Meat Science 234 (2026) 110045

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Bioactive compounds and fatty acid composition in raw and cooked beef with two different ageing periods

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ARTICLE INFO

Keywords:
Anserine
Carnosine
Coenzyme Q10
Glutathione
Lipids
Peptides

ABSTRACT

This study investigated the effects of ageing period (5 vs. 90 days) and meat status (raw vs. cooked) in a 2 × 2 factorial design on the concentration of bioactive compounds, intramuscular fat content and fatty acid composition of the *longissimus lumborum* muscle in beef. Samples were collected from 20 Angus steers finished on pasture. Significant ($P < 0.05$) interactions between ageing period and meat status were found for L-carnitine, glutathione, and taurine. Taurine and glutathione concentrations were greater ($P < 0.05$) in raw beef aged for 90 days, while L- carnitine content was lower in cooked meat aged for 5 days than the other treatments. Concentrations of coenzyme Q10, glutathione, L-carnitine, and taurine were higher ($P < 0.05$), and carnosine and anserine content were lower ($P < 0.05$) in meat aged for 90 days compared to 5 days. All bioactive compounds presented greater ($P < 0.05$) concentrations in raw than cooked meat. Beef aged for 90 days presented a greater ($P < 0.05$) proportion of intramuscular fat (IMF), and concentrations of saturated fatty acids (SFA), monounsaturated (MUFA), *n*-6 polyunsaturated fatty acids (PUFA)/*n*-3 PUFA ratio, atherogenic and thrombogenic indices. Cooking increased ($P < 0.05$) the concentrations of all fatty acids, however the PUFA/SFA ratio was greater ($P < 0.05$) in raw meat. Our findings show that ageing and cooking affect the bioactive compounds concentrations and fatty acids present in beef, and both factors should be considered to really know what meat provides under the conditions in which it is consumed.

1. Introduction

Meat is considered a high biological value protein, providing all the essential amino acids (Williams, 2007). Beef is also recognized as an important source of B vitamins (particularly vitamin B12), haem iron, zinc, phosphorus, long-chain fatty acids, and bioactive compounds with potential nutraceutical properties (Jairath et al., 2024; Leroy et al., 2023; Mora et al., 2017). Bioactive compounds are extra-nutritional components that exert physiological effects beneficial for human health (Teodoro, 2019). Different bioactive compounds have been reported in beef, such as L-carnitine, coenzyme Q10, taurine, anserine, carnosine, and glutathione (Chan & Decker, 1994; Purchas et al., 2004; Purchas et al., 2006; Rakowska et al., 2017; Rigault et al., 2008). L-carnitine plays a crucial role in energy generation, with the transportation of fatty acids into mitochondria (Rigault et al., 2008). Coenzyme Q10 is an important antioxidant which also participates in the mitochondrial electron transport chain (Overvad et al., 1999). Taurine plays a role in muscle contraction, regulation and defense against oxidative stress (Arihara & Ohata, 2008; Spriet & Whitfield, 2015). Among the different types of bioactive compounds, the peptides present

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https://doi.org/10.1016/j.meatsci.2026.110045
Received 30 June 2025; Received in revised form 23 January 2026; Accepted 25 January 2026
Available online 26 January 2026

in meat can be generated by endogenous enzymes in postmortem ageing or through other methods, such as heat treatment by cooking (Lafarga & Hayes, 2014; Mora et al., 2017; Sentandreu et al., 2002). In contrast, carnosine and anserine found in skeletal muscle are endogenous dipeptides with an important antioxidant function and buffering capacity at physiological pH (Boldyrev & Severin, 1990; Everaert et al., 2019). Glutathione is a tripeptide with a recognized antioxidant effect and is involved in cellular redox balance and detoxification (Marí et al., 2009; Schmidt & Dringen, 2011).

Ageing is the most recognized and widely used process to improve meat tenderness and palatability, which involves the intrinsic proteolytic systems of meat (Kim et al., 2018). A relevant amount of research has evaluated physicochemical, microbiological and sensory properties of beef during ageing (Holman et al., 2022; Lee et al., 2023; Lepper-Blilie et al., 2016; Sitz et al., 2006). However, there is limited literature that assesses the effects of meat ageing on its nutritional properties. Furthermore, beef nutritional value can also be influenced by cooking (Alfaia et al., 2010; Badiani et al., 2002) that causes meat shrinkage (Davey & Gilbert, 1974) and fluid losses, altering also protein and fat levels (Alfaia et al., 2010; Badiani et al., 2002; Purslow et al., 2016). From the nutritional standpoint, it is valuable to know the concentration of nutrients remaining in foods after cooking.

The present study aimed to evaluate the intramuscular fat content, levels of bioactive compounds: anserine, carnosine, L-carnitine, coenzyme Q10, glutathione and taurine, and the composition of fatty acids in raw and cooked beef aged for 5 or 90 days.

2. Materials and methods

2.1. Sampling and experimental treatments

Twenty Angus steers (under 30 months of age) reared and finished on pastures under commercial practices, were slaughtered (hot carcass weight: 257.5 kg) in a commercial meat packing plant according to the Uruguayan legislation. Twenty striploins (*longissimus lumborum* muscle) were removed from each left half carcass vacuum packaged and transported to the Meat Laboratory of the National Agricultural Research Institute of Uruguay. Subsequently, four 2.5 cm steaks were obtained from each striploin in a randomized order from the cranial to caudal direction. Steak samples were vacuum packaged (Cryovac®, Sealed Air Corporation, BB 2620, Brazil) and aged for 5 or 90 days at 2–4 °C. After each ageing period and for the cooked treatments, steaks were weighed before cooking using an electronic scale (EP-41KA, A&D Company, Tokyo, Japan). Steaks were cooked in a preheated clamshell-style grill (GRP100 The Next Grille ration, Spectrum Brands, Inc., Miami, USA) until the internal temperature at the geometric center reached 71 °C (AMSA, 2016) measured with a digital probe thermometer (Comark N9094, Norwich, UK). After cooking, the surface of each steak was slightly dried with a paper towel to remove the excess of liquid, cooled to room temperature, and weighed again to determine the cooking losses (CL) as: [(raw weight − cooked weight) / raw weight] × 100.

Experimental treatments were generated from the combination of two meat ageing periods (5 vs. 90 days) and meat status (raw vs. cooked). Therefore, four treatments were evaluated: raw meat aged for 5 days (R5d); cooked meat previously aged for 5 days (C5d); raw meat aged for 90 days (R90d); and cooked meat previously aged for 90 days (C90d).

Samples were homogenized using a Robot Coupe R2 (Robot Coupe®, Monteau-les-Mines, France). Approximately 15 g of meat was packed in individual whirl-pack bags (Nasca, Fort Atkinson, USA) and stored at −80 °C until analysis.

2.2. Moisture analysis

Moisture (%) was determined following AOAC method 950.46 (AOAC, 1991), by the losses of matter after drying. Approximately 2 g of sample were weighed and dried for 24 h at 100 °C in a forced-air oven (Thermo Scientific™ Heratherm™, Waltham, USA). Moisture was calculated according to the following formula: Moisture (%) = [wet weight − dry weight) / wet weight] × 100.

2.3. Bioactive compounds

The concentrations of anserine and carnosine were determined following the procedure described by Chamorro et al. (2014). For L-carnitine and glutathione, the quantification followed Hiemori-Kondo et al. (2022) protocol. In both protocols, a 0.5 g sample was extracted with 8% perchloric acid (PCA). Subsequently, samples were analyzed by high-performance liquid chromatography (HPLC; Shimadzu, Prominence, Kyoto, Japan) with an Asahipak NH2P-50 4E amino column (150 mm × 4.6 mm × 5 μm, Sodex, Tokyo, Japan). Quantification was performed using external calibration curves with pure analytical standards (Sigma-Aldrich, Milwaukee, USA). The retention times and UV–visible absorption spectra of the analytes in the samples were compared with those of the standards.

The analysis of coenzyme Q10 was based on the method described by Ercan and El (2011) with minor modifications. Briefly, 0.5 g of each sample was homogenized in a blender with 500 μL of NaCl (0.15 M), 1% of BHT, 750 μL of ethanol, and 250 μL of hexane. Each sample was vortexed for 1 min and centrifuged (Biocen 22R, Ortoalresa, Madrid, Spain) at 18,000 rpm for 10 min and then coenzyme Q10 was extracted with hexane. Samples were filtered and 10 μL was injected into the HPLC (Shimadzu, Prominence, Kyoto, Japan) equipped with a Luna C18 (150 mm × 4.6 mm × 3 μm, Phenomenex, Torrance, USA) column. Quantification was performed using a pure analytical standard for the calibration curves.

Taurine determination was performed using an adapted procedure of López-Fernández et al. (2022). Approximately 0.1 g of each meat sample was weighed, and 5% trichloroacetic acid (TCA) was added. Samples were then homogenized in a vortex (Bead Genie, Scientific Industries, NY, USA) and centrifuged (Biocen 22R, Ortoalresa, Madrid, Spain) at 4 °C for 20 min. After centrifugation, the resulting supernatant (500 μL of extract) was collected, and 1000 μL of acetonitrile (ACN) was added. Finally, samples were injected into an HPLC (Shimadzu, Prominence, Kyoto, Japan), equipped with a Supelcosil LC-18 column (150 mm × 4.6 mm × 3 μm, Sulpeco, Bellefonte, USA). Quantification was conducted using external calibration with a pure analytical standard.

The concentration of the bioactive compounds was expressed as mg/100 g.

2.4. Intramuscular fat and fatty acid composition

Intramuscular fat (IMF) proportion was determined gravimetrically using the chloroform-methanol method following the procedure described by Bligh and Dyer (1959). Fatty acids were cold methylated with methanolic potassium hydroxide solution (IUPAC, 1987), and the analysis was performed by gas chromatography (Shimadzu Nexis GC 2030, Tokyo, Japan) using a SLB – IL111 of 100 m capillary column (0.25 mm internal diameter and 0.2 μm film thickness; Supelco, Bellefonte, USA). Hydrogen was used as carrier gas at a flow rate of 1.28 mL/min with linear velocity control at 30 cm/s. The injection volume was 1 μL, and a flame ionization detector (FID) was used. The detector was kept at 260 °C, while the injector was at 250 °C; the temperature ramp was 150 °C for 5 min, with an increase of 4 °C/min until reaching 165 °C for 12 min, and increase of 3 °C/min until reaching 230 °C, with an increase of 20 °C/min until reaching 250 °C for 2 min, totaling 45.42 min. Fatty acid identification was carried out by comparing the retention times with those of the standard mixture of 37 compounds (FAME Supelco TM 37, Sigma, St. Louis, USA). In addition, conjugated linoleic acid (CLA; c9, t11) was identified using octadecadienoic acid, conjugated, methyl ester standard (No. O5632, Sigma, St. Louis, USA). An internal standard, 700 μL of 1 mg methyl heneicosanoate (C21:0) was

Table 1
Least-squares means ± standard error, of moisture and bioactive compound concentrations according to the ageing period (5 or 90 days) and meat status (raw or cooked) on a dry matter basis.

	Ageing period (AP)		Meat status (MS)		*P*-Values		
	5 days	90 days	Raw	Cooked	AP	MS	AP*MS
Moisture (%)	70.7 ± 0.27	69.8 ± 0.27	74.1 ± 0.27	66.4 ± 0.27	0.0003	<0.0001	0.1306
mg/100 g (dry basis)							
Anserine	339.3 ± 11.1	320.0 ± 11.1	378.9 ± 11.1	280.4 ± 11.1	0.0030	<0.0001	0.2514
Carnosine	1773.6 ± 32.5	1593.7 ± 32.5	1933.2 ± 32.5	1434.1 ± 32.5	<0.0001	<0.0001	0.2223
Coenzyme Q10	6.61 ± 0.14	7.14 ± 0.14	7.53 ± 0.14	6.23 ± 0.14	0.0003	<0.0001	0.8085
Glutathione	24.9 ± 1.76	42.3 ± 1.76	36.6 ± 1.76	30.6 ± 1.76	<0.0001	0.0072	0.0001
L-Carnitine	126.9 ± 3.54	137.7 ± 3.54	144.5 ± 3.54	120.0 ± 3.54	0.0097	<0.0001	0.0012
Taurine	89.0 ± 2.20	152 ± 2.25	144 ± 2.20	96.7 ± 2.25	<0.0001	<0.0001	<0.0001

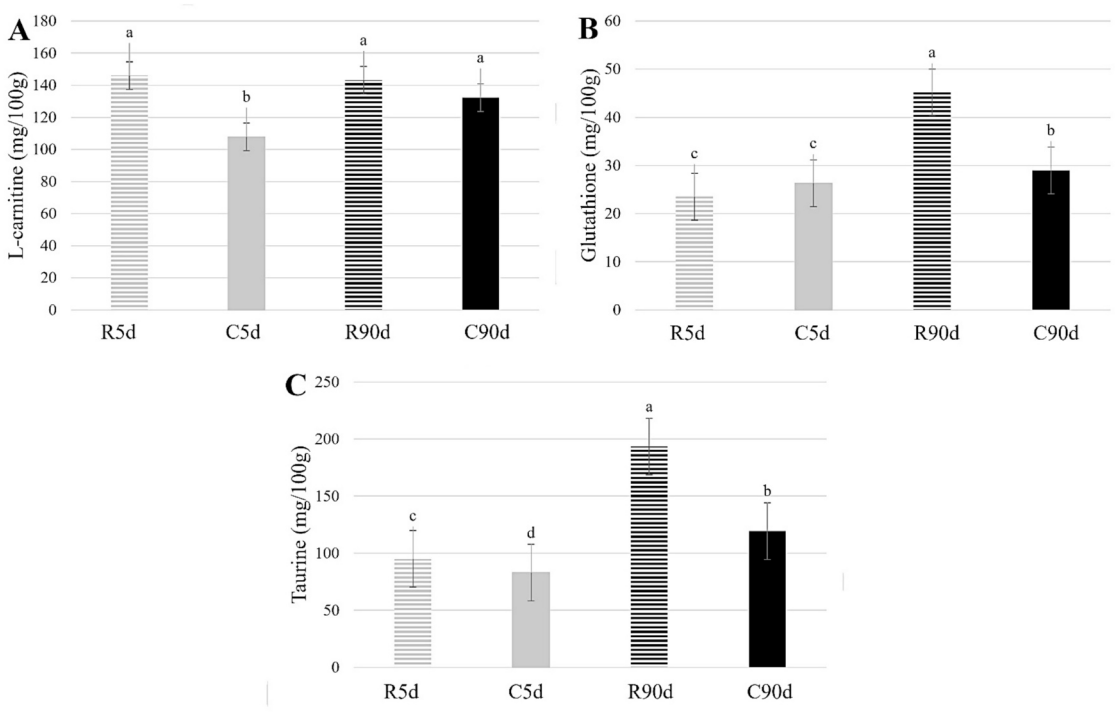

Fig. 1. Significant interactions ($P < 0.05$) between ageing period (5 or 90 days) and meat status (raw or cooked) on dry weight basis. [a - d] Bars with different superscript differ significantly ($P < 0.05$). A) L-carnitine. B) Glutathione. C) Taurine. R5d: raw meat aged for 5 days; C5d: cooked meat previously aged for 5 days; R90d: raw meat aged for 90 days; C90d: cooked meat previously aged for 90 days.

added before the methylating reagents. Fatty acids were reported in mg/100 g of meat.

2.5. True retentions of nutrients

True retentions (TR) of nutrients after cooking were calculated according to the equation of Murphy et al. (1975): TR (%) = [(nutrient content per g of cooked meat x g of meat after cooking / (nutrient content per g of raw meat x g of raw meat)] x 100].

2.6. Statistical analysis

Data analysis was conducted considering a mixed linear model using the MIXED procedure of the SAS software v. 9.4 (SAS Institute Inc., Cary, NC, USA). A 2 × 2 factorial design was used to evaluate two factors, meat status (raw vs. cooked) and ageing period (5 vs. 90 days). From 20 left striploins (representing each animal) four steaks were obtained for each treatment, i.e., raw and aged for 5 d, raw and aged for 90 days, cooked and aged for 5 d, and cooked and aged for 90 d, in a randomized order. The model included the meat status (raw vs. cooked) and ageing period (5 vs. 90 days) and their interaction as a fixed effect, and the striploin was considered as a random effect. Shapiro-Wilk test (Shapiro & Wilk, 1965) was performed to assess the normality of the data and studentized residuals were calculated to identify outliers. After variance analysis, the least-squares means were calculated for treatment comparisons with a significance level of $\alpha = 0.05$, using the PDIFF option of LSMEANS adjusted by Tukey, when F-tests were significant ($P < 0.05$).

3. Results and discussion

3.1. Moisture analysis

No interactions ($P > 0.05$) between ageing period and meat status were observed for moisture (Table 1). Extended ageing for 90 days presented a lower ($P < 0.05$) moisture content than 5 days, which agrees with Holman et al. (2022) who reported a decline in total moisture when the ageing period increased. Furthermore, raw beef presented a greater ($P < 0.05$) moisture content than cooked meat. In line with these results, cooking losses (%) were greater ($P < 0.05$) in beef aged for 90 days (20.2 ± 0.68%) than for 5 days (17.1 ± 0.68%).

3.2. Bioactive compounds

Results for bioactive compounds are presented on a dry matter basis. Significant ($P < 0.05$) interactions between ageing period and meat status were found for L-carnitine, glutathione, and taurine (Fig. 1). Glutathione concentration was greater ($P < 0.05$) in raw meat aged for 90 days (R90d) than the other treatments (C90d, C5d and R5d). Rakowska et al. (2017) reported that degradation products generated by postmortem proteolysis can be used as substrate in the production of glutathione which would explain the greater concentration in 90 d ageing. Taurine is a non-protein compound, highly soluble in water (Baliou et al., 2021) and may not be derived from protein proteolysis (Cornet & Bousset, 1999). Greater ($P < 0.05$) concentrations of taurine were found in R90d than C90d, R5d and C5d. The observed increases in taurine concentration in 90 days aged beef may reflect a greater extraction capacity rather than the new formation of this compound. The extensive structural degradation that occurs during prolonged ageing compromises the integrity of muscle tissue (Huff-Lonergan & Lonergan, 2005), which could increase the recoverability of soluble compounds. Our findings agree with Radzik-Rant et al. (2025), who reported greater concentrations of taurine in lamb meat (fresh meat) aged for 14 days than for 7 days. In addition, Purchas et al. (2004) observed a decrease in taurine content when lamb meat (dry matter basis) was cooked compared to raw meat, although they did not evaluate the effect of ageing period. For L-carnitine, greater ($P < 0.05$) concentrations were observed in R90d, R5d and C90d than C5d. Lee et al. (2023) reported that wet ageing of the *semimembranosus* muscle for 28 days significantly increased L-carnitine derivatives.

No significant ($P > 0.05$) interactions between raw vs. cooked and ageing period were observed for anserine, carnosine and coenzyme Q10 (Table 1).

Greater ($P < 0.05$) contents of anserine and carnosine were observed in meat aged for 5 days than for 90 days (Table 1). Anserine and carnosine are dipeptides with important antioxidant properties linked to their metal ion chelation, inhibition of protein glycation and scavenging of reactive carbonyl species effects (Everaert et al., 2019). Previous studies reported greater concentrations of carnosine than anserine in beef and swine, whereas anserine predominates in chickens and some fish, such as salmon (Arihara & Ohata, 2008; Chan & Decker, 1994; Jairath et al., 2024). Kim and Jang (2021) observed that carnosine concentration decreased with increasing ageing period in beef (wet weight) muscles, *longissimus dorsi* and *semimembranosus,* aged on a polystyrene tray. These authors also found a significant reduction in coenzyme Q10 content on day 19 compared to day 1. However, in the present study we observed greater ($P < 0.05$) concentrations of coenzyme Q10, glutathione, L-carnitine and taurine in beef aged for 90 days than for 5 days (Table 1). These greater concentrations of the above-mentioned compounds in meat aged for 90 days may be due to purge losses in prolonged ageing as reported by Holman et al. (2022), where water is lost to a greater extent than the bioactive compounds. In our study, purge losses during ageing were not measured. However, Yu et al. (2024) reported approximately 5% of purge losses after 28 days of ageing.

Regarding meat status (raw vs. cooked), raw samples presented greater ($P < 0.05$) concentrations of all bioactive compounds (Table 1). Our results agree with the findings of Purchas et al. (2006), who also observed a decrease in taurine, carnosine and coenzyme Q10 (on a fresh basis) in cooked beef (*longissimus lumborum* muscle). According to Aaslyng et al. (2003), cooking losses results from the liquids release and soluble compounds during meat heating, which occurs due to protein denaturation that decreases the ability of muscle fibers to retain water within their structure. Carnosine and anserine are dipeptides soluble in water, as a result, a lower concentration of these compounds can be found when subject to a cooking process (Jayasena et al., 2014; Peiretti et al., 2012). L-carnitine is highly water-soluble (Rigault et al., 2008) and prolonged heating or cooking in water can cause leaching and losses (Gokhisar & El, 2015; Harpaz, 2005). These losses vary depending on factors such as animal species, muscle type, and the specific heat treatment applied (Knuettel-Gustavsen & Harmeyer, 2011). In contrast, coenzyme Q10 is lipophilic, located mainly in cell membranes and fat, and its concentration may be influenced by both the fat content of the meat and the cooking method, which can lead to losses under certain conditions (Ercan & El, 2011; Podar et al., 2023).

Table 2
Least-squares means ± standard error of the true retention (%) of bioactive compounds according to the ageing period (5 or 90 days).

Bioactive compounds (%)	Ageing period		
	5 days	90 days	*P*
Anserine	78.3 ± 2.26	81.3 ± 2.26	0.3540
Carnosine	79.1 ± 1.61	77.3 ± 1.65	0.4572
Coenzyme Q10	90.5 ± 2.28	86.5 ± 2.34	0.2252
Glutathione	126.5 ± 6.77	79.5 ± 6.77	<0.0001
L-Carnitine	80.0 ± 3.78	100.8 ± 3.78	0.0004
Taurine	94.4 ± 3.55	64.4 ± 3.55	<0.0001

The recommended intakes of the bioactive compounds studied in the present work are not yet well defined in the literature (Arenas-Jal et al., 2020; Wu et al., 2016). No matter whether beef could be an interesting source of potentially bioactive compounds, the amounts present in 100 g of meat are generally lower than those available as dietary supplements (Purchas et al., 2004). According to Mattila and Kumpulainen (2001), the recommended daily intake of coenzyme Q10 is around 5.4 mg for men and 3.8 mg for women. Considering the concentrations that we obtained on a fresh basis (data not presented), a 100 g of meat would contribute to 34 (R5d) – 41 (C90d) % of the recommended intake for men and 49 (R5d) – 58 (C90d) % for women. However, Overvad et al. (1999) reported that dietary supplement manufacturers recommended a range of 10–30 mg/day of coenzyme Q10 for a healthy person, and they even showed that daily supplementation of up to 200 mg for 6–12 months and 100 mg daily for up to 6 years had no adverse health effects.

For L-carnitine, suggested daily intakes range from 0.3 to 1.9 mg/kg body weight/day (Demarquoy et al., 2004). In addition, it has been reported that taurine intake in people with omnivorous diets averages 58 mg/day (Rana & Sanders, 1986). Based on this intake, our results indicate that 100 g of meat (fresh basis) would provide between 42 (R5d) and 87% (R90d) of the suggested taurine intake (data not shown). Richie et al. (2015) found significant increases of glutathione in different body stores, in healthy adults supplemented with at least 250 mg/day of glutathione for 6 months. Although the concentrations of glutathione observed in our study (per 100 g on a fresh basis) were lower than 250 mg, they are aligned with the content found in beef muscles in previous studies (Jairath et al., 2024; Rakowska et al., 2017). Furthermore, it has been reported that beef steaks consumption or oral administration of L-carnosine (450 mg) increased the antioxidant capacity of human serum in aged people (Antonini et al., 2002). The consumption of red meat as part of a balanced diet can be beneficial to human health by helping to control oxidative stress and reduce the risk of diseases (Wu, 2020).

True retention (TR) of anserine, carnosine and coenzyme Q10 after cooking did not differ ($P > 0.05$) between meat aged for 5 or 90 days (Table 2). However, greater ($P < 0.05$) TR of glutathione and taurine were observed in meat aged for 5 than 90 days. This may be due to greater losses in cooking juices after 90 days of ageing since the post-mortem denaturation of myofibrillar proteins diminishes water retention capacity (Huff-Lonergan & Lonergan, 2005).

3.3. Intramuscular fat content and fatty acid composition

No interactions ($P > 0.05$) were observed between ageing period and raw vs. cooked for intramuscular fat and fatty acids composition (data not presented).

Intramuscular fat is an important attribute associated with meat

Table 3
Least-squares means ± standard error of intramuscular fat (IMF) content and fatty acid composition (mg fatty acid/100 g of meat) in the *longissimus lumborum* muscle according to the ageing period (5 or 90 days) and meat status (raw or cooked).

	Ageing period			Meat status		
	5 days	90 days	*P*	Raw	Cooked	*P*
IMF (%)	2.68 ± 0.20	3.37 ± 0.20	<0.0001	2.50 ± 0.20	3.55 ± 0.20	<0.0001
Fatty acids						
SFA[1]						
C12:0	3.45 ± 0.28	3.36 ± 0.28	0.7893	2.65 ± 0.28	4.16 ± 0.28	<0.0001
C14:0	42.2 ± 3.97	48.9 ± 4.01	0.0178	36.9 ± 3.98	54.3 ± 3.98	<0.0001
C15:0	8.88 ± 0.74	10.7 ± 0.74	0.0029	7.70 ± 0.74	11.9 ± 0.74	<0.0001
C16:0	553.8 ± 47.3	628.7 ± 47.8	0.0256	485.2 ± 47.5	697.3 ± 47.5	<0.0001
C17:0	16.3 ± 1.39	19.1 ± 1.39	0.0144	14.5 ± 1.39	20.9 ± 1.39	<0.0001
C18:0	386.8 ± 34.9	467.8 ± 34.9	0.0084	340.5 ± 34.9	514.0 ± 34.9	<0.0001
C20:0	3.29 ± 0.30	3.88 ± 0.30	0.0222	2.94 ± 0.30	4.23 ± 0.30	<0.0001
MUFA[2]						
C14:1 *c*9	7.43 ± 0.64	7.69 ± 0.64	0.5625	6.08 ± 0.64	9.04 ± 0.64	<0.0001
C16:1 *c*9	51.5 ± 4.30	58.3 ± 4.31	0.0058	46.2 ± 4.30	63.6 ± 4.31	<0.0001
C18:1 *c*9	819.4 ± 75.2	942.1 ± 75.2	0.0069	728.5 ± 75.2	1033.0 ± 75.2	<0.0001
C18:1 *c*10	29.7 ± 2.70	34.6 ± 2.70	0.0071	26.0 ± 2.70	38.4 ± 2.70	<0.0001
C18:1 *c*11	6.93 ± 0.56	3.36 ± 0.56	<0.0001	4.54 ± 0.56	5.75 ± 0.56	0.0026
C18:1 *c*12	4.72 ± 0.53	5.30 ± 0.53	0.1059	4.02 ± 0.53	6.00 ± 0.53	<0.0001
C18:1 *c*13	0.95 ± 0.15	0.71 ± 0.15	0.0367	0.68 ± 0.15	0.98 ± 0.15	0.0108
C18:1 *c*15	2.72 ± 0.25	3.42 ± 0.25	0.0003	2.53 ± 0.25	3.62 ± 0.25	<0.0001
C18:1 *t*8 + *t*9	1.24 ± 0.15	1.22 ± 0.15	0.9046	0.93 ± 0.15	1.53 ± 0.15	<0.0001
C18:1 *t*10	3.63 ± 0.41	5.56 ± 0.41	<0.0001	3.86 ± 0.41	5.33 ± 0.41	<0.0001
C18:1 *t*11	52.5 ± 5.13	70.0 ± 5.13	0.0007	47.4 ± 5.13	75.1 ± 5.13	<0.0001
C18:1 *c*14 + *t*16	0.79 ± 0.11	0.74 ± 0.11	0.5720	0.65 ± 0.11	0.87 ± 0.11	0.0171
C18:1 *t*6 + *t*7 + *c*5	1.87 ± 0.13	0.32 ± 0.13	<0.0001	0.99 ± 0.13	1.19 ± 0.13	0.1301
∑ all C18:1	924.6 ± 84.3	1067.7 ± 84.3	0.0066	820.1 ± 84.3	1172.2 ± 84.3	<0.0001
C20:1 *c*9	1.97 ± 0.21	2.18 ± 0.21	0.0631	1.75 ± 0.21	2.40 ± 0.21	<0.0001
PUFA[3]						
C18:2 *n*-6	49.7 ± 1.14	50.2 ± 1.12	0.7355	42.5 ± 1.11	57.4 ± 1.15	<0.0001
C18:2 *c*9, *t*11[4]	13.2 ± 1.16	15.0 ± 1.16	0.0444	11.2 ± 1.16	17.0 ± 1.16	<0.0001
C18:3 *n*-3	21.9 ± 0.86	22.5 ± 0.84	0.4702	18.4 ± 0.84	26.0 ± 0.86	<0.0001
C20:3 *n*-6	5.44 ± 0.19	5.46 ± 0.18	0.9305	4.74 ± 0.18	6.16 ± 0.19	<0.0001
C20:4 *n*-6	21.6 ± 0.53	20.8 ± 0.51	0.2447	18.6 ± 0.51	23.8 ± 0.53	<0.0001
C20:5 *n*-3	10.9 ± 0.34	10.1 ± 0.33	0.0348	9.00 ± 0.33	12.0 ± 0.34	<0.0001
C22:5 *n*-3	16.8 ± 0.56	16.7 ± 0.56	0.8509	14.4 ± 0.56	19.1 ± 0.56	<0.0001
C22:6 *n*-3	1.66 ± 0.07	1.74 ± 0.07	0.3479	1.44 ± 0.07	1.96 ± 0.07	<0.0001

[1] SFA: Saturated fatty acids.
[2] MUFA: Monounsaturated fatty acids.
[3] PUFA: Polyunsaturated fatty acids.
[4] CLA: Conjugated linoleic acid isomer *c*9, *t*11.

quality, that positively influences sensory characteristics (Frank et al., 2016; Hocquette et al., 2010; Hopkins et al., 2006; Platter et al., 2003; Vierck et al., 2018). Meat aged for 90 days showed a greater ($P < 0.05$) proportion of IMF than those steaks aged for 5 days (Table 3), which agrees with Holman et al. (2022) who reported that long term wet aged beef resulted in increases of IMF concentrations. However, this effect does not reflect lipid deposition during ageing but may occur because proteolysis during prolonged wet ageing leads to the degradation of cytoskeletal proteins, reducing water retention capacity and resulting in water losses (Holman et al., 2022).

The levels of fat present in the muscles of animals impact the fatty acid composition, often increasing the levels of saturated fatty acids as overall fat augment (Wood et al., 2008). Consumers are increasingly concerned about the risks of meat consumption (Font-i-Furnols, 2023; Realini et al., 2022), and several efforts are being made to produce meat with less SFA content and greater concentrations of *n*-3 PUFA (Chelopo et al., 2025). In the present study, concentrations of all SFA were greater ($P < 0.05$) in meat aged for 90 days than in beef aged for 5 days, except for C12:0 which did not differ ($P > 0.05$; Table 3). Concentrations of C16:1 *c*9 and total C18:1 were greater ($P < 0.05$) in meat aged for 90 than 5 days. However, in our study C18:1 *c*11, C18:1 *c*13 and C18:1 *t*6 + *t*7 + *c*5 contents were greater ($P < 0.05$) in the shorter ageing period. PUFA concentrations were not affected ($P > 0.05$) by the ageing period, except for the C18:2 *c*9, *t*11 isomer (CLA) that presented a greater ($P < 0.05$) content in meat aged for 90 than 5 days. Unlike our findings, Di Paolo et al. (2023) did not find differences in CLA content in wet ageing meat for 2, 15, 30 and 60 days from Charolais cattle. Gruffat et al. (2021) also reported no differences in total CLA content in meat aged for 1, 3 and 14 days from three muscles: *longissimus thoracis*, *longissimus lumborum*, and *triceps brachii*. However, these authors evaluated shorter ageing periods than in our study. CLA is an important fatty acid present in ruminant meat that has potential benefits to human health (Pariza, 2004; Wood et al., 2008). It can be formed in two ways, either through ruminal biohydrogenation of linoleic acid or by an endogenous synthesis pathway of trans-vaccenic acid (McAfee et al., 2010).

Regarding meat status, cooked meat presented a greater ($P < 0.05$) proportion of IMF and higher concentrations for all fatty acids than raw beef, except for C18:1 *t*6 + *t*7 + *c*5 (Table 3). Previous studies on fatty acid composition found that greater concentrations in cooked meat are a result of moisture loss during the cooking process (Badiani et al., 2002; Gruffat et al., 2021).

Greater ($P < 0.05$) concentrations of SFA, MUFA, *n*-6/*n*-3 ratios were observed in meat aged for 90 days than 5 days (Table 4). Preceding studies have reported increases in SFA proportion in meat aged for 14 days (Mahecha et al., 2009), although they did not observe differences in MUFA content (Kim & Jang, 2021; Mahecha et al., 2009). The ageing period did not affect ($P > 0.05$) the PUFA concentrations, even though meat aged for 5 days presented a greater ($P < 0.05$) PUFA/SFA ratio (Table 4). Neither raw and cooked beef nor 5- or 90-days ageing achieved a PUFA/SFA ratio greater than 0.45 for a healthy diet (British Department of Health, 1994). This Department also recommended that the *n*-6/*n*-3 ratio should not exceed 4.0, whose goal was achieved in all cases (Table 4). In addition, Simopoulos (2002) reported that the ideal balance between *n*-6/*n*-3 ratio ranges from 1:1 to 4:1 depending on the disease involved. The *n*-6/*n*-3 ratio is relevant because it affects brain

Table 4
Least-squares means ± standard error of total saturated, monounsaturated and polyunsaturated fatty acids (mg fatty acid/100 g of meat) and ratios in the *longissimus lumborum* muscle.

	Ageing period			Meat status		
	5 days	90 days	*P*	Raw	Cooked	*P*
∑SFA[1]	1014.9 ± 92.8	1202.8 ± 92.8	0.0058	901.8 ± 92.8	1315.9 ± 92.8	<0.0001
∑MUFA[2]	986.2 ± 89.6	1137.7 ± 89.6	0.0063	874.8 ± 89.6	1249.1 ± 89.6	<0.0001
∑PUFA[3]	141.8 ± 3.79	143.1 ± 3.70	0.7808	120.0 ± 3.70	164.6 ± 3.79	<0.0001
∑PUFA *n*-3[4]	51.6 ± 1.60	51.1 ± 1.57	0.7580	43.2 ± 1.57	59.4 ± 1.60	<0.0001
∑PUFA *n*-6[5]	90.3 ± 2.39	92.0 ± 2.33	0.5443	77.1 ± 2.33	105.2 ± 2.39	<0.0001
∑ unidentified	51.2 ± 3.82	57.4 ± 3.82	0.0493	44.7 ± 3.82	63.9 ± 3.82	<0.0001
PUFA/SFA	0.15 ± 0.01	0.13 ± 0.01	0.0016	0.15 ± 0.01	0.13 ± 0.01	0.0033
n-6/*n*-3	1.76 ± 0.03	1.81 ± 0.03	0.0002	1.79 ± 0.03	1.78 ± 0.03	0.6776
AI[6] index	0.63 ± 0.01	0.65 ± 0.01	0.0051	0.64 ± 0.01	0.65 ± 0.01	0.1159
TI[7] index	1.37 ± 0.03	1.48 ± 0.03	0.0009	1.39 ± 0.03	1.46 ± 0.03	0.0199

[1] SFA: Sum of C12:0, C14:0, C15:0, C16:0, C17:0, C18:0 and C20:0.
[2] MUFA: Sum of C14:1 *c*9, C16:1 *c*9, C18:1 *c*9, C18:1 *c*10, C18:1 *c*11, C18:1 *c*12, C18:1 *c*13, C18:1 *c*15, C20:1 *c*9, C18:1 *t*8 + *t*9, C18:1 *t*10, C18:1 *t*11, C18:1 *c*14 + *t*16 and C18:1 *t*6 + *t*7 + *c*5.
[3] PUFA: Sum of C18:2 *n*-6, C18:2 c9, t11, C18:3 *n*-3, C20:3 *n*-6, C20:4 *n*-6, C20:5 *n*-3, C22:5 *n*-3 and C22:6 *n*-3.
[4] PUFA *n*-3: Sum of C18:3 *n*-3, C20:5 *n*-3, C22:5 *n*-3 and C22:6 *n*-3.
[5] PUFA *n*-6: Sum of C18:2 *n*-6, C18:2 *c*9t11, C20:3 *n*-6 and C20:4 *n*-6.
[6] AI: Atherogenic index (Ulbricht & Southgate, 1991) = (C12:0 + (4 * C14:0) + C16:0) / (MUFA + (*n*-6 + *n*-3).
[7] TI: Thrombogenic index (Ulbricht & Southgate, 1991) = (C14:0 + C16:0 + C18:0) / (0.5 * MUFA) + (0.5 * *n*-6) + (3 * *n*-3) + (*n*-3/*n*-6).

development and function, partly due to factors such as increased membrane fluidity promoted by PUFA (Luchtman & Song, 2013; Yehuda et al., 2002). The atherogenic (AI) and thrombogenic (TI) indices were calculated to assess the influence of meat consumption on the incidence of coronary heart disease (Ulbricht & Southgate, 1991). The values of both indices (AI and TI) were greater ($P < 0.05$) in beef aged for 90 days than for 5 days. However, although these differences were statistically significant, they were numerically small and not biologically relevant. Furthermore, the AI index was below 1 in all cases, which is the recommended value for a healthy diet (Sinanoglou et al., 2013). Although Gruffat et al. (2021) observed no differences in the AI and TI values in meat aged for 1, 3 and 14 days, a longer ageing period (90 days) could lead to some oxidation of UFAs, affecting these indices.

Meat fatty acids profile has been studied for years because of its relationship with human health, beef palatability and eating quality (Jiang et al., 2010). Comparing raw and cooked meat, greater ($P < 0.05$) concentrations of SFA, MUFA, PUFA, *n*-3 PUFA, and *n*-6 PUFA, and TI index were found in the latter. In line with our findings, Alfaia et al. (2010) reported that SFA and MUFA increased when beef was grilled compared to raw samples, due to the loss of moisture and lower susceptibility to oxidative degradation compared to PUFA. Raw beef presented greater ($P < 0.05$) PUFA/SFA ratio than cooked meat due to PUFA oxidation during the cooking process, which agrees with the study conducted by Alfaia et al. (2010).

4. Conclusions

Studies on bioactive compounds present in beef analyzing how ageing and cooking affect their stability are still scarce. In this context, direct comparisons with previously published data are limited. The ageing of meat over extended periods (90 days in our study) indicates that not all bioactive compounds respond in the same way to extended chilling storage. On the other hand, comparing raw vs. cooked beef, we ascertain that the cooking process reduced consistently the concentration of all bioactive compounds. Regarding fatty acid composition, the concentrations of SFA, MUFA and PUFA were greater in cooked than raw meat. Further studies are warranted to evaluate beef production systems (pasture vs. concentrate), meat ageing methods (dry vs. wet), ageing periods, and cooking techniques to better understand how they affect bioactive compound and fatty acid concentrations in beef.

CRediT authorship contribution statement

Ana Carolina T.S. Cougo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Guillermo de Souza:** Methodology. **Florencia Bonjour:** Methodology. **Bruno A. Irigaray:** Methodology. **Iván Jachmanián:** Methodology. **Gustavo Brito:** Writing – review & editing. **María del Mar Campo:** Writing – review & editing, Supervision. **Facundo Ibáñez:** Writing – review & editing, Supervision, Methodology. **Santiago Luzardo:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge funding support from the National Agency for Research and Innovation of Uruguay – ANII (FMV_3_2022_1_172315). The first author acknowledges PhD financial support from ANII (POS_NAC_2023_2_177395).

Data availability

Data will be made available on request.

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