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Effects of freezing and thawing on microbiological and physical-chemical properties of dry-aged beef

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ABSTRACT

This study evaluated the effects of freezing, prior to and after dry aging, on the microbiological and physical-chemical quality of beef. Strip loins ($n = 24$) from 12 carcasses were assigned to four treatments: non-frozen dry aging (**Dry**); dry aging, steak fabrication, freezing and slow thawing (**Dry + ST**); freezing, fast thawing (FT; 20 °C/15 h) and dry aging (**FT + Dry**); freezing, slow thawing (ST; 4 °C/48 h) and dry aging (**ST + Dry**). Freezing conditions were − 20 °C/28 days and dry aging conditions were 2 °C/70% relative humidity, for 28 days. Freezing prior to dry aging did not affect the microbial counts compared to Dry. However, FT + Dry and ST + Dry increased (16%) total process loss ($P < .05$) compared to Dry and Dry + ST. Moreover, freezing changed volatile compounds profile. Thus, freezing prior to dry aging was not a feasible process due to increased process loss, while freezing after dry aging was considered a viable alternative to preserve the steaks without compromising beef physical-chemical traits.

1. Introduction

Dry-aged beef is the product of aging unpackaged beef under controlled conditions of temperature, relative humidity and air velocity. This aging process improves tenderness and contributes to "dry-aged beef" flavor (Savell, 2008). However, dry aging is a costly process (DeGeer et al., 2009; Miller, Davis, & Ramsey, 1985; Smith et al., 2008) as it requires strictly controlled conditions, larger spaces in chambers (Smith et al., 2014), and causes higher weight loss compared to wet-aging (Dikeman, Obuz, Gök, Akkaya, & Stroda, 2013; Oreskovich, McKeith, Carr, Novakofski, & Bechetel, 1988; Parrish, Boles, Rust, & Olson, 1991; Warren & Kastner, 1992).

Additionally, dry-aged beef is usually produced with high-quality beef from British cattle, which presents high marbling and thick subcutaneous fat. However, in Brazil, about 80% of the cattle has influence of zebu breeds (Ferraz & Felício, 2010) raised on grass-fed system, and known for their low marbling and thin subcutaneous fat. Thus, the Brazilian production of high-quality beef is limited and can vary during the year, making dry-aged beef a seasonal and costly product. Therefore, freezing could be an alternative to store high-quality beef, avoiding the seasonality in supply, allowing negotiations for best prices, and increasing the availability of dry-aged beef in the market.

Freezing is one of the most common and efficient methods to preserve and market beef (Kim, Meyers, Kim, Liceaga, & Lemenager, 2017). Several studies have reported that freezing may increase tenderness when applied prior to aging in a vacuum bag (Crouse & Koohmaraie, 1990; Grayson, King, Shackelford, Koohmaraie, & Wheeler, 2014; Lagerstedt, Enfält, Johansson, & Lundström, 2008; Shanks, Wulf, & Maddock, 2002) and after the dry aging process (Kim et al., 2017). However, freezing and thawing can increase the weight loss by purging (Kim et al., 2017), and may reduce the sensory quality due to fat and protein oxidation, caused by damage in the muscle cells that release pro-oxidative enzymes (Leygonie, Britz, & Hoffman, 2012).

Although several studies have reported the effects of freezing and thawing on wet-aged beef (Crouse & Koohmaraie, 1990; Grayson et al., 2014; Kim, Liesse, Kemp, & Balan, 2015; Lagerstedt et al., 2008; Shanks et al., 2002), few studies have investigated freezing and thawing prior to or after dry aging and how this procedure impacts dry-aged beef

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quality attributes. Therefore, this study assessed the viability to produce dry-aged beef with previously frozen loins and analyzed the effects of thawing at two different rates (temperature x time) on the yield, physical-chemical traits, volatile compounds, and microbial characteristics. In addition, it was evaluated the impact of freezing steaks after dry aging on physical-chemical quality attributes.

2. Material and methods

2.1. Raw material preparation and aging conditions

Twelve pairs of strip loins (left and right-side) from grass-fed zebu steers (around 3 years old; 288 kg ± 35 kg average carcass weight; and 3.3 ± 0.3 mm of subcutaneous fat measured between the 12th and 13th ribs) were collected at 3 days postmortem from a commercial beef plant.

The strip loins were vacuum packaged in bags (50 μm thick; O_2 permeability of 20 cm^3 O_2/m^2/24 h at 23 °C, atmospheric pressure and 0% relative humidity; maximum CO_2 permeability of 100 cm^3 CO_2/m^2/24 h at 23 °C, atmospheric pressure and 0% relative humidity; Cryovac® BB 2620, Cryovac Brasil Ltda.), placed on ice, and transported to the Meat Laboratory at the University of Campinas, São Paulo, Brazil.

At the laboratory, one steak was cut out from the middle of each strip loin for sample characterization analyses (pH, moisture and fat content, Warner-Bratzler shear force, and volatile compounds), providing other two loin sections per strip loin (four per animal). Each section position (anterior and posterior) was balanced allocated into one of the four treatments, avoiding any position effect among the treatments: dry aging for 28 days, steak fabrication and analyses on non-frozen steaks (Dry); dry aging for 28 days, followed by steak fabrication, freezing of steaks at −20 °C for 30 days, slow thawing (ST) at 4 °C/24 h and analyses (Dry + ST); freezing of loins at −20 °C for 30 days, fast thawing (FT) at 20 °C/15 h (controlled room temperature), dry aging for 28 days, followed by steak fabrication and analyses (FT + Dry); freezing of loins at −20 °C for 30 days, slow thawing (ST) at 4 °C/48 h, dry aging for 28 days, followed by steak fabrication and analyses (ST + Dry).

The sections assigned to FT + Dry and ST + Dry were vacuum packaged, and evenly distributed in a commercial freezer (BVR28 model, Brastemp - Whirlpool, Brazil) used exclusively for this experiment and set to −20 °C. The remaining sections were placed in an adapted aging chamber (VN50R model, Metalfrio 2010 ©, Brazil) at 2 °C, 70% relative humidity and 2.5 m/s of air velocity.

2.2. Weight loss and steak preparation

The strip loins sections were weighed prior to and after aging and the percentage of evaporation loss was calculated as: (initial weight – post-aging weight)/initial weight x 100. After aging, the dried surface was trimmed (approximately 5 mm-thick) and weighed. The percentage of trimming loss was calculated as: (weight of trimmings/initial weight) x 100. The percentage of thawing loss was also determined by weighing the strip loin sections before and after the freezing/thawing process: (initial weight – post-thawing weight/initial weight) x 100.

After the trimming process, steaks (2.54 cm-thick) were cut out from the loin sections, using a ruler to measure the thickness of each steak. The steaks were assigned to moisture content and pH, thiobarbituric acid-reactive substances, shear force, color display, and volatile compounds. Samples for microbiological analyses were collected from the dehydrated lean surface, avoiding subcutaneous fat.

2.3. pH and water activity

The pH was measured in duplicate by inserting a calibrated puncture pH electrode (MP125 portable pH meter, Mettler Toledo, Brazil) directly into the steaks assigned to this analysis, cut from both fresh (non-frozen and non-aged) and aged samples. The water activity was measured in the dried trimmed crust (2 mm-thick), using a water activity analyzer (Aqualab 4TE, Decagon, São Paulo, Brazil).

2.4. Moisture, fat and thiobarbituric acid-reactive substances

The internal moisture content was determined for both non-aged and aged samples, in triplicate, by grinding lean beef and drying it in a forced air convection oven, according to AOAC (1990) procedures. The fat content was determined according to the Bligh and Dyer (1959) procedures, in non-aged samples. The thiobarbituric acid-reactive substances (TBARS) were determined, in quadruplicate, on the internal lean beef after aging, according to Bruna, Ordóñez, Fernández, Herranz, and De La Hoz (2001), modifying the procedure by adding 20 mL of 5% TCA instead of 15 mL of 0.38 M $HClO_4$.

2.5. Shear force

Steaks (2.54 cm-thick) assigned for the shear force analysis were cooked using some adaptations from the American Meat Science Association cookery guidelines (AMSA, 2015). The steaks were placed on a metal rack over and aluminum tray and cooked in an electric oven (Fritomaq, Brazil) at 170 °C until the internal temperature reached 71 °C measured by copper-constantan thermocouples (E5CWL Omron, CSW) inserted into the geometric center of the steaks. Each steak was weighed, prior to and after cooking, and the cooking loss was calculated according to the following equation: (raw weight – cooked weight/raw weight) × 100.

After cooking, the steaks were kept at room temperature to cool (about 30 min) and then overwrapped in polyvinyl chloride film and chilled overnight at 4 °C according to AMSA (2015) procedures. Six round cores of 1.27 cm diameter were removed from each steak parallel to the muscle fibers using a handheld coring device. The shear force (N) was measured by shearing each core in the center using a Warner-Bratzler blade attached to a Texture Analyzer (TA-XT Plus, Texture Technologies Corp./Stable Micro Systems, UK) with a crosshead speed of 250 mm/min (AMSA, 2015).

2.6. Color

Each steak assigned for the measurement of instrumental color was placed on a polystyrene tray covered with a polyvinyl chloride film and placed in a cooler at 4 °C, without light exposure, for 5 days. The instrumental color was measured every day in triplicate using a portable colorimeter (CM 508-d, Hunter MiniScan TMXE) with attached moisture protector accessory and calibrated using white and black tile standards. The color was determined by CIE L*, a*, and b* values using the illuminant D65 source and the standard observer of 10°, according to AMSA (2012) protocol. Chroma and hue angle were calculated by using the CIE L*, a*, and b* values, according to the following formulas; Chroma = $(a^{*2} + b^{*2})^{1/2}$ and hue angle = $\tan^{-1}(b^*/a^*)$ (AMSA, 2012).

2.7. Microbiological analyses

Samples for microbiological analyses were collected prior to aging and freezing (fresh samples), after thawing (FT + Dry and ST + Dry), and after dry aging (Dry, FT + Dry and ST + Dry). Ten grams of the beef surface, measuring ± 2 mm-thick, were aseptically collected from three loin sections of each step and treatment mentioned above. Each sample was homogenized with 90 mL of 0.1% peptone water (Difco, MD, USA) in a stomacher (Stomacher 400 circulator, Seward, UK) for 2 min at 230 rpm. When necessary, decimal dilutions were performed in 0.1% peptone water (Difco). Samples were analyzed for total bacterial count (TBC), psychrotrophic microorganisms (PSY), *Enterobacteriaceae* (ENT), lactic acid bacteria (LAB) and yeasts and molds (YM).

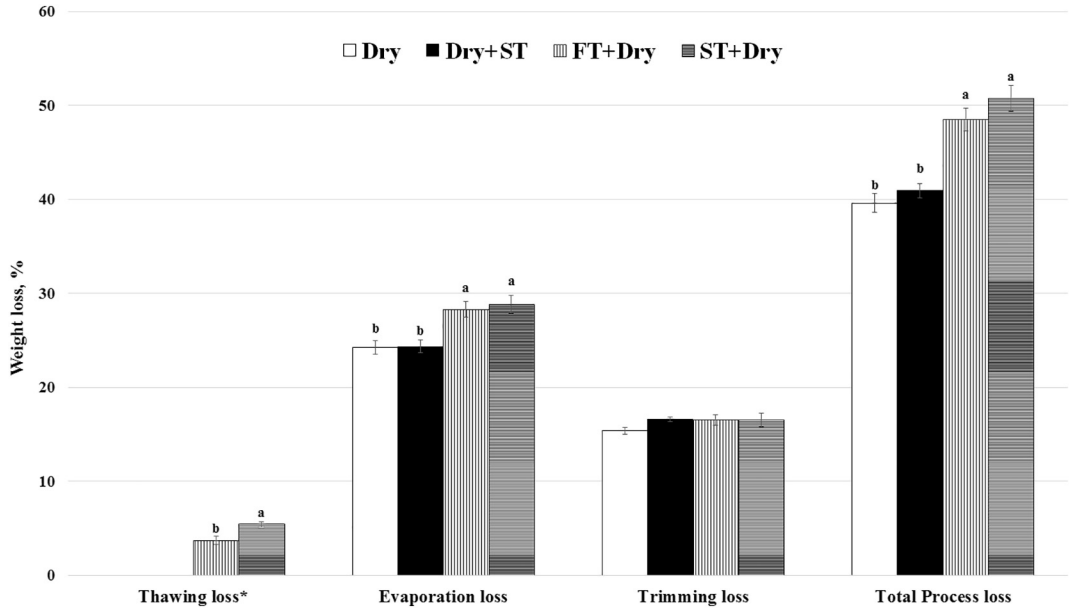

Fig. 1. Effect (mean ± SEM) of treatments on thawing, evaporation, trimming and total process losses on beef samples (n = 48). a, b Different letters among treatments within each weight loss parameter (thawing, evaporation, trimming and total process loss) indicate significant differences ($P < .05$). * Thawing loss of Dry + ST was measured on thawed steaks (4 °C/10 h) and had 1.1 ± 0.1%. (□) Dry: dry aging for 28 days; (■) Dry + ST: dry aging for 28 days, steaks fabrication, freezing at −20 °C and thawing at 4 °C/24 h; (▥) FT + Dry: freezing at −20 °C, fast thawing at 20 °C/15 h and dry aging for 28 days; (▤) ST + Dry: freezing at −20 °C, slow thawing at 4 °C/48 h and dry aging for 28 days.

The TBC and PSY counts were determined in Plate Count Agar (PCA, Acumedia, MI, USA) with incubation at 35 °C for 48 h and 7 °C for 10 days, respectively (Ryser & Schuman, 2015; Vasavada & Critzer, 2015). The LAB was determined using Man, Rogosa and Sharpe agar (MRS, Difco) incubated under anaerobic conditions (Probac, Brazil) at 35 °C for 72 h (Njongmeta, Hall, Ledenbach, & Flowers, 2015). To quantify the *Enterobacteriaceae* family, Violet Red Bile Glucose agar (VRBG, Acumedia) in pour plate with overlay and incubation at 35 °C for 24 h was used (Kornacki, Gurtler, & Stawick, 2015). The YM counts were determined in Dichloran Rose Bengal Chloramphenicol agar (DRBC, Acumedia) incubated at 25 °C for 5 days (Ryu & Wolf-Hall, 2015). The molds isolated from dry aged samples were cultivated in Czapek Yeast Autolysate agar (CYA) at 25 °C for 7 days and identified by morphological characteristics (Pitt & Hocking, 2009).

2.8. Volatile compounds qualitative profile

A pool of 12 steaks (2.5 cm-thick) was obtained for each treatment. The steaks were cooked, separately, in a Combi oven (Tedesco, Santa Maria, RS, Brazil), pre-heated at 180 °C, until the samples reached an internal temperature of 75 °C. After cooking, the 12 steaks of each treatment were ground all at once, obtaining a pooled sample. Ten grams of this pool were weighed in 60 mL flasks in triplicate. The solid-phase microextraction technique was used for volatile compounds extraction, using a CAR/PDMS (Carboxen/polydimethylsiloxane, Sigma, Bellefonte, PA, USA) fiber as stationary phase. The extraction was performed at 60 °C for 10 min in a water bath, and then the fiber was exposed in the headspace for 65 min. Gas Chromatography coupled to Mass Spectrometry (GC–MS) (QP-2010 model, Shimadzu® Kyoto, Japan) was used to separate and identify beef volatile compounds. Thermal desorption was performed at 300 °C in a splitless mode injector for 1 min. The volatile compounds were separated by a DB- 5 MS (5% phenyl, 95% dimethylpolysiloxane) of 60 m × 0.25 mm internal diameter and 1 µm width of the stationary phase (J&W Scientific®, Santa Clara, CA, USA). The column temperature started at 40 °C for 2 min, increasing the temperature at the rate of 4 °C/min until 180 °C, followed by a rate of 60 °C/min up to 300 °C, remaining at this temperature for 5 min. The rate of 60 °C/min was used to reach the final temperature faster and cleaning the column, as the volatile compounds of interest are detected up to 180 °C. Helium was the carrier gas, at a flow rate of 1 mL/min. A quadrupole mass detector was operated in the following conditions: ionization energy 70 eV, interface temperature of 300 °C, ions source temperature 200 °C. The mass spectrum was set at the scanning mode, monitoring the range from 35 to 350 *m/z*. Compounds were identified by their mass spectra, compared to the library database of GC–MS (NIST, 2002). To confirm identification, a n-alcane (C_7-C_{30}) (Supelco, Bellefonte, PA, USA) solution was injected into the equipment under the same conditions as the samples to obtain the LTPRI (linear temperature programmed retention index of the volatile compounds). The experimental identification was performed by comparing the LTPRI and the mass spectra with the reports from the literature, with a similarity of a minimum of 85% and maximum variation of ± 10.

Specific compounds of each volatile compound were selected, transformed to log10 and a principal component analysis (PCA) was applied to check any separation between groups within the effects studied based on the volatile compounds.

2.9. Data analyses

This experimental design allocated 24 loins from 12 beef carcasses into 4 balanced treatments considering four freezing/thawing methods: non-frozen; frozen steaks after dry aging; frozen before dry aging and thawed at 20 °C/15 h; frozen prior to dry aging and thawed at 4 °C/48 h. The data were analyzed using the software Statistica 10.0 (StatSoft, 2010) for ANOVA one-way. For color, the data were analyzed using the variance analysis for repeated measures ANOVA. The means of interest were analyzed by the Tukey test at 5% level of significance.

3. Results and discussion

3.1. Sample characterization

Samples were characterized prior to aging. On average, raw samples had 3.3 ± 0.3 mm thickness of subcutaneous fat, a pH of 5.43 ± 0.02,

moisture and fat content of 75.1 ± 0.2% and 1.9 ± 0.1%, respectively, and Warner-Bratzler shear force of 52.87 ± 2.83 N.

3.2. Thawing, trimming, evaporation and total process losses

Thawing losses were lower for FT + Dry compared to ST + Dry ($P < .05$; Fig. 1). According to Haugland (2002) fast thawing (lower thawing time) reduces exudate formation. In addition, Gonzalez-Sanguinetti, Añon, and Calvelo (1985) indicated that the lower exudate formation is related to water flow into intracellular spaces and water reabsorption by the muscle fibers, caused by increased water activity due to the melting of ice crystal formed in the extracellular spaces during the freezing process. The Dry + ST steaks, that were frozen after dry aging, had on average 1.1 ± 0.1% of thawing loss. The trimming loss reached about 16% and no difference was observed between the treatments ($P = .258$; Fig. 1).

The FT + Dry and ST + Dry had higher evaporation and total process losses compared to Dry and Dry + ST ($P < .05$; Fig. 1). Evaporation loss was about 4 percentage points higher for FT + Dry and ST + Dry and, adding to thawing loss, these samples had up to 11 percentage points more of total process loss than Dry and Dry + ST did. This increase in evaporation loss, and consequently in total process loss, is related to muscle fiber disruption due to the formation of ice crystals that damages the fiber structure and concentrates solutes leading to protein denaturation (Leygonie et al., 2012) and to a decrease in the water-holding capacity of meat (Añón & Calvelo, 1980; Leygonie et al., 2012; Ngapo, Barbare, Reynolds, & Mawson, 1999; Vieira, Diaz, Martínez, & García-Cachán, 2009). Therefore, in this study, the freezing of the loins prior to dry aging was not considered a viable procedure to store high-quality beef. Increasing total process loss could also result in higher production costs, making the process inviable.

3.3. pH, water activity, moisture and thiobarbituric acid-reactive substances

The final pH was not affected by the treatments ($P = .274$; Table 1). Previous studies have reported similar results, where the freezing process did not influence the pH either prior to or after aging (DeGeer et al., 2009; Kim et al., 2015; Kim et al., 2017).

Regarding the effects of treatments on the water content, the FT + Dry and ST + Dry showed lower values of surface water activity and moisture content compared to Dry and Dry + ST ($P < .05$; Table 1). These results were expected as freezing reduces the meat water-holding capacity due to muscle fiber disruption caused by ice crystals formation (Leygonie et al., 2012).

No differences were observed in TBARS values between the treatments ($P = .971$; Table 1). Moreover, the values obtained in the TBARS analysis were lower than the threshold for detection of rancid flavor, which is 2 mg MDA/kg (Campo et al., 2006; Greene & Cumuze, 1981; Vieira et al., 2009; Watts, 1962). These low TBARS could be related to the high levels of vitamin E found in beef from grass-fed cattle. Studies have demonstrated that beef from grass-fed cattle has higher vitamin E contents, which increases the beef stability against lipid oxidation (Nuernberg et al., 2005; Warren et al., 2008). Additionally, Nuernberg et al. (2005) indicated that beef from grass-fed cattle showed more oxidative stability than from cattle fed with concentrate diet, even for previously frozen beef. Although it was not determined, it seems that the beef from grass-fed cattle used in the current study had high Vitamin E contents, which provided the oxidative stability. Further investigation is necessary to evaluate the effects of Vitamin E and other potential antioxidants on freezing and dry aging processes.

3.4. Shear force and cooking loss

No differences were observed in shear force values for the different treatments ($P = .449$; Table 1). Aroeira (2014) also found no differences in shear force values between samples frozen prior to aging and non-frozen samples, both aged for the same period. Wheeler, Miller, Savell, and Cross (1990) reported no differences in shear force values of samples frozen after 14 days of aging compared to samples aged for 13 days and non-frozen samples. However, several studies indicate that freezing prior to aging increases beef tenderness (Crouse & Koohmaraie, 1990; Kim et al., 2017; Lagerstedt et al., 2008; Shanks et al., 2002) which can be associated to muscle fiber disruption through ice crystal formation (Leygonie et al., 2012; Petrović, Grujić, & Petrović, 1993) and/or by the release of proteolytic enzymes that increase the aging rate (Vieira et al., 2009). According to Setyabrata and Kim (2019), this divergence between studies could be related to the various intrinsic and extrinsic factors that affect shear force values; therefore, further investigations are necessary to evaluate the decrease in shear force due to freezing on sensory perception.

In this study, FT + Dry and ST + Dry samples had lower cooking loss compared to Dry and Dry + ST samples ($P < .05$; Table 1). This result was expected due to the lower moisture content found in the FT + Dry and ST + Dry after aging thus less water was available to be lost during the cooking procedure.

3.5. Color

No differences were observed in L* values (lightness) during 4 days of display ($P > .05$; Table 2). However, on the 5th day of display the FT + Dry had slightly lower L* values ($P < .05$; Table 2) compared to the other treatments, although this difference was less than three units, which was considered minimal. Even though several studies have described that freezing and thawing resulted in lower L* values (darker color) compared to non-frozen beef (Aroeira et al., 2017;Kim et al., 2017; Vieira et al., 2009), the results of this current study indicated that the beef lightness was not affected by freezing. This suggests that the dry aging process had more impact on the L* values than the freezing process did.

The Dry showed higher a* (redness), b* (yellowness), and chroma (color intensity) values ($P < .05$; Table 2) compared to the other treatments during the display period, except on the 2nd day, when Dry had sharp decrease in a*, b*, and chroma values ($P < .05$; Table 2). These observations indicated that freezing, either prior to or after dry

Table 1
Effect (mean ± SEM) of treatments on pH, surface a_W, moisture, TBARS, Warner-Bratzler shear force (WBSF) and cooking loss ($n = 48$) of beef samples.

	Dry	Dry + ST	FT + Dry	ST + Dry	P-value
pH	5.59 ± 0.02	5.53 ± 0.02	5.51 ± 0.02	5.45 ± 0.09	0.274
Surface a_W	0.9419 ± 0.0017^{a}	0.9384 ± 0.0030^{ab}	0.9269 ± 0.0030^{bc}	0.9255 ± 0.0046^{c}	< 0.05
Moisture, %	72.80 ± 0.29^{a}	72.91 ± 0.28^{a}	70.02 ± 0.27^{b}	69.61 ± 0.31^{b}	< 0.0001
TBARS, mg MDA/kg	0.24 ± 0.03	0.25 ± 0.02	0.24 ± 0.02	0.24 ± 0.03	0.971
WBSF, N	34.43 ± 1.85	31.00 ± 2.03	31.69 ± 1.04	32.29 ± 1.07	0.449
Cooking loss, %	17.18 ± 0.57^{a}	17.03 ± 0.72^{a}	13.10 ± 0.51^{b}	12.62 ± 0.71^{b}	< 0.0001

^{a,b}Different letters in a row indicate significant differences ($P < .05$).
Dry: dry aging for 28 days; Dry + ST: dry aging for 28 days, steaks fabrication, freezing at −20 °C and thawing at 4 °C/24 h; FT + Dry: freezing at −20 °C, fast thawing at 20 °C/15 h and dry aging for 28 days; ST + Dry: freezing at −20 °C, slow thawing at 4 °C/48 h and dry aging for 28 days.

Table 2
Effect (mean ± SEM) of treatments on L*, a*, b*, chroma and hue parameters of color stability during 5 days of display (n = 48).

Treatment	Color parameter	Display period (days)				
		1	2	3	4	5
Dry	L*	33.95 ± 0.68	29.19 ± 0.66	33.48 ± 1.04	32.71 ± 0.82	32.47 ± 0.90^{ab}
Dry + ST		32.65 ± 0.78	29.88 ± 0.66	31.22 ± 0.65	30.83 ± 0.62	31.30 ± 0.51^{ab}
FT + Dry		34.56 ± 0.61	28.93 ± 0.62	31.22 ± 0.82	31.68 ± 1.03	29.97 ± 0.75^{b}
ST + Dry		35.45 ± 0.87	31.19 ± 0.64	32.39 ± 0.71	33.55 ± 0.59	32.94 ± 0.61^{a}
P-value		0.070	0.084	0.168	0.102	< 0.05
Dry	a*	23.84 ± 0.54^{a}	16.73 ± 0.83^{ab}	21.69 ± 0.95^{a}	20.82 ± 0.73^{a}	21.29 ± 0.79^{a}
Dry + ST		19.25 ± 0.66^{b}	19.29 ± 0.47^{a}	17.79 ± 0.43^{b}	16.61 ± 0.50^{b}	15.49 ± 0.40^{b}
FT + Dry		18.49 ± 0.81^{b}	18.76 ± 0.65^{ab}	15.48 ± 0.75^{bc}	15.48 ± 0.82^{bc}	14.46 ± 0.53^{c}
ST + Dry		16.65 ± 0.83^{b}	16.43 ± 0.80^{b}	14.59 ± 0.79^{c}	13.28 ± 0.54^{c}	12.12 ± 0.64^{c}
P-value		< 0.0001	< 0.05	< 0.0001	< 0.0001	< 0.0001
Dry	b*	20.67 ± 0.42^{a}	9.56 ± 0.77^{b}	17.73 ± 0.94^{a}	17.36 ± 0.53^{a}	17.65 ± 0.64^{a}
Dry + ST		15.55 ± 0.57^{b}	16.32 ± 0.44^{a}	16.14 ± 0.34^{ab}	14.44 ± 0.49^{b}	12.94 ± 0.41^{b}
FT + Dry		13.63 ± 0.71^{bc}	15.81 ± 0.71^{a}	13.41 ± 0.75^{bc}	13.44 ± 0.82^{b}	12.14 ± 0.69^{b}
ST + Dry		11.60 ± 0.79^{c}	12.25 ± 0.93^{b}	11.77 ± 1.02^{c}	10.76 ± 0.65^{c}	8.94 ± 0.72^{c}
P-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Dry	Hue angle	40.93 ± 0.25^{a}	29.49 ± 0.90^{c}	39.14 ± 0.30^{bc}	39.86 ± 0.41	39.69 ± 0.42^{a}
Dry + ST		38.93 ± 0.52^{a}	40.22 ± 0.51^{a}	42.24 ± 0.33^{a}	40.96 ± 0.47	39.83 ± 0.60^{a}
FT + Dry		36.30 ± 0.62^{b}	40.02 ± 0.52^{a}	40.80 ± 0.54^{ab}	40.83 ± 0.87	39.73 ± 0.72^{a}
ST + Dry		34.59 ± 0.78^{b}	36.25 ± 0.96^{b}	38.29 ± 1.03^{c}	38.73 ± 1.06	35.88 ± 1.20^{b}
P-value		< 0.0001	< 0.0001	< 0.0001	0.141	< 0.05
Dry	Chroma	31.56 ± 0.67^{a}	19.30 ± 1.07^{b}	28.02 ± 1.32^{a}	27.12 ± 0.89^{a}	27.66 ± 0.99^{a}
Dry + ST		24.75 ± 0.84^{b}	25.28 ± 0.60^{a}	24.02 ± 0.53^{b}	22.02 ± 0.67^{b}	20.19 ± 0.54^{b}
FT + Dry		22.98 ± 1.05^{bc}	24.55 ± 0.94^{a}	20.48 ± 1.05^{bc}	20.52 ± 1.12^{b}	18.91 ± 0.84^{b}
ST + Dry		20.31 ± 1.11^{c}	20.52 ± 1.18^{b}	18.78 ± 1.25^{c}	17.12 ± 0.78^{c}	15.09 ± 0.92^{c}
P-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

^{a,b} Different letters in a column, for each color parameter, indicate significant differences ($P < .05$).
Dry: dry aging for 28 days; Dry + ST: dry aging for 28 days, steaks fabrication, freezing at −20 °C and thawing at 4 °C/24 h; FT + Dry: freezing at −20 °C, fast thawing at 20 °C/15 h and dry aging for 28 days; ST + Dry: freezing at −20 °C, slow thawing at 4 °C/48 h and dry aging for 28 days.

Table 3
Microbial counts (log CFU/g ± SEM) on beef samples.

Microbial groups	Dry		FT + Dry			ST + Dry		
	Fresh sample	After dry aging	Fresh sample	After thawing	After dry aging	Fresh sample	After thawing	After dry aging
TBC[1]	2.79 ± 0.24^{a}	3.27 ± 0.25^{a}	3.34 ± 0.37^{a}	3.36 ± 0.35^{a}	3.54 ± 0.16^{a}	2.88 ± 0.44^{a}	2.70 ± 0.32^{a}	3.18 ± 0.08^{a}
PSY[2]	2.58 ± 0.29^{b}	4.07 ± 0.34^{ab}	2.55 ± 0.28^{b}	3.04 ± 0.23^{b}	4.79 ± 0.59^{a}	2.58 ± 0.29^{b}	2.98 ± 0.06^{b}	5.05 ± 0.14^{a}
EB[1]	< 1.00 ± 0.00^{a}	< 1.00 ± 0.00^{a}	< 1.00 ± 0.10^{a}	< 1.10 ± 0.10^{a}	< 1.83 ± 0.49^{a}	< 1.00 ± 0.00^{a}	< 1.00 ± 0.00^{a}	< 1.16 ± 0.16^{a}
LAB[1]	< 1.26 ± 0.14^{b}	< 1.53 ± 0.53^{b}	< 1.16 ± 0.16^{b}	< 1.26 ± 0.14^{b}	< 1.80 ± 0.41^{ab}	< 1.42 ± 0.28^{b}	< 1.10 ± 0.10^{b}	2.56 ± 0.24^{a}
YM[2]	< 2.00 ± 0.00^{a}	3.02 ± 0.47^{a}	< 2.00 ± 0.00^{a}	< 2.10 ± 0.10^{a}	2.96 ± 0.32^{a}	< 2.00 ± 0.00^{a}	< 2.00 ± 0.00^{a}	< 2.73 ± 0.37^{a}

^{a,b} Different letters in a row indicate significant differences ($P < .05$).
Dry: dry aging for 28 days; FT + Dry: freezing at −20 °C, fast thawing at 20 °C/15 h and dry aging for 28 days; ST + Dry: freezing at −20 °C, slow thawing at 4 °C/48 h and dry aging for 28 days.
TBC: total bacterial count; PSY: psychrotrophic microorganisms; EB: *Enterobacteriaceae*; LAB: lactic acid bacteria; YM: yeasts and molds.
[1] Limit of detection: 1 log CFU/g; [2] Limit of detection: 2 log CFU/g.

aging, reduced the beef color stability. Similar results were also observed in other studies, where frozen beef had lower a* values than non-frozen (Kim et al., 2015; Kim et al., 2017; Setyabrata & Kim, 2019; Vieira et al., 2009). The freezing of meat causes muscle fiber disruption and concentration of solutes, which results in protein denaturation, including myoglobin (Leygonie et al., 2012; Setyabrata & Kim, 2019). Thus, freezing increases the susceptibility of myoglobin oxidation (Kim et al., 2015; MacDougall, 1982; Renerre, 1990; Setyabrata & Kim, 2019), and consequently reduces color stability of the frozen/thawed meat compared to non-frozen one. However, in the current study the ST + Dry had the lowest hue angle values ($P < .05$; Table 2) during display compared to the other treatments, excepting on the 4th day where there was no difference between the treatments ($P = .141$; Table 2). For meat color analysis, the increase of hue values during display storage correspond to more discoloration (Kim et al., 2017; Vieira et al., 2009). Overall, hue angle showed similar values throughout the display period within each treatment.

3.6. Microbiological analyses

The samples analyzed prior to aging and freezing (fresh samples) showed low microbial load, TBC ranged from 2.79 to 3.34 log CFU/g, PSY was around 2.55 log CFU/g and LAB from < 1.16 to < 1.42 log CFU/g. *Enterobacteriaceae* and yeasts and molds were below the limit of detection (1 log CFU/g for ENT; 2 log CFU/g for YM). These results are similar to others reported in the literature (DeGeer et al., 2009; Li et al., 2014; Li, Babol, Wallby, & Lundström, 2013).

Favorable conditions for microbial growth may occur during meat thawing, due to cell disruption and destruction of muscle fibers caused by freezing (Choe, Stuart, & Kim, 2016). The temperature increase and exudate release create an ideal environment for microbial development (Gill, 2014). However, in the current study no statistical difference ($P > .05$) was observed among fresh samples and the samples analyzed immediately after thawing.

After 28 days of dry aging, for TBC and PSY, the highest counts obtained were 3.54 log CFU/g in FT + Dry samples and 5.05 log CFU/g in ST + Dry samples, respectively, corroborating with Ahnström,

Table 4
Volatile compounds identified in cooked beef samples regard aging and freezing treatments (pooled sample).

Groups	Class	Compound	Fresh sample	Dry	Dry + ST	FT + Dry	ST + Dry
All treatments	Alcohols	1-Penten-3-ol	x	x	x	x	x
	Alcohols	1-Hexanol	x	x	x	x	x
	Alcohols	1-Hexanol, 2-ethyl-	x	x	x	x	x
	Alcohols	1-Pentanol	x	x	x	x	x
	Alcohols	Ethanol, 2-butoxy-	x	x	x	x	x
	Aldehydes	Butanal, 2-methyl-	x	x	x	x	x
	Aldehydes	Butanal, 3-methyl-	x	x	x	x	x
	Aldehydes	Octanal	x	x	x	x	x
	Aldehydes	Heptanal	x	x	x	x	x
	Aldehydes	Nonanal	x	x	x	x	x
	Aldehydes	Pentanal	x	x	x	x	x
	Aldehydes	Hexanal	x	x	x	x	x
	Aldehydes	Decanal	x	x	x	x	x
	Aromatic C.	Ethylbenzene	x	x	x	x	x
	Aromatic C.	Furan, 2-pentyl-	x	x	x	x	x
	Aromatic C.	Toluene	x	x	x	x	x
	Aromatic C.	Benzaldehyde	x	x	x	x	x
	Carboxylic Ac.	Pentanoic acid	x	x	x	x	x
	Ketones	5-Hepten-2-one, 6-methyl-	x	x	x	x	x
	Ketones	2-Heptanone	x	x	x	x	x
	Ketones	2-Pentanone	x	x	x	x	x
	Ketones	2,3-Octanedione	x	x	x	x	x
	Ketones	2-Butanone, 3-hydroxy-	x	x	x	x	x
	Lactone	Butyrolactone	x	x	x	x	x
	Sulfur C.	Dimethyl trisulfide	x	x	x	x	x
	Sulfur C.	Disulfide, dimethyl	x	x	x	x	x
Fresh sample (non-aged)	Alcohols	1-Undecanol	x				
	Alcohols	1-Octanol	x				
	Aldehydes	Undecanal	x				
	Esters	Formic acid, pentyl ester	x				
	Hydrocarbons	2-Octene, (*Z*)-	x				
	Hydrocarbons	1-Octene	x				
	Hydrocarbons	Tridecane	x				
	Hydrocarbons	Hexane	x				
	Hydrocarbons	Octane	x				
	Ketones	2-Heptanone, 6-methyl-	x				
Never frozen	Alcohols	6-Hepten-1-ol		x			
	Carboxylic Ac.	Nonanoic acid		x			
	Hydrocarbons	1,3-Octadiene		x			
Dry-aged	Alcohols	1-Butanol		x	x	x	x
	Alcohols	1-Butanol, 3-methyl-		x	x	x	x
	Alcohols	2,3-Butanediol		x	x	x	x
	Aldehydes	2-Butenal, 2-methyl-, (*E*)-		x	x	x	x
	Carboxylic acid	Hexanoic acid		x	x	x	x
	Esters	Ethyl Acetate		x	x	x	x
	Hydrocarbons	Ethane, (methylthio)-		x	x	x	x
	Hydrocarbons	Nonane, 5-butyl-		x	x	x	x
	Ketones	2,3-Butanedione		x	x	x	x
Frozen	Alcohols	2-Propanol, 1-methoxy-			x		x
	Alcohols	1-Butanol, 2-methyl-, (.+/−.)-				x	
	Alcohols	2,3-Butanediol, [R-(R@,R@)]-				x	x
	Alcohols	Benzyl Alcohol			x		
	Aromatic C.	Thiazole				x	
	Carboxylic Ac.	Octanoic Acid			x		
	Esters	Propanoic acid, 2-hydroxy-, ethyl ester			x		
	Ether	n-Butyl ether			x	x	x
	Ketones	2-Heptanone, 3-methyl-					x
	Ketones	2-Heptanone, 4,6-dimethyl-				x	x
Not grouped	Alcohols	3-Buten-1-ol, 3-methyl-	x	x	x	x	
	Alcohols	1-Heptanol	x	x	x		
	Alcohols	1-Octen-3-ol	x	x	x		
	Alcohols	1-Propanol, 2-methyl-		x	x	x	
	Aldehydes	Butanal		x			
	Aldehydes	2-Butenal, 3-methyl-		x		x	x
	Aldehydes	Methional/propanal, 3-methylthio		x		x	x
	Carboxylic Ac.	Butanoic acid, 3-methyl-		x		x	x
	Hydrocarbons	Nonane	x	x	x		x
	Hydrocarbons	1-Heptene	x	x		x	x
	Hydrocarbons	Decane	x	x			
	Hydrocarbons	Pentadecane		x		x	x
	Ketones	2,3-Pentanedione	x	x		x	x
	Ketones	2-Butanone	x		x		

(*continued on next page*)

Table 4 (*continued*)

Groups	Class	Compound	Fresh sample	Dry	Dry + ST	FT + Dry	ST + Dry
	Terpenoids	.alpha.-Pinene	x		x	x	x

Dry: dry aging for 28 days; Dry + ST: dry aging for 28 days, steaks fabrication, freezing at −20 °C and thawing at 4 °C/24 h; FT + Dry: freezing at −20 °C, fast thawing at 20 °C/15 h and dry aging for 28 days; ST + Dry: freezing at −20 °C, slow thawing at 4 °C/48 h and dry aging for 28 days.

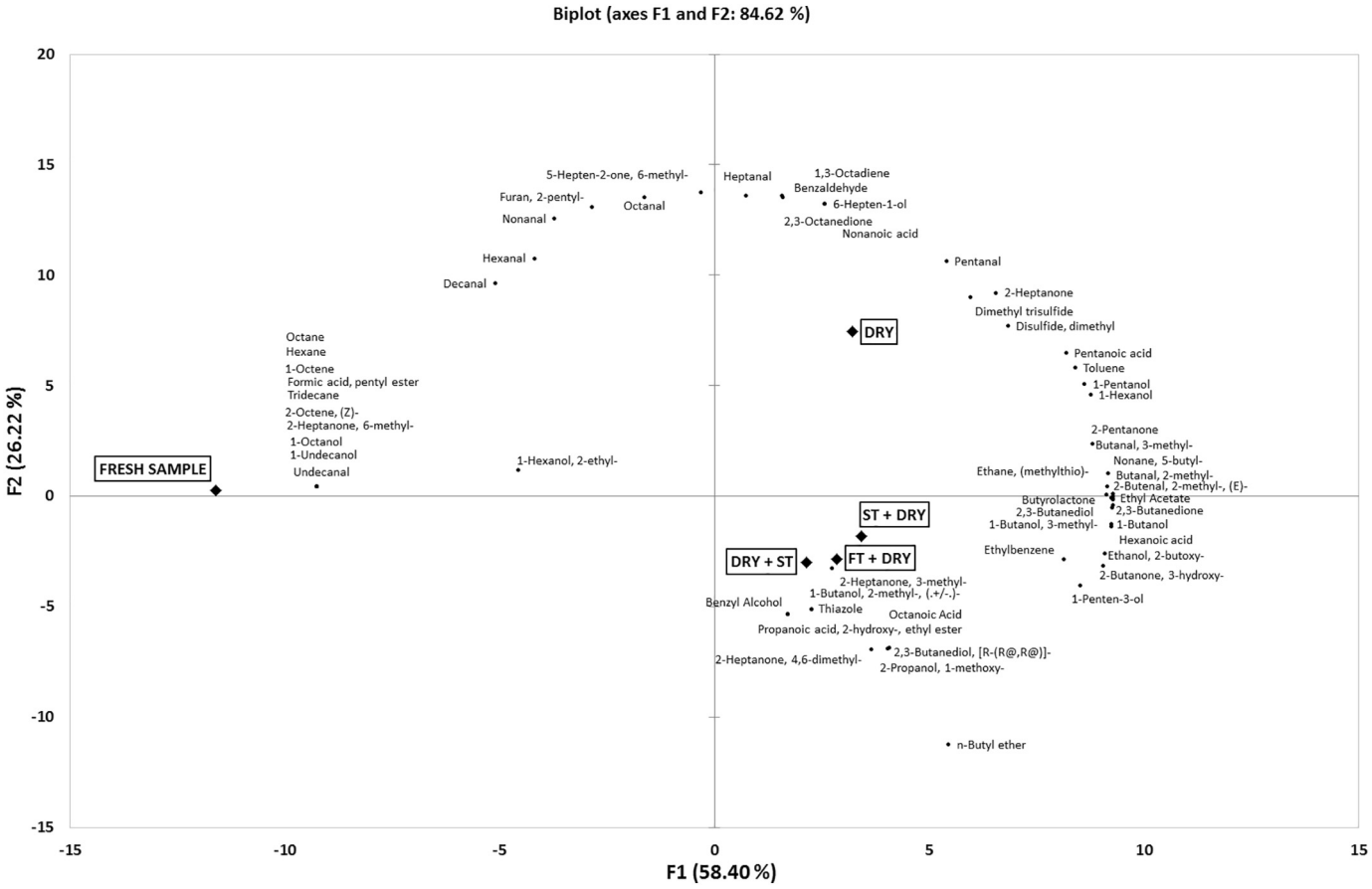

Fig. 2. Principal component analysis (PCA) score plot of cooked beef samples and volatile compounds.
Fresh Sample: non-aged beef; Dry: dry aging for 28 days; Dry + ST: dry aging for 28 days, steaks fabrication, freezing at −20 °C and thawing at 4 °C/24 h; FT + Dry: freezing at −20 °C, fast thawing at 20 °C/15 h and dry aging for 28 days; ST + Dry: freezing at −20 °C, slow thawing at 4 °C/48 h and dry aging for 28 days.

Seyfert, Hunt, and Johnson (2006), Gudjónsdóttir et al. (2015), and Li et al. (2013). For LAB, 2.56 log CFU/g was achieved in the ST + Dry samples, similar values have been reported in dry-aged beef (Hulánková, Kameník, Saláková, Závodský, & Borilova, 2018). *Enteroba÷cteriaceae* were < 2 log CFU/g and YM ≤ 3 log CFU/g. The PSY counts obtained in FT + Dry and ST + Dry were approximately 1 log CFU/g higher than Dry; nevertheless, the results were not statistically different ($P > .05$; Table 3). LAB counts were higher for ST + Dry compared to Dry ($P < .05$), but similar to FT + Dry counts. *Aspergillus* sp. was isolated in 75% of the FT + Dry samples. In addition, *Cladosporium* sp. was identified in 25% of the FT + Dry and ST + Dry samples. Although commonly found in refrigerated meats (Fung, 2014), there were no previous reports on the presence of these molds in dry-aged beef. Therefore, the data indicated that freezing prior to dry aging had no major impact on the microbial load. In addition, all the processes resulted in microbiological counts ≤5 log CFU/g, i.e., had an acceptable microbiological quality (Feiner, 2006; Hulánková et al., 2018).

3.7. Volatile compounds qualitative profile

Altogether, 73 volatile compounds were identified and classified as alcohols ($n = 19$), aldehydes ($n = 13$), ketones (n = 13), hydrocarbons ($n = 12$), aromatic compounds ($n = 5$), carboxylic acids ($n = 4$), esters ($n = 3$), sulfur compounds ($n = 2$), ether (n = 1) or terpenoid (n = 1). Twenty-six of these compounds were found in all samples analyzed. Many of these compounds detected in all samples are commonly related to cooked beef, for example 1-Hexanol, 2-ethyl- (green); 1-Pentanol (mild, fruit); Octanal (fatty, green); Heptanal (fatty); Pentanal (almond, pungent, malt); Hexanal (grassy taste); 2-Heptanone (fruity); Benzaldehyde (burning aromatic taste) (Calkins & Hodgen, 2007; Faridnia et al., 2015; Insausti, Beriain, Gorraiz, & Purroy, 2002). Several volatile compounds found in cooked meat include products of lipid oxidation, for example, hydrocarbons, aldehydes, ketones, alcohols, carboxylic acids, and esters (Mottram, 1998).

In this study, 10 volatile compounds were found only in non-aged samples (fresh) and nine compounds in aged samples, frozen or non-frozen (Table 4). Therefore, it can be suggested that the volatile compounds found in non-aged samples were transformed into other products, when submitted to aging, as they did not appear in the aged

samples, frozen or non-frozen. Generally, hydrocarbons do not have odoriferous importance and many of them were isolated in non-aged samples (fresh): 2- octene (*Z*)-, 1- octene, tridecane, hexane, octane. While in dry-aged samples, only two hydrocarbons were found: ethane (methylthio)- and nonane 5-butyl.

Three volatile compounds were only detected in non-frozen dry-aged samples (Table 4). From these compounds, nonanoic acid is found in both dry and wet-aged beef (King, Matthews, Rule, & Field, 1995), and 1,3 octadiene is found in beef (Calkins & Hodgen, 2007). Studies indicated that, in aged beef, volatile compounds from fatty acid oxidation increases and affect the beef flavor intensity (Gorraiz, Beriain, Chasco, & Insausti, 2002; Khan, Jo, & Tariq, 2015).

In relation to the frozen samples, thiazoles, a product formed from lipid oxidation products combined with Maillard reaction compounds were found. Additionally, other compounds found included ketones (2-heptanone 3 methyl and 2-heptanone, 4,6 dimethyl - both associated to "green" flavor); octanoic acid (fatty, soapy, rancid, cheesy); benzyl alcohol (bitter); 2,3 butanediol (buttery); 1-butanol, 2-methyl (+/−) (roasted, onion); 2-propanol, 1-methoxy (compound found in coffee).

Several studies have reported that the freezing/thawing process increases lipid oxidation compared to non-frozen aged meat (Ali, Rajput, Li, Zhang, & Zhou, 2016; Hansen et al., 2004; Setyabrata & Kim, 2019; Xia, Kong, Liu, & Liu, 2009). Lipid oxidation is the major form of deterioration in frozen meat and results in the accumulation of volatile carbonyls, alcohols, and acids, which are responsible for off-flavors (Utrera, Parra, & Estévez, 2014). Moreover, ketones are some of the main aroma compounds derived from lipid oxidation (Resconi, Escudero, & Campo, 2013). However, in this study, TBARS values were low, under the threshold for detection of rancid flavor, and no differences between treatments were observed (Table 1), although different compounds related to lipid oxidation were identified in the aged samples, frozen or non-frozen (Table 4). This suggested that, regardless of the treatment, volatile compounds were formed due to the lipid oxidation, possibly in low quantities. These results could be better explained by further investigations about the effects of vitamin E and other potential antioxidants on beef from grass-fed cattle submitted to freezing and dry aging processes, and by the quantification of volatile compounds identified in this study.

A PCA (Fig. 2) was created to illustrate the differences between each treatment based on individual volatile compounds. The first principal component (PC1) described 58.40% and the second principal component (PC2) described 26.22% of the total variation. The PC1 indicated a distinct difference between non-aged (fresh samples) and the other treatments, while the PC2 explained the differences between non-frozen and frozen treatments. The frozen samples, either prior to or after aging, were located in the lower right quadrant clearly separated from the dry-aged samples, located in the top right quadrant. These results indicated that both aging and freezing process modify the volatile compounds profile compared to non-aged beef. In addition, volatile compounds from lipid and protein degradation were identified in this study, which could affect the meat flavor. Therefore, further investigations are suggested to evaluate the effects of freezing and dry aging processes on the beef sensory attributes.

4. Conclusion

Freezing, prior to or after dry aging, changed the volatile compounds profile compared to non-frozen samples, although no differences were observed in lipid oxidation. Freezing prior to dry aging had no significant effect on the microbiological counts compared to non-frozen dry-aged samples. However, freezing prior to dry aging greatly increased the evaporation and total process loss, reducing moisture content, water activity, and cooking loss. Therefore, freezing the loins prior to dry aging was not considered a viable process. The freezing after dry aging did not have a major impact on the physical-chemical traits; however, further studies on sensory attributes are recommended to validate the consumer acceptance and the viability of freezing after dry aging, to preserve dry-aged beef for a longer period and, consequently, increase the opportunities for this high value market.

Declaration of Competing Interest

The authors declare no conflict of interest.

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