luminosity. However, the display storage period made the samples lighter. The yellow intensity was lower in samples aged with lipid coatings and was also affected by the display time, decreasing in intensity as the display time increased. Aging type interacted with display time for the red intensity, wherein the $a*$ value decreased with an increase in the display storage days (Table 6).

When analyzing the interaction effects for the $a*$ coordinate, samples from the butter- and lard-aged treatments exhibited similar behavior within the same

Table 6. Color and lipid stability of beef loins aged under vacuum, coated with butter, or with lard for 28 d

	$L*$	$a*$	$b*$	MMD (µg/g)
Treatment (T)				
Wet-aged	39.01 ± 0.62^a	23.12 ± 0.63^a	21.28 ± 0.42^a	0.26 ± 0.04^b
Butter-aged	38.24 ± 0.53^a	17.67 ± 1.06^b	17.26 ± 0.63^b	0.90 ± 0.10^a
Lard-aged	39.14 ± 0.43^a	15.96 ± 1.05^b	15.71 ± 0.51^c	0.31 ± 0.03^b
P value	0.44	<0.001	<0.001	<0.001
Display (D)				
Day 1	37.83 ± 0.55^b	24.42 ± 0.39^a	21.32 ± 0.46^a	0.27 ± 0.06^c
Day 3	39.90 ± 0.43^a	18.73 ± 0.85^b	17.40 ± 0.50^b	0.52 ± 0.09^b
Day 6	38.70 ± 0.56^{ab}	13.71 ± 0.93^c	15.61 ± 0.59^c	0.72 ± 0.11^a
P value	<0.05	<0.001	<0.001	<0.001
Interaction (T × D)				
P value	0.78	<0.001	0.37	<0.001

[a–c]Means (± standard error of mean) with different letters in the same column differ from each other by analysis of variance ($P < 0.05$).

$L*$ = luminosity; $a*$ = red-green coordinate; $b*$ = yellow-blue coordinate; MMD = malondialdehyde.

day of analysis, both being more oxidized than the wet-aged samples (Figures 1 and 2). The color oxidation arose because, in both lipid-coated aging groups, cracks formed in the fat layer, which increased the oxygen pressure in the meat-fat system, potentially decreasing the metmyoglobin reducing activity.

Metmyoglobin is reduced to oxymyoglobin by metmyoglobin reductase, an endogenous enzyme in meat. This process occurs by consuming nicotinamide adenine dinucleotide (NADH) as a substrate for reducing iron ions, resulting in a color change from brown (oxidized iron: Fe^{3+}) to bright red (reduced iron: Fe^{2+}) (Suman and Joseph, 2014). When the energy substrate is depleted, the iron reduction process is halted, and the color of the meat remains brown (Suman and Joseph, 2014; Mitacek et al., 2018). Factors such as the presence of oxygen and aerobic microorganisms can accelerate this reduction (Mitacek et al., 2018). These conditions were observed in the lipid-coated aged meats, explaining their lower color stability compared with the wet-aged meats.

Lipid oxidation

An interaction effect for lipid oxidation was observed between the types of aging and display storage (Table 6). The butter-aged samples exhibited the highest degree of oxidation compared with the other treatments on days 3 and 6 of display (Figure 1). As discussed in the yield (Table 3) and water activity sections (Table 4), the samples coated with butter retained liquid between the meat-fat system, whereas the lard-coated samples lost water by dehydration, without concentrating exudate. The higher water concentration near the meat, along with the presence of oxygen from microfissures in the fat, is what influenced the higher degree of lipid oxidation in the butter-aged samples. This occurs because of the phospholipid membrane of the meat matrix containing a high amount of polyunsaturated fatty acids, which are exposed when the meat is cut (Domínguez et al., 2019). Consequently, pro-oxidizing agents like oxygen and high humidity exert a stronger influence on the process of lipid oxidation.

Cooking loss and instrumental tenderness

The cooking loss was higher ($P < 0.05$) in wet-aged samples compared with samples aged with the coatings, which did not differ from each other (Table 4). The coated samples underwent a prior drying process for 48 h, which resulted in water reduction by drying and released exudate, as demonstrated by the
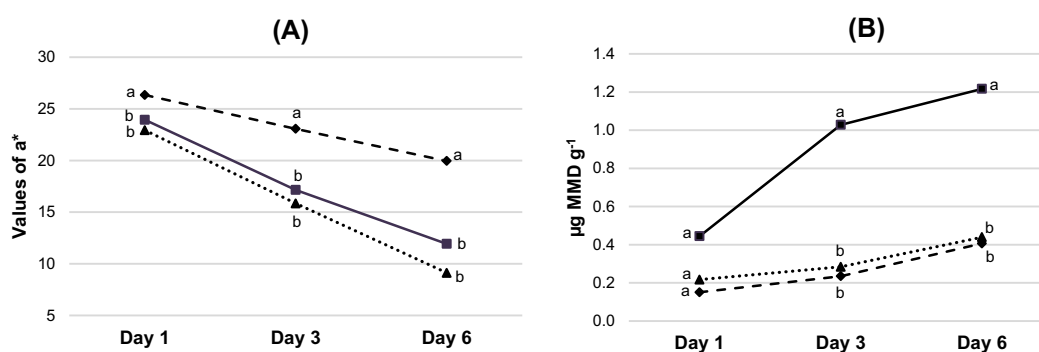
Figure 1. Interaction of the effects of the type of aging with the display storage in the *a** color coordinate (A), and of the malondialdehyde content (B), of beef striploin aged under vacuum (–◆– WA) and with coating of butter (–■– BA) and lard (···▲··· LA). [a,b]Different letters on the same display day differ from each other by Tukey test ($P < 0.05$).
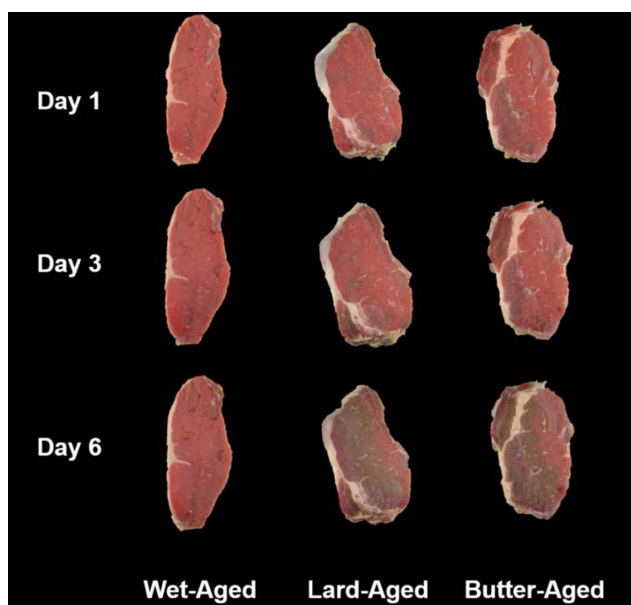

Figure 2. Visual discoloration of wet-, lard-, and butter-aged steaks stored on display for 6 d at 2°C.

yield parameters (Table 3) and surface water activity (Table 4).

On the other hand, instrumental tenderness was not affected by the different aging types ($P = 0.20$; Table 4). However, in all treatments, the samples exhibited tenderness lower than 3.9 kg, categorizing the samples as very tender (ASTM, 2011).

Volatile compounds

A total of 148 volatile compounds were identified in the beef samples, chemically classified as carboxylic acids ($n = 9$), alcohols ($n = 17$), aldehydes ($n = 22$), ketones ($n = 24$), aromatic compounds ($n = 8$), heterocyclic compounds ($n = 2$), sulfur compounds ($n = 1$), esters ($n = 26$), furans ($n = 6$), hydrocarbons ($n = 25$), lactone ($n = 1$), pyrazines ($n = 5$), and terpenes ($n = 2$).

The volatile compound content in meat can be modulated by various factors, including the meat's quality, which encompasses the degree of marbling and aged time (Tucker et al., 2019; Mallick et al., 2021). Additionally, the cooking process conditions, such as steak thickness, surface temperature, and degree of cooking, can exert a significant influence (Miller et al., 2019; Kerth et al., 2022). It is worth noting that the microbiological quality of meat can have a direct impact on its volatile profile (Casaburi et al., 2015).

Individually, the nonaged samples (fresh meat) had 123 identified compounds, whereas wet-aged samples had 107 compounds, butter-aged had 118 compounds, and lard-aged had 99 compounds. These volatile compounds are responsible for the flavor and aroma of the meat and are influenced by processing, storage, and cooking (Calkins and Hodgen, 2007; Kerth et al., 2022). The flavors and aromas associated with beef are generally developed during heating, and the main reactions that occur during this process are lipid oxidation, Maillard reaction, and thiamine degradation (Mottram, 1998).

In total, 14 compounds were found exclusively in the fresh meat (nonaged samples). Among the total of 14 compounds, the chemical groups of alcohols, aromatic compounds, heterocyclic compounds, sulfur compounds, and ester presented only 1 compound each, whereas aldehydes and pyrazines presented 2 compounds each, and finally hydrocarbons presented with 5 compounds.

Seven compounds were exclusively detected in the wet-aged samples (Table 5). Among these, esters and ketones were identified, associated with lipid degradation and the Maillard reaction involving free amino acids and reducing sugars (Macleod, 1994; Barker et al., 2023), and may also be related to microbial degradation reactions (Dainty et al., 1985). The chemical

Table 7. Microbiological counts of beef loins aged under vacuum, coated with butter, or with lard for 28 d

Parameter (log CFU g^{-1})	Wet-aged	Butter-aged	Lard-aged	Valor P
Psychrotrophic bacteria	7.18 ± 0.08^b	8.03 ± 0.22^a	7.42 ± 0.06^b	<0.01
Mesophilic bacteria	5.68 ± 0.14^b	7.45 ± 0.21^a	6.32 ± 0.11^b	<0.001
Lactic acid bacteria	5.75 ± 0.14^c	7.65 ± 0.15^a	6.70 ± 0.05^b	<0.001
Enterobacteria	3.32 ± 0.63^a	4.10 ± 0.59^a	3.40 ± 0.15^a	0.53
Molds and yeasts	3.16 ± 0.08^a	4.12 ± 0.58^a	4.56 ± 0.38^a	0.12

[a,b]Means (± standard error of mean) with different letters in the same row differ from each other by analysis of variance ($P < 0.05$).

CFU = colony-forming unit.

category of hydrocarbons was notably prevalent in the wet-aged samples. These compounds are generated through the caramelization or degradation of sugars and are known for their characteristic meaty or syrupy flavor profile (Hodge, 1953; Kerth et al., 2023).

The butter-aged samples exhibited the presence of 5 specific compounds (Table 5). Within this set, the chemical groups of alcohol and ester were discerned, both of which are associated with indicators of microbial degradation and Maillard reaction (Casaburi et al., 2015; Barker et al., 2023). The pyrazine chemical group was also efficient in distinguishing the volatile profiles among the treatments and is connected to lipid degradation and Maillard reaction pathways (Macleod, 1994; Lund and Ray, 2017). In contrast, the lard-aged samples did not display distinct volatile compounds. These variations in compound composition across treatments underscore the interplay between the aging method and the ultimate composition of volatile compounds in aged beef, thereby defining a specific volatile profile for each treatment.

Microbiological quality

The wet- and lard-aged samples did not differ in terms of psychrotrophic and mesophilic counts, with both treatments showing lower counts, for both microbial groups, compared with the butter-aged samples ($P < 0.01$; Table 7). These microbial groups are strongly related to the sanitary quality of meat, especially regarding deterioration, with only a few pathogens being found among the psychrotrophic group (Silva et al., 2017; Wei et al., 2019). Even with an average count of 7.18 logarithmic units for psychrotrophics, the wet-aged samples showed no signs of deterioration. However, with a slightly higher average count of 7.42 logarithmic units, some lard-aged samples exhibited signs of deterioration, such as the presence of surface slime and acidified aroma. On the other hand, the butter-aged samples had higher psychrotrophic counts as well as apparent signs of deterioration,

including the formation of surface mucus in some samples.

The butter-aged samples showed a higher count of lactic acid bacteria, followed by the lard- and wet-aged treatments (Table 7). The presence of liquid between the meat and the butter layer may have helped, because it is an easily available substrate for microbial growth, along with the microaerophilic condition promoted by cracks in the fat layer. This microbial group is classified as facultative anaerobes, with an optimal pH range for growth between 5.5 and 6.2 (Silva et al., 2017), which is the natural condition of meat. The ideal pH and the absence of anaerobic conditions may have favored the increase in lactic acid bacteria counts, which may have been responsible for more apparent deterioration in the meats covered with fats, particularly in the butter-aged samples. Furthermore, certain psychrotrophic bacteria can utilize non-carbohydrate substrates as a carbon source. This metabolic ability results in the production of secondary metabolites that enhance the development of traits associated with meat putrefaction (Wei et al., 2019).

The counts of enterobacteria and molds and yeasts showed no difference in the samples from all treatments (Table 7). Enterobacteria are indicator microorganisms of hygiene conditions in processes and products, whereas molds and yeasts are related to environmental hygiene (Silva et al., 2017), specifically in this study, in the atmospheric air in the aging chamber.

Conclusions

Applying lipid coating in the aging process results in products with physicochemical characteristics close to those of wet-aged. Using the same amount of fat in the meat coating, samples with butter showed a higher final yield. However, color and microbiological quality were more satisfactory in samples aged with lard coating compared with those with butter. Although lard has better conditions of use compared with butter, the aging

process with lipid coating clearly does not have advantages over the wet-aged process, under the conditions applied to the study, because it results in samples with problems in color and presents a potential health risk. Therefore, new studies are suggested in which a shorter aging time is applied for commercial viability of these products.

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