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Dry-aged and wet-aged beef: effects of aging time and temperature on microbiological profile, physicochemical characteristics, volatile compound profile and weight loss of meat from Nellore cattle (*Bos indicus*)

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Abstract

Context. Dry-aged beef is a value-added product with a unique flavour. The effects of the dry aging process, in terms of chamber temperature and aging time, have not been described previously for *Bos indicus* meat.

Aims. This study evaluated the effects of aging conditions (method, temperature, time) on the microbiological, physicochemical, volatile compound profile and weight loss characteristics of dry-aged and wet-aged beef from Nellore cattle (*Bos indicus*).

Methods. Beef loins (n = 16) were cut into eight portions and assigned to treatments in a complete block design combining aging method (dry or wet), temperature (2°C or 7°C) and time (21 or 42 days).

Key results. Samples dry-aged at 7°C had higher ($P < 0.05$) weight loss than samples dry-aged at 2°C. Although storage temperature did not affect ($P > 0.05$) the yield of wet-aged samples, Enterobacteriaceae counts increased ($P < 0.05$) in the samples stored at 7°C compared with 2°C. Aging for 42 days resulted in greater ($P < 0.05$) process losses (drip + evaporation + trimming) for both aging methods than aging for 21 days. The pH, moisture content and Warner–Bratzler shear force values were not affected ($P > 0.05$) by aging method, temperature or time. Qualitative analysis indicated that volatile compounds were affected by aging method and time, but not by aging temperature.

Conclusions. The results indicate that higher temperature and longer aging periods cannot be not recommended for either dry or wet aging, owing to the increase in process losses of dry-aged samples, and growth of Enterobacteriaceae and psychrotrophic bacteria in wet-aged samples.

Implications. This study highlights the importance of controlling conditions for dry aging to produce a safe product and obtain higher yields. Processing plants or retailers that produce dry-aged or wet-aged meat could use these results to adjust their production systems.

Keywords: dry aging, wet aging, aging time, temperature, beef quality, Nellore cattle, *Bos indicus*.

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Introduction

Zebu cattle (*Bos indicus*), mainly grass-fed Nellore breed, account for 80% of the Brazilian herd (Ferraz and Felício 2010). *Bos indicus* cattle such as Nellore are known to have lower aging rates (Wheeler *et al*. 1994) than British cattle breeds. In addition, grass-based diets hinder fat deposition in carcasses, compromising beef sensory quality (Shorthose and Harris 1990; Koohmaraie 1994). However, after 7–14 days of aging, Nellore cattle beef can achieve an acceptable tenderness level (Ferraz and Felício 2010). Therefore, aging is one of the main techniques used to improve the sensory attributes of beef such as flavour and tenderness, and can be performed in a vacuum package (wet aging) or without packaging (dry aging).

Dry aging has gained the interest of consumers because it purportedly has desirable flavours (Li *et al*. 2013; Stënström *et al*. 2014; Lepper-Blilie *et al*. 2016) such as roasted beef

flavour (Warren and Kastner 1992) and umami taste (Li *et al.* 2014). However, the effectiveness of the dry aging process is questionable, and some studies have found no sensory differences between dry-aged and wet-aged samples (Parrish *et al.* 1991; Laster *et al.* 2008). Production of dry-aged beef requires strict control of the aging conditions, including temperature, relative air humidity and air speed. Control and monitoring of these parameters will reduce weight loss and inhibit microbial growth (Kim *et al.* 2016; Savell 2008), resulting in a consistent and high-quality product.

Several studies have compared dry and wet aging techniques (Parrish *et al.* 1991; Warren and Kastner 1992; Laster *et al.* 2008; Smith *et al.* 2008; Dikeman *et al.* 2013; Lepper-Blilie *et al.* 2016; Vilella *et al.* 2019); however, those studies were conducted at temperatures between 1°C and 3°C, using beef of British cattle such as Angus or Hereford. These breeds have a thicker fat cover and higher marbling levels (O'Connor *et al.* 1997) and they have higher aging rates, and consequently, greater tenderness than beef of Nellore cattle (Whipple *et al.* 1990). Additionally, under commercial conditions, temperature can reach higher values than those recommended in the literature (0–4°C; Dashdorj *et al.* 2016). High temperatures could increase beef tenderness through enzymatic processes; however, they might increase microbiological growth (Dashdorj *et al.* 2016; Choe *et al.* 2018).

We evaluated the impact and viability of two aging methods (dry and wet aging) under two different temperatures (2°C and 7°C) and two aging times (21 and 42 days) using beef of Nellore cattle. Microbiological and physicochemical characteristics (moisture, water activity, pH, shear force), as well as volatile compounds and weight loss, were assessed.

Materials and methods

Sample collection and aging conditions

We collected 16 bone-in loins (*longissimus thoracis et lumborum* from the sixth thoracic vertebra to the third lumbar vertebra) from both sides of eight carcasses of Nellore intact male cattle (~36 months of age, carcass weight 245 ± 25 kg, average pH 5.46 ± 0.01, subcutaneous fat thickness measured between 12th and 13th ribs 5.33 ± 0.71 mm) at a commercial packing plant at 2 days post-mortem (data are presented as mean ± standard deviation). The bone-in loins were placed on ice and transported to the Meat Laboratory, Food Technology Department, University of Campinas.

In the laboratory, each loin was divided into four sections (eight loin sections per animal) plus a steak 1.5 cm thick. This steak was vacuum-packaged, wrapped in aluminium foil, frozen at –18°C, and used to characterise the volatile compounds of fresh samples (non-aged). Each loin section was then assigned to one of the eight treatments comprising wet or dry aging, storage at 2°C or 7°C, and aging for 21 or 42 days, according to a predetermined complete block design (Fig. 1). The aging time for dry-aged beef varies greatly and periods of 14–35 days are normally cited in the literature (Ahnström *et al.* 2006; Berger *et al.* 2018; Hulánková *et al.* 2018). However, in Brazil, on average, dry-aged processors use a minimum of 21 days and a maximum of 42 days.

The sections assigned for the wet aging process were weighed, deboned, reweighed and vacuum packaged in Cryovac BB 2620 packages (Cryovac Brasil, São Paulo) of 50 µm thickness; O_2 permeability 20 cm^3/m^2, 24 h, 1 bar (1 kPa) at 23°C and 0% relative humidity; and maximum CO_2 permeability of 100 cm^3/m^2, 24 h, bar at 23°C and 0% relative

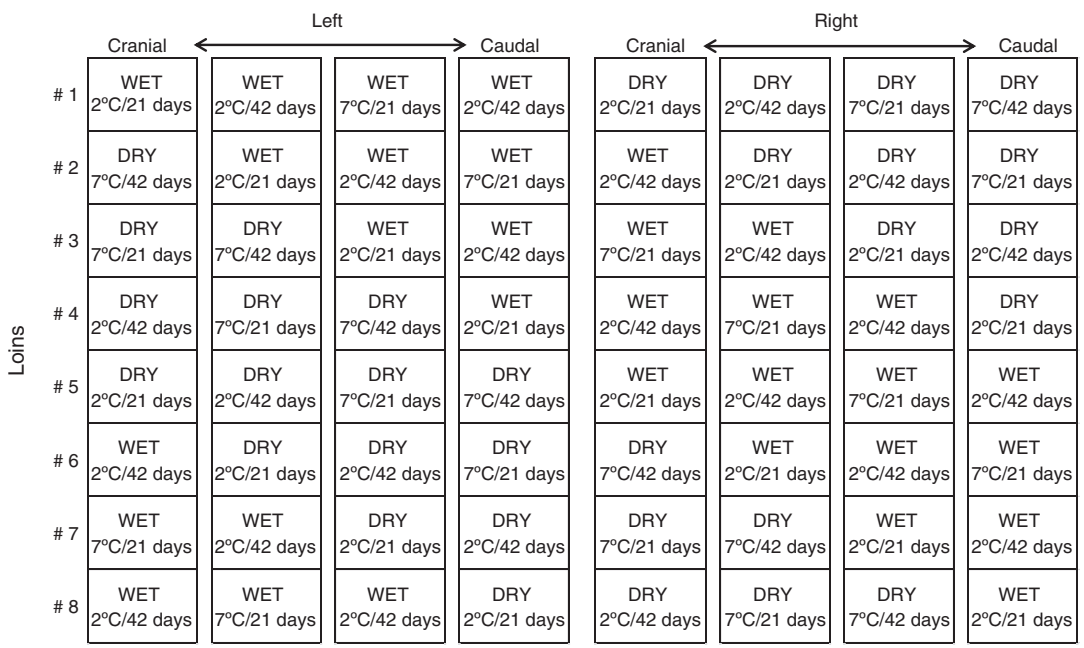

Fig. 1. Schematic illustrating treatment allocation to each section of paired-loins from eight carcasses based on a balanced complete block design: wet-aging (Wet) and dry-aging (Dry) at two chamber temperatures (2°C or 7°C) and for two aging times (21 and 42 days).

humidity. For the dry-aged treatments, the bone-in loin sections were weighed and placed in the aging chamber with the subcutaneous fat facing up. Every 3 days, the samples were rotated inside the chamber to avoid location effects. The aging chambers (model VN50R; MetalFrio, São Paulo) were set to work at 75 ± 5% relative humidity and 2.5 m/s airspeed.

After aging and trimming, the loin sections were weighed and cut into steaks. The steaks were sequentially assigned to the analyses, following the respective order (anterior to posterior): pH and moisture contents (~2.0 cm thick), volatile compounds (~1.5 cm thick), and Warner–Bratzler shear force (~2.5 cm thick).

Weight loss

Samples (n = 64) were weighed before and after aging to determine (*i*) weight losses due to drip (for wet-aged samples only) according to the equation ((weight before aging – weight after aging)/weight before aging) × 100; and (*ii*) evaporation (for dry-aged samples only), calculated as ((weight of bone-in loin sections before aging – weight of aged bone-in loin sections)/weight of bone-in loin sections before aging) × 100. Respective weight losses due to deboning and trimming were determined by the following equations: (weight of bones/weight before aging) × 100, and (weight of trimmings/weight before aging) × 100. Process loss was defined as drip loss for wet-aged samples, and the sum of evaporation and trimming losses for dry-aged samples.

Microbiological analyses

Microbiological analyses were performed on three loins from each treatment. At each point of analysis, a sample (10 g) was collected, using tweezers and a scalpel, from five different locations of the external surface of each loin (samples 2 mm thick were collected to represent only the external area), avoiding subcutaneous fat. The samples were aseptically placed in sterile plastic bags (Twirl'em; Labplas, Montreal, Canada). The same amount of material was collected from the internal part (lean beef) of dry-aged samples after trimming. Each sample was homogenised with 90 mL 0.1% peptone water (Difco, Sparks, MD, USA) in a Stomacher 400 Circulator (Seward, Worthing, UK) for 2 min at 230 r.p.m. Decimal dilutions were made with 0.1% peptone water when necessary.

Psychrotrophic (PSY) and total (TBC) bacterial counts were determined on plate count agar (Acumedia, Lansing, MI, USA) with incubation at 7°C for 10 days for PSY (Vasavada and Critzer 2015) and at 35°C for 48 h for TBC (Ryser and Schuman 2015). Lactic acid bacteria (LAB) were counted by using de Man, Rogosa and Sharpe agar (Difco), incubated at 35°C for 72 h under anaerobiosis (Probac do Brasil, São Paulo) (Njongmeta *et al*. 2015). Enterobacteriaceae (EB) were quantified by using Violet Red Bile Glucose agar (Acumedia) in a pour plate with overlay and incubation at 35°C for 24 h (Kornacki *et al.* 2015).

Mould and yeast counts (MYC) were determined in Dichloran Rose Bengal Chloramphenicol agar (Acumedia) at 25°C for 5 days (Ryu and Wolf-Hall 2015). The moulds obtained in the samples were isolated in Czapek Yeast Autolysate agar at 25°C for 7 days and identified by morphological characteristics (Pitt and Hocking 2009).

Gram staining and catalase test were performed to confirm colonies of LAB, EB and yeasts. The results were expressed as log colony forming units (CFU)/g.

Moisture content, pH and water activity

Moisture content was determined in triplicate from each steak by grinding and drying the lean beef in a forced air convection oven, according to AOAC methodology (Official Methods of Analysis 2000)). The pH was measured in duplicate by inserting a calibrated potentiometer (MP125 portable pH meter; Mettler Toledo, Brazil) directly into the sample. Water activity (a_w; ratio of vapour pressure of the sample in an undisturbed balance with the surrounding air media and vapor pressure of distilled water under identical conditions) was determined in duplicate on the surface portion (3 mm) of both dry-aged and wet-aged samples by using a water activity analyser (AquaLab 4TE; Decagon, São José dos Campos, SP, Brazil).

Warner–Bratzler shear force and cooking loss

For the shear force analysis, one steak from each section was cooked following the protocol described by AMSA (2015), with adaptations. The steaks were cooked in an electric oven (FRITOMAQ, São Paulo) set at 170°C until the samples reached an internal temperature of 71°C measured by a copper–constantan thermocouple (E5CWL; OMRON, Kyoto), inserted into the geometric centre of the steaks. Cooking loss was determined according to the equation: (weight of raw sample – weight of cooked sample)/weight of raw sample × 100.

After cooking, the steaks were cooled at room temperature, wrapped in polyvinyl chloride film, and chilled overnight at 4°C according to AMSA protocol (AMSA 2015). Six cylinders, each of 1.27 cm diameter, were removed from each steak parallel to the muscle fibre orientation by using a coring device. The cylinders were sheared in a texturometer (TA-XT plus; Stable Micro Systems, Godalming, UK), equipped with a Warner–Bratzler Blade 1 mm thick (AMSA 2015).

Volatile compound qualitative profile

Volatile compounds were analysed for fresh (non-aged) samples and aged samples. The analyses were performed according to the method described by da Silva Bernardo *et al*. (2020). The samples were first kept under refrigeration for 24 h and then cooked for the analyses of volatile compounds. For each treatment, as well as for fresh samples, four random steaks (1.5 cm thick) were broiled in a pre-warmed electric oven (NKS, 9 L, 800 W; Rio de Janeiro), until an internal temperature of 75°C was reached. The four steaks were then cut into pieces and ground in a food processor (Walita Viva RI1364/06; Philips, Rio de Janeiro) and a pool of samples for each treatment was obtained. A sample of this pool

Table 1. Weight loss due to drip, evaporation, trimmings, process and deboning of aged beef samples
Values are means ± s.e.m. Definitions: drip (D), relation between final weight and initial weight of boneless samples in the wet aging process; evaporation (E), relation between final weight and initial weight of bone-in samples in the dry aging process; trimmings (T), relation between weight of trimmings (dried surfaces) and weight of sample after aging and deboning at the end of the dry aging process; process (P), relation between final weight and initial weight in the aging process (for dry-aged P = E + T; for wet-aged P = D); deboning, relation between weight of bones removed from the loin (after the dry aging process and at the beginning of the wet aging process) and initial weight

	Drip	Evaporation	Trimmings (%)	Process	Deboning
			Aging method		
Wet (n = 32)	2.64 ± 0.21	–	–	2.64 ± 0.21	33.34 ± 1.17
Dry (n = 32)	–	18.71 ± 0.62	5.69 ± 0.23	24.41 ± 0.75	34.09 ± 0.91
P-value	–	–	–	<0.05	0.61
			Aging temperature		
2°C (n = 32)	2.50 ± 0.26	17.95 ± 0.88	5.41 ± 0.32	12.93 ± 1.95	34.33 ± 1.11
7°C (n = 32)	2.78 ± 0.34	19.47 ± 0.86	5.98 ± 0.32	14.12 ± 2.10	33.09 ± 0.97
P-value	0.53	0.23	0.22	0.68	0.40
			Aging time		
21 days (n = 32)	1.78 ± 0.12	16.02 ± 0.52	5.01 ± 0.34	11.40 ± 1.76	34.32 ± 1.10
42 days (n = 32)	3.50 ± 0.27	21.40 ± 0.59	6.38 ± 0.19	15.64 ± 2.20	33.10 ± 0.98
P-value	<0.05	<0.05	<0.05	0.14	0.41
			Interaction effects		
Method × temperature	–	–	–	<0.05	0.51
Method × time	–	–	–	<0.05	0.40
Temperature × time	0.42	0.82	0.35	0.95	0.63
Method × temperature × time	–	–	–	0.63	0.30

Table 2. Effect of aging time (21 and 42 days) and temperature (2°C and 7°C) on process loss in wet-aged and dry-aged beef
Values are means ± s.e.m. Pairs of means followed by the same letter are not significantly different (P > 0.05): within rows for lowercase letters; within columns for uppercase letters. Process: sum of losses due to drip, evaporation and trimmings

	Wet (n = 32)	Dry (n = 32)
	Aging time	
21 days (n = 32)	1.78 ± 0.12bB	21.03 ± 0.67aB
42 days (n = 32)	3.50 ± 0.27bA	27.79 ± 0.58aA
	Aging temperature	
2°C (n = 32)	2.50 ± 0.26bA	23.36 ± 1.06aB
7°C (n = 32)	2.78 ± 0.34bA	25.45 ± 1.03aA

(10 g) was weighed in a flask (capacity 60 mL). Analyses were performed in triplicate for each treatment. We used the solid-phase microextraction technique with a CAR/PDMS fibre (Carboxen/polydimethylsiloxane; 1 cm length, d_f 75 µm, for use with manual holder, needle size 24 gauge; Supelco, Bellefonte, PA, USA) as the stationary phase to extract the volatile compounds. The extraction was performed at 60°C for 10 min in a water bath. The stationary phase then was exposed in the headspace for 65 min. Gas chromatography coupled to a mass spectrometry (QP-2010 model; Shimadzu, Kyoto) was used to separate and identify the 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 column (5% phenyl, 95% dimethylpolysiloxane; 60 m length, 0.25 mm internal diameter and 1 µm width of the stationary phase; J&W Scientific, Santa Clara, CA, USA). The mass spectrum was set at the scanning mode and the monitoring range from 35 to 350 m/z. Compounds were first identified by their mass spectra compared with the equipment library database (GC-MS Solution software; Shimadzu). An n-alkane (C7–C30) solution (Supelco) 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 for confirmation of the identification of compounds. Identification was confirmed by comparing the LTPRI and the mass spectra with data in the literature (NIST Mass Spectrometry Data Center 2020). The criteria adopted were a minimum of 85% similarity and a maximum variation of ±10%.

Statistical analyses

The experimental design was a 2 × 2 × 2 factorial (2 aging methods × 2 temperatures × 2 aging times), with eight biological replicates (n = 8; 8 animals × 2 carcass sides × 4 sections = 64 sections for 4 treatments). The treatments were distributed along two loins from the same animal according to an extended Latin square design in a randomised order of location on the first animal (Fig. 1). Data from physicochemical and instrumental results were statistically analysed using the factorial analysis of variance (ANOVA) in Statistica 10.0 software (StatSoft, Tulsa, OK, USA) using a model with the fixed main effects of aging method, aging temperature and aging time, and the random effects of animal,

Table 3. Effects of beef aging method, temperature and time on microbial counts

Values are means ± s.e.m. Within a column and treatment, means followed by the same letter are not significantly different ($P > 0.05$). TBC, Total bacteria count; PSY, psychrotrophic bacteria; EB, Enterobacteriaceae; LAB, lactic acid bacteria; MYC, mould and yeast count. Limit of detection 1 $\log_{10}$ CFU/g for TBC, EB, LAB; 2 $\log_{10}$ CFU/g for PSY, MYC

	TBC	PSY	EB ($\log_{10}$ CFU/g)	LAB	MYC
			Aging method		
Wet (n = 12)	7.22 ± 0.24a	8.20 ± 0.14a	5.35 ± 0.45a	6.93 ± 0.14a	2.10 ± 0.10
Dry, surface (n = 12)	3.52 ± 0.27b	4.43 ± 0.34b	1.02 ± 0.02 b	2.16 ± 0.24b	2.50 ± 0.29
Dry, internal (n = 12)	3.31 ± 0.19b	4.59 ± 0.37b	1.36 ± 0.14 b	2.64 ± 0.24b	2.18 ± 0.18
P-value	<0.05	<0.05	<0.05	<0.05	0.25
			Aging temperature		
2°C (n = 18)	4.42 ± 0.42b	5.56 ± 0.50	2.18 ± 0.42b	3.86 ± 0.54	2.00 ± 0.00b
7°C (n = 18)	4.95 ± 0.51a	5.92 ± 0.46	2.97 ± 0.60a	3.96 ± 0.55	2.52 ± 0.23a
P-value	<0.05	0.19	<0.05	0.69	<0.05
			Aging time		
21 days (n = 18)	4.56 ± 0.43	5.41 ± 0.47b	2.41 ± 0.41	3.96 ± 0.46	2.36 ± 0.21
42 days (n = 18)	4.81 ± 0.51	6.07 ± 0.48a	2.74 ± 0.61	3.86 ± 0.61	2.16 ± 0.12
P-value	0.33	<0.05	0.13	0.71	0.32
			Interaction effects		
Method × temperature	0.44	<0.05	<0.05	0.42	0.25
Method × time	<0.05	0.28	<0.05	0.12	0.10
Temperature × time	0.22	0.10	0.17	0.80	0.32
Method × temperature × time	0.88	0.07	<0.05	0.34	0.10

carcass side and section, with eight replicates. Microbiological data were evaluated using a 3 × 2 × 2 factorial ANOVA because the dry-aged samples were analysed from the surface and internal areas (3 treatments × 2 temperatures × 2 aging times), using a model with the fixed main effects of aging method, aging temperature and aging time, with three repetitions. Mean values (±s.e.m.) were analysed by the Tukey test at $P = 0.05$. The profile of volatile compounds was analysed by using principal component analysis (PCA). Each volatile compound was selected and its area values were adjusted and used to perform the PCA, in order to determine separation between groups. The values for the peak areas do not characterise the real amounts of the volatiles in the samples and are only a parameter for associating the aging-related variations in the volatile compounds of a single sample.

Results and discussion

Sample characterisation

The non-aged samples had pH 5.46 ± 0.01 (mean ± standard deviation). Contents of fat and moisture were 3.11 ± 0.48% and 74.15 ± 0.41%, respectively, and subcutaneous fat thickness was 5.33 ± 0.71 mm. Water activity (a_w) was 0.993 ± 0.001 and shear force was 4.97 ± 0.56 kg.

Weight loss

No interactions ($P > 0.05$) among aging method, temperature and time were observed for drip, evaporation, trimming or deboning losses (Table 1).

Drip loss was not affected ($P > 0.05$) by temperature, evaluated only on the wet-aged samples, although it was affected by aging time, with samples aged for 42 days presenting higher ($P < 0.05$) drip loss than samples aged for 21 days (Table 1). Similarly, evaporation and trimming losses, evaluated only on the dry-aged samples, were not affected ($P > 0.05$) by temperature; however, both evaporation and trimming losses increased ($P < 0.05$) with aging time (Table 1). Higher evaporation and trimming losses after 42 days of aging were to be expected, because lean beef continues to lose weight from dehydration during the dry aging process. In addition, these results suggest that evaporation could increase the dried surface (i.e. thickness of the dried surface), which in turn resulted in a greater trimming loss. Likewise, Ahnström *et al*. (2006) indicated that a longer period (21 vs 14 days) of dry aging increased weight loss. Aging method had no effect ($P > 0.05$) on the amount of bone to be removed after aging (Table 1). This result agrees with other studies that reported no difference in weight loss due to deboning between dry-aged and wet-aged samples (Kim *et al*. 2016; Berger *et al*. 2018; Vilella *et al*. 2019).

There was a significant ($P < 0.05$) aging method × time interaction for the process loss (Table 1). Furthermore, samples aged for 42 days, both dry-aged and wet-aged, showed a higher ($P < 0.05$) process loss than those aged for 21 days, and regardless of aging time, dry-aged samples showed a higher ($P < 0.05$) process loss than wet-aged samples. Other studies comparing dry and wet aging also indicated higher weight loss on dry-aged samples (Laster *et al*. 2008; Dikeman *et al*. 2013; Li *et al*. 2014; Kim *et al*. 2016; Berger *et al*. 2018; Vilella *et al*. 2019). There was a significant ($P < 0.05$) aging method × temperature interaction for process loss (Table 1), in which no difference ($P > 0.05$)

Table 4. Microbial counts (mean $\log_{10}$ CFU/g ± s.e.m.) after aging for 21 and 42 days at 2°C and 7°C for wet-aged beef samples and for dry-aged samples before (surface) and after trimming (internal)

TBC, Total bacteria count; PSY, psychrotrophic bacterial; EB, Enterobacteriaceae; LAB, lactic acid bacteria; MYC, mould and yeast counts. Limit of detection 1 $\log_{10}$ CFU/g for TBC, EB, LAB; 2 $\log_{10}$ CFU/g for PSY, MYC

	21 days						42 days					
	2°C			7°C			2°C			7°C		
	Dry, surface	Dry, internal	Wet	Dry, surface	Dry, internal	Wet	Dry, surface	Dry, internal	Wet	Dry, surface	Dry, internal	Wet
TBC	3.40 ± 0.17	2.75 ± 0.05	6.29 ± 0.34	4.38 ± 0.74	3.28 ± 0.39	7.31 ± 0.51	3.02 ± 0.29	3.81 ± 0.00	7.29 ± 0.30	3.30 ± 0.68	3.45 ± 0.62	8.01 ± 0.34
PSY	3.53 ± 0.20	3.66 ± 0.26	7.82 ± 0.48	5.10 ± 0.81	4.27 ± 0.36	8.12 ± 0.11	3.75 ± 0.38	6.30 ± 0.78	8.31 ± 0.13	5.36 ± 0.70	4.15 ± 0.78	8.56 ± 0.12
EB	<1.00 ± 0.00	<1.16 ± 0.16	4.36 ± 0.50	<1.10 ± 0.10	1.93 ± 0.08	4.94 ± 0.47	<1.00 ± 0.00	<1.00 ± 0.00	4.61 ± 0.76	<1.00 ± 0.00	<1.38 ± 0.66	7.49 ± 0.58
LAB	2.41 ± 0.48	2.75 ± 0.07	6.48 ± 0.04	2.48 ± 0.32	2.91 ± 0.12	6.74 ± 0.19	<1.43 ± 0.22	2.87 ± 1.03	7.25 ± 0.38	<2.34 ± 0.73	2.07 ± 0.84	7.26 ± 0.14
MYC	<2.00 ± 0.00	<2.00 ± 0.00	<2.00 ± 0.00	3.79 ± 0.91	<2.00 ± 0.00	<2.41 ± 0.41	<2.00 ± 0.00	<2.00 ± 0.00	<2.00 ± 0.00	2.24 ± 0.14	<2.72 ± 0.72	<2.00 ± 0.00

was observed for process loss between wet-aged samples at 2°C and 7°C, whereas dry-aged samples showed greater ($P < 0.05$) process loss at 7°C than 2°C (Table 2), mainly due to the greater evaporation loss (Table 1). Higher temperature provides a greater dehydration rate in many foods (Krokida *et al*. 2003). In this regard, greater moisture loss was previously reported in beef samples dry-aged at 8°C than 2°C (Muniz Da Silva *et al*. 2019).

Microbiological analyses

Aging method had a significant effect ($P < 0.05$) on all microbial counts except MYC. In general, wet-aged samples showed higher counts than dry-aged samples (Table 3). For dry aging, no differences ($P > 0.05$) were observed between microbial counts obtained on the external surface (before trimming) and internal portion (after trimming) (Table 3). Meanwhile storage time (21 and 42 days) influenced only PSY, and aging temperature (2°C and 7°C) significantly affected ($P < 0.05$) TBC, EB and MYC (Table 3).

Although the statistical analysis showed that temperature had no influence on PSY, there was a significant ($P < 0.05$) aging method × temperature interaction influencing this microbial group (Table 3). PSY counts in dry-aged samples were 1.6 $\log_{10}$ CFU/g higher at 7°C than at 2°C, whereas for wet-aged samples the difference between temperature treatments was much smaller (Table 4). For TBC, a significant ($P < 0.05$) aging method × time interaction was observed (Table 3). On the surface of the dry-aged samples at both temperatures, the count decreased from 21 to 42 days of aging, whereas for the wet-aged samples the count increased from 21 to 42 days of aging. In addition, after 21 days, there was a higher count on the surface of dry-aged samples at 7°C than at 2°C (4.38 vs 3.40 $\log_{10}$ CFU/g); however, after 42 days of aging, the difference was reduced and the final counts were 3.30 and 3.02 $\log_{10}$ CFU/g for 7°C and 2°C, respectively (Table 3). The TBC decrease on the surface of the dry-aged samples at 7°C after 42 days may have occurred because of moisture loss and consequent decrease in water activity (a_w) of the surface of the sample over time (Table 5). Some studies have shown an increase over time in TBC on dry-aged beef at 4°C (Li *et al*. 2014; Gudjónsdóttir *et al*. 2015), whereas other studies showed accelerated microbial growth in the first 2 weeks of aging, reducing afterward (Ahnström *et al*. 2006).

The TBC and PSY counts in wet-aged samples were 2-fold higher ($P < 0.05$) than in dry-aged samples after 42 days; on average, wet-aged samples at 2°C had 7.29 and 8.31 $\log_{10}$ CFU/g of TBC and PSY, respectively, and at 7°C, the counts were 8.01 and 8.56 $\log_{10}$ CFU/g (Table 4). These higher counts are compatible with deteriorated sample and may be related to high water activity of the wet-aged samples ($a_w \geq 0.98$; Table 5). Conversely, other studies reported higher total bacteria counts in dry-aged beef (Li *et al*. 2014; Gudjónsdóttir *et al*. 2015), or no difference between dry-aged and wet-aged methods (Berger *et al*. 2018).

The LAB counts on the surface of dry-aged samples were ≤2.48 $\log_{10}$ CFU/g (Table 4). Other studies reported LAB counts ~3.00 $\log_{10}$ CFU/g (Ahnström *et al*. 2006; Li *et al*. 2014; Gudjónsdóttir *et al*. 2015; Berger *et al*. 2018; Hulánková

Table 5. The pH, water activity (a_w), moisture content, cooking loss and Warner–Bratzler shear force (WBSF) of aged beef samples
Values are means ± s.e.m.

	pH	Water activity	Moisture (%)	Cooking loss (%)	WBSF (kg)
		Aging method			
Wet aging (n = 32)	5.45 ± 0.02	0.9917 ± 0.0007	72.98 ± 0.18	21.10 ± 0.42	3.11 ± 0.09
Dry aging (n = 32)	5.46 ± 0.01	0.9285 ± 0.0022	72.11 ± 0.38	19.38 ± 0.47	3.12 ± 0.09
P-value	0.49	<0.05	<0.05	<0.05	0.96
		Aging temperature			
2°C (n = 32)	5.45 ± 0.01	0.9600 ± 0.0060	72.64 ± 0.31	19.89 ± 0.46	3.13 ± 0.09
7°C (n = 32)	5.46 ± 0.02	0.9602 ± 0.0060	72.46 ± 0.31	20.58 ± 0.47	3.10 ± 0.09
P-value	0.89	0.99	0.68	0.30	0.82
		Aging time			
21 days (n = 32)	5.45 ± 0.01	0.9615 ± 0.0050	72.80 ± 0.30	19.51 ± 0.41	3.08 ± 0.09
42 days (n = 32)	5.46 ± 0.02	0.9587 ± 0.0070	72.29 ± 0.31	20.96 ± 0.49	3.14 ± 0.08
P-value	0.56	0.73	0.23	<0.05	0.64
		Interaction effects			
Method × temperature	0.76	0.44	0.86	0.96	0.72
Method × time	0.18	<0.05	0.27	0.23	0.35
Temperature × time	0.44	0.69	0.92	0.99	0.80
Method × temperature × time	0.47	0.53	0.58	0.87	0.42

et al. 2018). On the other hand, wet-aged samples showed counts of 6.48 and 6.74 $\log_{10}$ CFU/g at 2°C and 7°C after 21 days (Table 4), ~3-fold higher than counts of dry-aged samples. Similar result was reported by Gudjónsdóttir *et al*. (2015). Although LAB can grow in both storage conditions (with and without oxygen), it is the predominant group of bacteria in wet-aged samples (Pennacchia *et al*. 2011; Hernández-Macedo *et al*. 2012; Pothakos *et al*. 2015). In our study, he LAB count on the wet-aged samples was close to the threshold count (7 $\log_{10}$ CFU/g) capable of causing spoilage signals such as off-flavour (Hulánková *et al*. 2018).

Owing to their capacity to metabolise amino acids into volatile compounds such as diamines and sulfur compounds, EB are considered meat spoilage bacteria (Säde *et al*. 2013). In addition, many foodborne pathogens belong to the Enterobacteriaceae family. EB counts were influenced (P < 0.05) by higher order interactions, method × time, method × temperature and method × temperature × time. In dry-aged samples, both before and after trimming, EB counts were ≤1.93 $\log_{10}$ CFU/g. However, in wet-aged samples, after 42 days stored at 2°C EB counts were 4.61 $\log_{10}$ CFU/g, and at 7°C the count was 7.49 $\log_{10}$ CFU/g (Table 4). Therefore, wet aging at 7°C is not recommended, because EB counts >7 $\log_{10}$ CFU/g can cause meat deterioration and risk consumer health.

As noted above, temperature was the only aging factor that affected MYC. Yeasts were found in wet-aged and dry-aged samples, whereas moulds were found only in dry-aged samples (data not shown). The presence of moulds in dry-aged samples is related to the aerobic environment, lower water activity, and consequent lower microbial competition (Fung 2014). Gudjónsdóttir *et al*. (2015) also reported higher mould counts in dry-aged samples. The moulds isolated from the external region (before trimming) of samples aged at 2°C and 7°C were identified as *Cladosporium* sp., *Penicillium brevicompactum* and *Fusarium* sp. After trimming, at the internal area, *Penicillium citrinum* and *Talaromyces funiculosus* were identified in samples aged at 2°C. None of these species had been previously reported in dry-aged beef. Nevertheless, *Cladosporium* and *Penicillium* are commonly found in refrigerated meat, which may indicate their high incidence in the meat production environment (Fung 2014). Other authors reported the presence of *Cochliobolus* sp, *Cochliobolus sativus*, *Mucor racemosus* (Tapp 2016), *Rhodotorula glutinis*, *Rhodotorula mucilaginosa*, *Penicillium camembert* and *Debaryomyces hansenii* (Ryu *et al*. 2018) in dry-aged samples. According to Fung (2014), the presence of *Thamnidium* may be beneficial for dry-aged beef because its development enhances flavour and tenderness; however, we did not detect *Thamnidium* in this study.

pH, water activity, and moisture content

The pH values were not affected (P > 0.05) by aging method, temperature or time (Table 5). Other studies reported similar results with no difference in final pH among dry-aged and wet-aged beef samples under different conditions (temperature, time, relative humidity) of aging (Li *et al*. 2013; Kim *et al*. 2016; Berger *et al*. 2018; Oh *et al*. 2018; Zhang *et al*. 2019). Thus, our results suggest that the aging condition had no major effect on the pH of dry-aged and wet-aged samples.

Water activity (a_w) values were lower (P < 0.05) in the dry-aged than the wet-aged samples (Table 5). This result was expected because water activity was determined on the surface of samples and dry-aged samples had a dryer surface than wet-aged samples. In addition, there was a significant (P < 0.05) aging method × time interaction for water activity (data not shown in tabular form). Comparing 21 and 42 days of aging, the a_w values of dry-aged samples decreased (from 0.934 to 0.923; P < 0.05), whereas those of wet-aged samples increased (from 0.989 to 0.995; P < 0.05). The evaporation process during dry aging may explain the lower water activity in the

Table 6. Volatile compounds identified in cooked beef samples and their association with experimental treatments

RT, Retention time; LTPRI, linear temperature programmed retention index. Another 36 compounds were found in all nine treatments: alcohols (1-butanol, 1-octen-3-ol, 1-pentanol, 1-penten-3-ol and 3-heptanol); aldehydes (2-methylbutanal, 3-methyl-2-butenal, 3-methylbutanal, decanal, heptanal, hexanal, nonanal, octanal and pentanal); aromatic compounds (benzaldehyde, ethylbenzene and toluene); carboxylic acids (hexanoic acid); hydrocarbons (1-octene, 2,3,3-trimethylpentane, 2-octene, decane, pentadecane, tetradecane and tridecane); ketones (2,3-butanedione, 2,3-octanedione, 2,3-pentanedione, 2-butanone, 2-heptanone, 2-pentanone, 3-hydroxy-2-butanone and 6-methyl-5-hepten-2-one); lactone (butyrolactone); sulfur compounds (ethyl methyl sulfide); terpenes (alpha-pinene)

RT (min)	Compound	LTPRI	Class	Treatments (Peak area[A])							
				Fresh	Wet aging				Dry aging		
					21 days		42 days		21 days		42 days	
					2°C	7°C	2°C	7°C	2°C	7°C	2°C	7°C
			Compounds found in all aged treatments									
15.98	2-Methyl-2-butenal	746	Aldehydes		395 114	315 319	193 547	193 905	189 156	223 540	450 913	538 428
24.13	Methional	912	Aldehydes		798 050	574 121	627 273	257 534	401 953	485 226	807 991	854 955
13.41	1-Heptene	691	Hydrocarbons		35 852	35 770	47 837	40 303	19 124	24 420	30 566	34 385
9.56	Hexane	600	Hydrocarbons		662 670	498 561	473 788	730 790	385 775	385 155	556 404	606 993
16.32	Dimethyl disulfide	752	Sulfur comp.		428 662	647 140	771 502	892 936	171 621	178 038	435 030	477 665
			Compounds found only in dry-aged treatments									
16.57	2-Pentenal	757	Aldehydes						2164	13 617	39 940	15 990
30.28	Benzyl alcohol	1045	Aromatic comp.									244 988
26.15	6-Methyl-2-heptanone	955	Ketones								84 898	74 397
20.13	Methylpyrazine	830	Pyrazine						144 884			81 441
28.63	Trimethyl-pyrazine	1009	Pyrazine						77 136	41 714	96 499	90 377
19.23	2-Methylthiazole	811	Sulfur comp.								163 935	243 822
			Compounds found only in wet-aged treatments									
15.66	2-Methyl-1-butanol	738	Alcohols					197 283				
21.43	2-Hexenal	856	Aldehydes		68 347	53 273						
21.01	2-Methylbutanoic acid ethyl ester	848	Ester					45 730				
36.38	Ethyl ester of butanoic acid	1191	Ester					545 045				
			Compounds found only in treatments aged at 2°C									
10.15	2-Methylthiirane	614	Sulfur comp.		25 062						52 888	
			Compounds found only in treatments aged at 7°C									
19.81	3-Methylbutanoic acid	822	Carboxylic acid					404 116				106 032
			Compounds found only in treatments aged for 21 days									
22.65	*n*-Butyl ether	881	Ether		348 784	295 867				210 050		
			Compounds found only in treatments aged for 42 days									
15.8	Ethylcyclopentane	741	Hydrocarbons				26 935					46 071
15.4	Methylcyclohexane	733	Hydrocarbons				36 551					66 770
			Compounds that could not be associated with specific group of treatments									
26.83	1-Heptanol	970	Alcohols	18 844		33 261	81 244	88 503	33 962	43 069	79842	61 936
21.49	1-Hexanol	867	Alcohols	62 688	245 462	242 932	425 965	620 005		220 105	468473	421 404
18.16	2,3-Butanediol	790	Alcohols	179 130		886 831	1 646 928					
10.68	2-Methyl-1-propanol	626	Alcohols			48 929		67 143	54 851	45 894	91814	74 888
15.44	3-Methyl-1-butanol	734	Alcohols			189 011		1 049 311	20 727	33 836	43937	
15.33	3-Methyl-3-buten-1-ol	731	Alcohols	81 320		53 973		56 261	54 123	98 442	59900	
11.73	2-Butenal	651	Aldehydes		75 639						51023	207 940
35.33	2-Nonenal	1167	Aldehydes		15 845						21573	18 684
31.03	2-Octenal	1063	Aldehydes		45 612	36 383					41 502	35 511
30.79	Benzeneacetaldehyde	977	Aromatic comp.		423 682	315 792				273 233	476 736	237 046
16.26	Pyrrole	750	Aromatic comp.	67 353		34 148			124 667	136 744		139 310
15.73	Thiazole	740	Aromatic comp.		113 553						110 301	255 255
15.63	2-Methylpropanoic acid	736	Carboxylic acid					98 544	76 993	49 838	77 635	
17.10	Butanoic acid	768	Carboxylic acid			983 840	208 903	1 784 765			1 045 308	1 170 108
38.17	Nonanoic acid	1260	Carboxylic acid		29 189	18 799				3022		37 728
34.82	Octanoic acid	1153	Carboxylic acid			69 530					77 831	175 128

21.69	Pentanoic acid	862	Carboxylic acid	69 297	141 779	213 836		349 825	166 482	112 220	165 500	179 091
23.84	2-Butoxy-1-ethanol	906	Ether	40 507		62 104	161 281	211 349	211 353	206 957	286 002	310 776
9.73	2-Methylfuran	604	Furan		37 143			29 501			27 500	40 090
27.94	2-Pentylfuran	933	Furan	130 334	351 906	293 162		212 681		185 144	232 371	184 349
20.14	1,3-Octadiene	830	Hydrocarbons		87 438	33 134					45 924	
14.15	2-Heptene	708	Hydrocarbons		40 361	35 554	55 086	84 716	22 990		6889	
18.52	3-Ethyl-3-hexene	797	Hydrocarbons		72 057		84 605		57 441		111 496	141 628
19.11	3-Methyl-2-heptene	809	Hydrocarbons		47 899		58 017	123 453	15 261			91 527
36.67	Dodecane	1200	Hydrocarbons	32 098	424 763	133 003	62 720	162 661	43 915	48 911	47 505	
18.66	Octane	800	Hydrocarbons		1 822 588		1 991 975	1 731 825	600 346		2 013 805	2 384 367
13.65	3-pentanone	696	Ketones			73 414		73 372	66 792	72 579	130 266	
27.83	Dimethyl trisulfide	991	Sulfur comp.		317 690	365 601	444 912	579 071			306 552	385 066
14.65	Methyl propyl sulfide	717	Sulfur comp.		46 009	39 429	37 417	38 243		30 462	40 219	42 356
28.04	Beta-pinene	995	Terpenes	52 080		114 883	30 400					52 813
30.11	Limonene	1042	Terpenes	22 057	856 903	289 484	72 256	260 270	45 950	117 647		26 765

[A]Peak area obtained by GC-FID (detector response factors equal to unity). It corresponds to the absolute area of the chromatographic peaks.

samples dry-aged for 42 days; it is a continuous process that could reduce the water activity on the meat surface. The higher water activity in the samples wet-aged for 42 days might be explained by water redistribution, because free water may increase in conditions where immobilised water has moved within the meat structures. During post-mortem aging, muscle structures become loose as a result of myofibrillar degradation and cytoskeletal proteins (Huff-Lonergan and Lonergan 2005).

As expected, dry-aged samples had lower ($P < 0.05$) moisture content than wet-aged samples (Table 5). This result is related to evaporation that occurred in dry-aged samples, which reduces moisture compared with wet-aged samples. Other studies also indicated lower moisture content in dry-aged than in wet-aged samples (Dikeman *et al*. 2013; Li *et al*. 2014).

Warner–Bratzler shear force and cooking loss

No interactions ($P > 0.05$) among aging method, temperature and time were found for cooking loss and Warner–Bratzler shear force (Table 5).

Aging temperature did not affect ($P > 0.05$) cooking loss (Table 5); however, differences were found with aging method and time. Dry-aged samples had lower cooking loss values than those wet-aged, and samples aged for 21 days had lower values than those aged for 42 days (both $P < 0.05$; Table 5). With increasing aging time, immobilised water likely changed into to free water, which is easily lost during cooking (Kołczak *et al*. 2007). Laster *et al*. (2008) and Smith *et al*. (2014) also reported lower cooking loss for dry-aged than wet-aged samples. According to Kim *et al*. (2016), water that is lost during ageing cannot be lost during cooking.

The Warner–Bratzler shear force was not affected ($P > 0.05$) by aging method, temperature or time (Table 5). According to Ferraz and Felício (2010), even in zebu breeds, after 7–14 days of aging, the *longissimus* muscle achieves an acceptable tenderness level. Other studies also found no differences in Warner–Bratzler shear force values between dry-aged and wet-aged samples under different aging conditions: 14 and 21 days of aging at 3°C (Vilella *et al*. 2019); 28 days at 2°C (Berger *et al*. 2018; Oh *et al*. 2018); 14, 21, 28, 35, 42 and 49 days of aging at 1°C (Lepper-Blilie *et al*. 2016); 21 days of aging at 2.2°C (Dikeman *et al*. 2013); and 14, 21, 28 and 35 days of aging at 1°C (Smith *et al*. 2008). On the other hand, Jose *et al*. (2020) found wet-aged samples to be tenderer than dry-aged ones after 28 days of aging. Furthermore, some of these studies indicated that the shear force values decreased with aging time (Smith *et al*. 2008; Lepper-Blilie *et al*. 2016; Vilella *et al*. 2019). No differences were observed in shear force values between 21 and 42 days of aging, or between 2°C and 7°C temperature treatments. Likewise, an increase in tenderness is temperature-dependent; higher temperature accelerates the enzymatic process, which enhances tenderness and beef palatability. Nevertheless, higher temperature can also increase microbiological growth, compromising beef quality (Dashdorj *et al*. 2016). In addition, all treatments showed a low shear force value (Warner–Bratzler shear force $\leq$3.9 kg), with samples

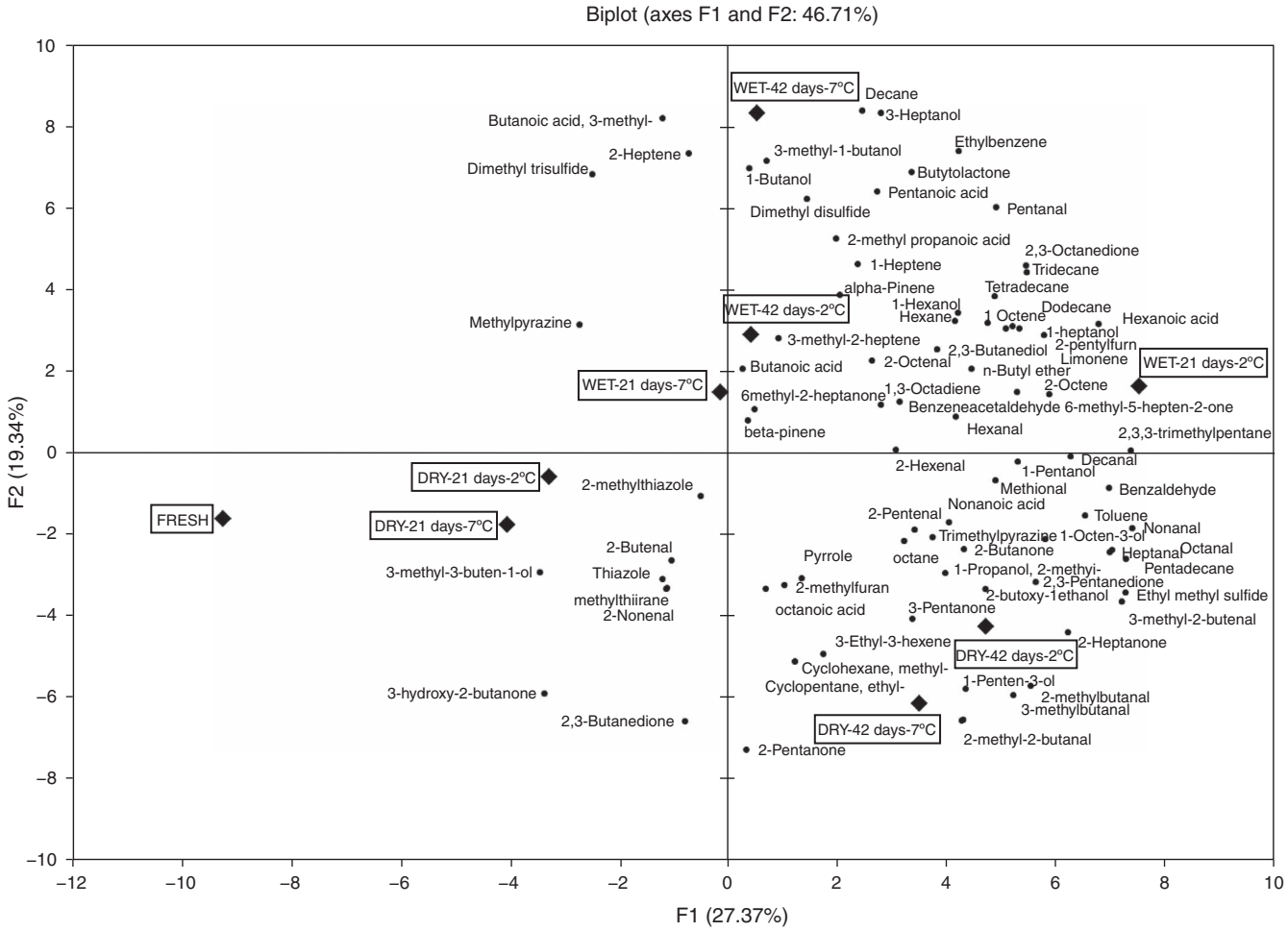

Fig. 2. Principal component analysis (PCA) score plot of cooked beef samples and volatile compounds.

considered very tender (ASTM 2011), regardless of the aging conditions (method × temperature × time).

Thus, even using beef from Nellore cattle with thin fat cover and low marbling, this study showed that both aging methods (dry and wet aging) resulted in tender beef.

Qualitative profile of volatile compounds

Overall, we recognised 86 volatile compounds (Table 6) and classified as them hydrocarbons (n = 17), aldehydes (n = 16), alcohols (n = 12), ketones (n = 10), aromatic compounds (n = 7), carboxylic acids (n = 7), sulfur compounds (n = 6), terpenoids (n = 3), esters (n = 2), furans (n = 2), pyrazine (n = 2), ether (n = 1) and lactone (n = 1).

The compounds 2-methyl-1-butanol, 2-hexenal, ethyl ester of butanoic acid and 2-methylbutanoic acid ethyl ester were found only in wet-aged samples (Table 6). Presence of 2-hexenal is related to linolenic acid oxidation, and the ethyl ester of butanoic acid and 2-methylbutanoic acid ethyl ester are related to fruit aromas (reaction between ethanol and butyric acid) (NCBI 1988; Belitz *et al.* 2009). Trimethylpyrazine and 2-pentenal, from pyrazine and aldehydes groups respectively, were found in dry-aged samples from both aging times (21 and 42 days) and temperatures (2°C and 7°C). These compounds are related to the Maillard reaction and linked to peanut, nut and roasted aromas. The compound 2-methylthiazole, also related to the Maillard reaction (DeMan 1999; De Maria *et al.* 1999), was found in samples that were dry-aged longer (42 days) at both temperatures (Table 6).

A PCA was created to show variances for each treatment based on individual volatile compounds (Fig. 2). The first principal component (PC1) described 27.37% and the second (PC2) described 19.34% of the total variation. Generally, in the PCA biplot, a larger number of volatile compounds can be observed in the wet-aged samples at both temperatures, followed by dry-aged samples at 42 days regardless of temperature. The fresh (non-aged) samples and those dry-aged for 21 days, at both temperatures, were in the same quadrant with fewer volatile compounds (Fig. 2).

The PC1 is explained by some important odoriferous meat compounds such as the hexanoic acid (goaty), 1-heptanol (oily/fatty) and 2-pentylfuran (butter) (Calkins and Hodgen 2007), which are compounds closely related to the sample wet-aged for 21 days at 2°C. This specific sample was separated

from the other wet-aged samples, although wet-aged samples are located practically in the same quadrant. Samples wet-aged for 42 days at 7°C were located close to the following compounds: pentanoic acid, which is related to lipid oxidation and described as rancid (Mottram 1998); 3-methyl butanoic acid, related to the Strecker degradation (parmesan cheese) (Parker 2015); and 3-methyl dimethyl disulfide and dimethyl trisulfide (cabbage-like aroma), compounds derived from amino acid degradation and which could undesirably affect meat flavour (Belitz *et al*. 2009).

The samples wet-aged for 21 days at 7°C and 42 days at 2°C are related to methylpyrazine (earthy aroma) (Belitz *et al*. 2009) and butanoic acid (rancid) (Kerth and Miller 2015). PC2 explained the differences between wet-aged and dry-aged samples, located in the superior and inferior parts of the PCA plot, respectively. The dry-aged samples were separated by aging time but not by temperature (at 42 days, the number of volatile compounds was higher, similar to wet-aged samples), which means that volatile compounds were not affected by temperature. Samples dry-aged for 42 days, at both temperatures, were related to lipid oxidation compounds such as 2-heptanone (fruity/spicy), octanal (fatty), nonanal (fatty/green) and 1-octen-3-ol (mushrooms) (Calkins and Hodgen 2007), and thiamine degradation compounds 2-methylbutanal and 3-methylbutanal (malty, nuts and roasted), characteristics of roasted beef (Macleod 1994; Belitz *et al*. 2009).

Conclusions

In this study, regardless of aging method, temperature and time, beef of Nellore cattle, characterised by thin fat cover and low marbling, was tender (WBSF <4.4 kg), indicating that both aging methods increased beef tenderness, and could improve product acceptability. The dry-aged samples presented a greater reduction in surface water activity with lower internal moisture content than wet-aged samples. Although aging for 42 days at high temperature did not affect the physicochemical traits, it increased process loss for dry-aged samples and allowed further growth of Enterobacteriaceae and psychrotrophic bacteria, as well as formation of sulfur-based and oxidation-related volatile compounds linked to off-flavour in wet-aged samples, suggesting that these conditions should be avoided for both aging methods. The present study suggests that aging for 21 days at 2°C is the best processing method of the treatments trialled, mainly because it resulted in higher yield in dry-aged samples, and lower microbial counts and undesirable volatile compounds in wet-aged samples.

Conflicts of interest

The authors declare no conflict of interests.

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