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Evaluation of quality and biochemical properties of Bovine meat from different rearing systems

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PAPER

Abstract

The quality of meat plays an important role in maintaining human health. The aim of this study was to characterize the quality traits (color, marbling, and tenderness) of the *Longissimus thoracis* muscle and to compare the antioxidant capacity, phospholipid (PL) composition, and advanced glycation end products (AGEPs) in the diaphragm of beef from organic and traditional farming. Additionally, the effects of short supply chains (SSCs) and long supply chains (LSCs) were compared. For statistical analysis, t-tests and ANOVA were used. The results demonstrated that meat from organically raised animals had 1.7 times higher antioxidant power, richer in PL content by 1.25 and 1.12 times, and cholesterol levels were 3.38 and 2.84 times higher than meat from traditionally raised animals in both SSCs and LSCs, respectively. Conversely, the LSC doubled the value of AGEPs in organic meat.

Keywords: advanced glycation end products; antioxidant properties; long supply chain; organic meat; traditional meat; phospholipids; short supply chain

Introduction

Meat is one of the most important components in the Mediterranean diet as it is considered an optimal and complete food (Kanaan and Tarek, 2022). Meat quality is essential for human health, so controlling the supply chain in terms of services, processes, and products is crucial in maintaining this quality (Bastas and Liyanage, 2018).

Initiatives aimed at improving supply by reducing physical and social distances began in the 1960s, with a shift from global to local food systems, where alternative models started to replace traditional supply systems (Edwards, 2016). Since then, the short supply chain (SSC) has been a valid alternative to the long supply chain (LSC). SSCs offer a significant advantage, as meat is highly perishable and has a short shelf life, particularly when considering potential sources of microbial contamination due to the complexity of habitats in the preharvest, harvest, and postharvest phases of the food supply chain (Foster *et al.*, 2011).

Additionally, local food networks of bovine farms that use organic methods are proliferating across Italy and worldwide (Vitali *et al.*, 2018). As a result, the supply of meat can be influenced by various economic and quality choices. The market is certainly shaped by numerous factors, and the COVID-19 pandemic has underscored the adaptability and resilience of meat supply chains in the medium to long term (Hobbs, 2021).

Color and tenderness are the well-established parameters for evaluating meat quality (Cenci Goga *et al.*, 2020; Grispoldi *et al.*, 2022). However, several new approaches have been explored, such as assessing heavy metal content (Kamouh *et al.*, 2024), as well as proteomic (Purslow *et al.*, 2021) and metabolomic profiles (Wang *et al.*, 2024).

Friesian, Chianina, Polish crossbreed, and Piemontese cattle breeds are highly sought after by meat shops and butchers, each offering distinct characteristics that appeal to specific consumer segments. Friesian cattle, primarily dairy-focused, provide lean, mild-flavored beef, even though it typically lacks marbling. Chianina, one of Italy's oldest and largest breeds, is prized for its tender, high-quality meat, especially in premium cuts, making it popular in gourmet markets. The Polish crossbreed, a mix of native and European genetics, offers lean meat with robust flavor, serving both domestic and export markets, particularly in Eastern Europe. Piemontese cattle, known for their double-muscle trait, yield lean, tender beef with low fat and high protein content, appealing to health-conscious consumers and niche markets. Collectively, these breeds occupy diverse market segments, from high-end to more affordable offerings, each contributing unique value to the global beef industry (Cenci Goga *et al.*, 2020; Grispoldi *et al.*, 2022).

The aim of this study was to characterize the quality traits (color, marbling, and tenderness) of the *Longissimus thoracis* muscle from strip loins and to compare the antioxidant capacity (AC), phospholipid (PL) composition, and advanced glycation end product (AGEP) content in the diaphragm of beef from organic and traditional farming. Additionally, the effects of SSC and LSC were compared. Meat samples were analyzed from the traditional short supply chain (TSSC), organic short supply chain (OSSC), traditional long supply chain (TLSC), and organic long supply chain (OLSC).

Material and Methods

Experimental design

Four different bovine groups were considered: TSSC (Friesian), OSSC (Chianina breed), TLSC (Polish crossbreed), and OLSC (Piemontese breed). For each genetic type, seven batches were analyzed; each batch was obtained from a different animal and consisted of three meat cuts. All animals involved in the study were uncastrated males aged between 12 and 24 months (Category A according to the European classification). All data provided by the producers were recorded in a database.

The meat cut analyzed was the entrecote, or boneless rib, obtained from the muscles located between the fifth and eighth ribs of the loin. Specifically, all tests for quality traits (color, marbling, and tenderness) were conducted on the *Longissimus thoracis* muscle. Each cut was analyzed in triplicate for color, tenderness, and marbling, as described below.

All samples were vacuum skin-packed after cutting and sent to the laboratory, where they were stored at refrigerator temperatures (0–4°C) and kept in the dark for a standardized period of approximately 10 days between packaging and analysis.

The diaphragm was also collected from the same animals for the evaluation of AC, PLs, and AGEPs; it was selected due to its higher oxidative phosphorylation capacity compared to the boneless rib (Ramos *et al.*, 2021).

Colorimetric analysis

The ColorMeter RGB Colorimeter app (White Marten GmbH, Stuttgart, Germany) was used to measure the color of the samples using an iPhone XS running iOS 13.7, as described in a study by Grispoldi *et al.* (2022). Conventional colorimeters are designed to determine the color of a single point in a uniform area; however, the average color of each sample was measured to better replicate the consumer's perception in this study.

The app was calibrated against a reference colorimeter, the Minolta CR 200 Chroma Meter (Konica Minolta Inc., Tokyo, Japan), as described by Grispoldi *et al.* (2022). Briefly, the Minolta CR 200 Chroma Meter was used to measure a series of red/reddish calibration plates (specifically, the CR-A47 DP, CR-A47 R, and CR-A47 B) along with a standard white plate to determine the CIELAB color spaces: L* (lightness), a* (redness), and b* (yellowness). These results were then used to calibrate the readout of the app.

The Minolta CR 200 Chroma Meter was set to measure under the CIE (International Commission on Illumination) Standard Illuminant D65, which approximates the average midday light in Western and Northern Europe, including both direct sunlight and diffused light from a clear sky. Consequently, D65 is also known as a daylight illumination standard, with a correlated color temperature of approximately 6500 K.

To ensure consistent lighting conditions for the ColorMeter RGB Colorimeter app, a 6500 K light source (Godox Led 64, Godox, Shenzhen, China) was used under controlled conditions in a photographic lightbox. CIELAB, a color space system, describes colors visible to the human eye based on hue and chroma (position on the a* and b* axes) and lightness (L*), which corresponds to a position on a black-to-white scale.

Marbling

To determine the marbling of the samples, the meat cuts were photographed using a professional photographic setup illuminated with 6500 K LED lighting. The camera (Nikon D850) was mounted on a fixed stand to maintain a consistent distance between the lens and the sample.

The images were processed using Adobe Photoshop CS6 (version 13.0 × 64) on a MacBook Pro Mid 2012 (2.7 GHz Intel Core i7 with a NVIDIA GeForce GT 650M 1GB graphics card). In each image, a square area of 750 × 750 pixels was selected and the number of white pixels within this area was calculated. By calculating the percentage of white pixels relative to the total, it was possible to quantify the amount of visible intramuscular fat (marbling).

Tenderness

A Sauter FL 100 digital dynamometer (Sauter Italia, Cinisello Balsamo, Milan, Italy) was used to measure meat tenderness. The device was mounted on a test bench for traction and compression measurements equipped with a digital caliper (Sauter Italia, Cinisello Balsamo, Milan, Italy). The methodology applied was a modified version of the Warner-Bratzler method, the most commonly used technique for instrumentally measuring meat tenderness. Briefly, from each slice of meat, six cubes measuring 1.5 cm^2 were collected using a handheld coring device oriented parallel to the longitudinal muscle fibers.

Three cubes were used to measure resistance to the compressive force, applied using a flat head on the dynamometer, and the other three cubes were used to measure resistance to the shear force, applied using a wedge-shaped head. Both tests were conducted with a head speed of 250 mm/min, and the forces were applied perpendicular to the fiber orientation. The resistance curve to the applied force was digitally recorded for each sample and the peak force was used for statistical analysis.

Antioxidant assay by oxygen radical absorbance capacity

The AC was analyzed using the oxygen radical absorbance capacity (ORAC) method, as reported by Codini *et al.* (2020). A duplicate extraction was performed for each sample to evaluate both lipophilic (L-ORACFL) and hydrophilic (H-ORACFL) ORACFL (oxygen radical absorbance capacity fluorescein) values. Evaluations of the lipophilic and hydrophilic ORACFL in the LBB (lipid-rich breast milk) samples were performed separately and the total antioxidant capacity (TAC) was calculated by summing the L-ORACFL and H-ORACFL values.

The ORACFL assays were carried out on a FLUOstar OPTIMA microplate fluorescence reader (BMG LABTECH, Offenburg, Germany) with an excitation wavelength of 485 nm and an emission wavelength of 520 nm.

AAPH (2,2'-azobis [2-amidinopropane] dihydrochloride) was used as a peroxyl radical generator, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as a reference antioxidant standard, and fluorescein as the fluorescent probe. The data are expressed as micromoles of Trolox equivalents (TE) per gram of the sample (μmol TE/g).

Phospholipid analysis

Lipids were extracted using a chloroform-methanol mixture (2:1, v/v), followed by filtration and treatment with 0.5% NaCl, as described by Albi and Magni (2002). The total PLs were evaluated by measuring organic phosphorus where they were separated on the thin-layer silica gel chromatography. Each spot was collected for the measurement of organic phosphorus, as outlined by Albi and Magni (2002).

Advanced glycation end product assay

The AGEP content was quantified using the AGEP ELISA (enzyme-linked immunosorbent assay) kit (ELK Biotechnology, Denver, CO, USA) employing a competitive enzymatic immunoassay following the manufacturer's instructions.

Meat samples (0.1 g) were homogenized in phosphate-buffered saline (PBS; 900 μL) and centrifuged at 2000 rpm for 30 minutes at 4°C before being used in the assay. Briefly, samples were added to the AGEP-precoated microtiter plate, followed by the addition of a biotin-conjugated antibody specific to AGEPs. After incubation, avidin conjugated to horseradish peroxidase was added. Following another incubation, 3,3′,5,5′-tetramethylbenzidine (TMB) solution was added. After 20 minutes, the reaction was stopped by adding sulfuric acid, which caused the solution to change color from yellow to blue. The microplate was read at 450 nm. A logarithmic standard curve was created and the sample concentration was determined by comparing the sample optical density (OD) to the standard curve.

Statistical analysis

Statistical analysis of the data was performed using StatView 5.0.1 software (SAS Institute, Cary, NC, USA). Initially, a t-test for unpaired data with a 95% confidence limit was conducted. Analysis of variance (ANOVA) was also performed using Fisher's PLSD (protected least significant differences). The graphical representation of the results was generated with Prism 8.4.3 software for Mac OS (GraphPad Software, Boston, MA, USA).

Results

Colorimetric analysis

The results of the colorimetric analysis are presented in Figure 1 and Table 1. The analysis revealed that meat from the OSSC, TSSC, and TLSC were significantly redder than meat from the OLSC, with higher a* coordinate values (26.64 ± 3.02, 22.44 ± 2.04, and 21.37 ± 2.11, respectively). Regarding the b* coordinate, which indicates the tendency toward yellow or cyan (higher values indicate yellow, lower values indicate cyan), the same three groups (OSSC, TSSC, and TLSC) exhibited higher values (23.49 ± 3.24, 21.00 ± 1.94, and 18.92 ± 2.33, respectively). Finally, meat from the OSSC (34.79 ± 5.30) and TSSC (33.78 ± 1.26) appeared brighter, showing higher L* coordinate values, especially in comparison to the OLSC (26.39 ± 3.18), which had a darker shade.

Marbling

The results of the marbling evaluation are presented in Figure 1 and Table 1. The meat from the TLSC (18.83 ± 5.75) had the highest marbling, followed by the TSSC (17.51 ± 5.18), OSSC (14.60 ± 6.28), and OLSC (10.23 ± 4.05), with statistically significant differences observed between the groups.

Tenderness

Regarding tenderness, the results are presented in Figure 1 and Table 1. The lowest resistance to compressive force was recorded for the TSSC (43.44 ± 18.75 N), followed by the OLSC (59.23 ± 22.17 N), TLSC (63.23 ± 18.71 N), and OSSC (66.18 ± 21.61 N).

Similar but more consistent results were observed in the shear force test. The lowest values were recorded for the OLSC (15.22 ± 6.69 N), OSSC (15.24 ± 6.49 N), and TSSC (15.26 ± 6.63 N), while the TLSC had the highest values (21.62 ± 8.86 N).

Specific characteristics of bovine diaphragm from TSSC, OSSC, TLSC, and OLSC

To further investigate the diaphragm of different animals as a potential food source, we next analyzed the AC. As shown in Figure 2, the organic samples exhibited higher AC than the traditional samples, whether from the OSSC or OLSC. No significant differences were observed between the TSSC and TLSC or between the OSSC and OLSC.

It was then important to analyze whether there were differences among the samples in terms of proteins and PLs. The results indicated that the protein content was similar across all samples (Figure 3).

The total PLs content was higher in both organic samples (OSSC and OLSC) compared to their respective traditional samples (TSSC and TLSC) (Figure 4). No significant differences were observed between the TSSC and TLSC or between the OSSC and OLSC.

We then analyzed each PL separately. The content of phosphatidylserine (PS) plus phosphatidylinositol (PI), sphingomyelin (SM), and phosphatidylethanolamine (PE) was higher in both organic samples (OSSC and OLSC) compared to their respective traditional samples (TSSC and TLSC) (Figure 5). Additionally, phosphatidylcholine (PC) levels were higher in OSSC than in TSSC. Intriguingly, the SM content was lower in the LSC compared to the SSC in both organic and traditional samples. No differences were observed for PC between TLSC and OLSC. The PE content in TLSC was higher than in TSSC (Figure 5). Furthermore, the cholesterol (Chol) levels were analyzed and the results clearly demonstrated a higher cholesterol content in the organic samples compared to the traditional samples (Figure 5).

Finally, we conducted a test to find out whether AGEPs would be higher in the traditional product compared to the organic product or in the LSC compared to the SSC. The results showed that the mean values were slightly lower in OSSC than in TSSC. Interestingly, the LSC had a more significant impact on the organic product than on the traditional one. Specifically, OLSC exhibited statistically significantly higher values compared to both OSSC and TLSC (Figure 6).

Discussion

The meat on our tables comes from a wide variety of farming systems. Traditional methods require the animals to have little opportunity to move and eat prepackaged feed until slaughter. Alternatively, organic farming

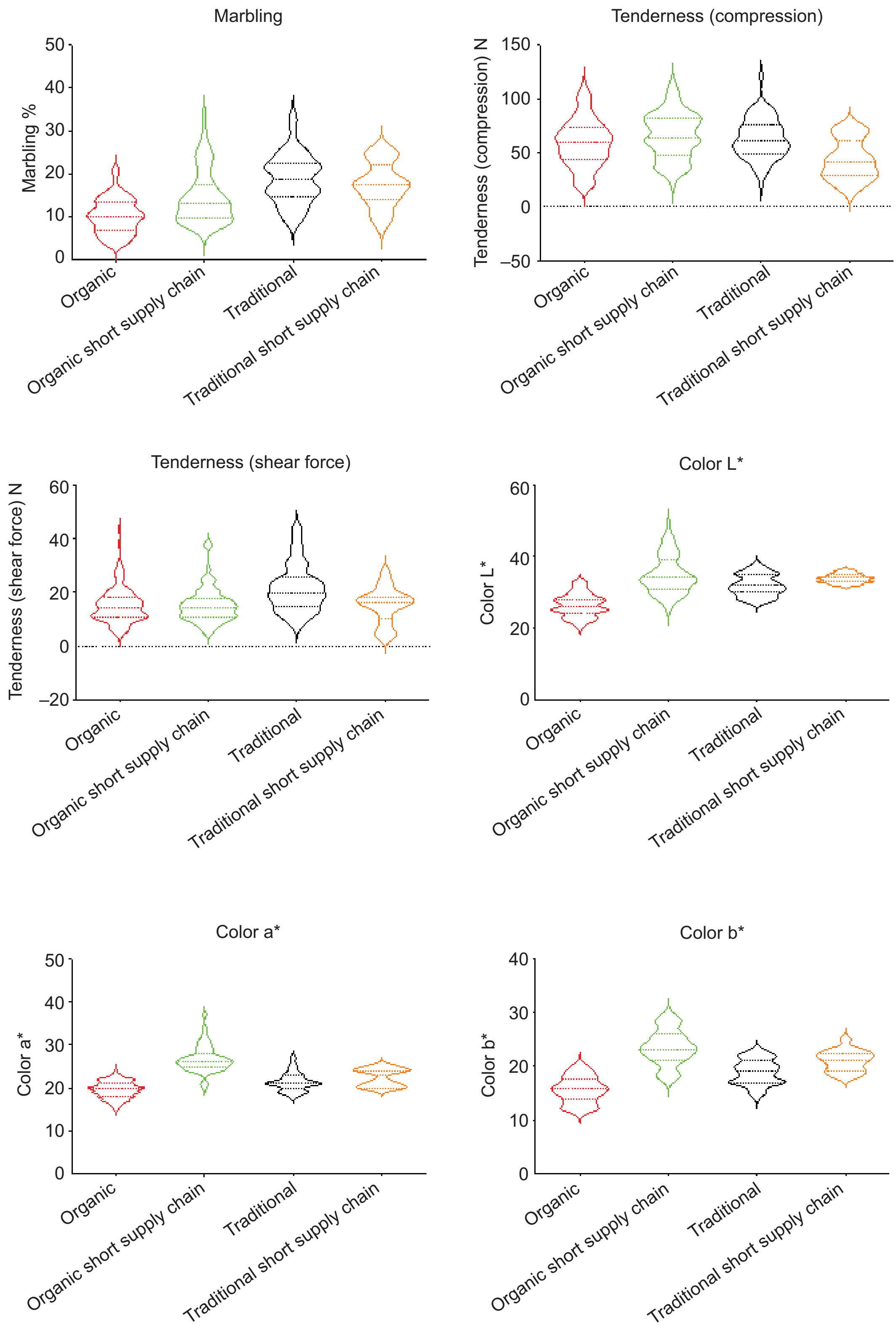

Figure 1. Violin plots showing data distribution.

Table 1. Results of colorimetric, tenderness, marbling, and total lipids evaluations expressed as mean, standard deviation (SD), and minimum and maximum values.

	L*				a*				b*			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
OSSC	34.79	5.30	25.00	49.00	26.64	3.02	20.00	37.00	23.49	3.24	17.00	30.00
OLSC	26.39[a]	3.18	20.00	33.00	19.55[a]	1.93	15.00	24.00	15.59[a]	2.48	11.00	21.00
TLSC	31.89[a]	2.95	27.00	38.00	21.37[b]	2.11	18.00	27,00	18.92	2.33	14.00	23.00
TSSC	33.78	1.26	32.00	36.00	22.44[b]	2.04	20.00	25.00	21.00	1.94	18.00	25.00

	Tenderness (compression)[1]				Tenderness (shear force)[1]				Marbling[2]			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
OSSC	66.18[a]	21.61	20.70	115.80	15.24[b]	6.49	4.90	37.90	14.60[b]	6.28	5.69	33.70
OLSC	59.23[c]	22.17	15.65	114.15	15.22[ab]	6.69	4.00	43.55	10.23[a]	4.05	3.00	21.41
TLSC	63.23[a]	18.71	20.55	121.45	21.62[c]	8.86	7.45	44.20	18.83[b]	5.75	7.93	33.72
TSSC	43.44[bc]	18.75	14.60	73.05	15.26[bc]	6.63	3.35	27.50	17.51[b]	5.18	8.13	25.73

*L**, *a**, *b**: colorimetric coordinates; [1]tenderness expressed in Newton (N); [2]visible marbling expressed in percentage on the total surface of the sample. Different letters in the same column indicate means with statistically significant differences ($p < 0.05$).

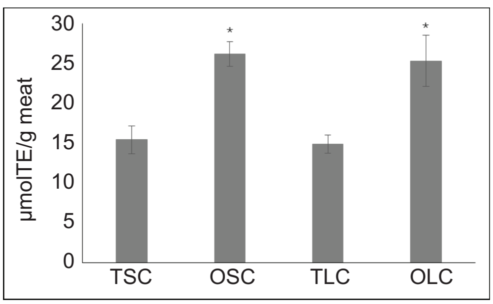

Figure 2. Antioxidant potential of bovine diaphragm. TSSC, traditional short supply chain; OSSC, organic short supply chain; TLSC, traditional long supply chain; OLSC, organic long supply chain. Data are expressed as mean ± SD. *, significance of OSSC versus TSSC and OLSC versus TLSC. *P* < 0.05.

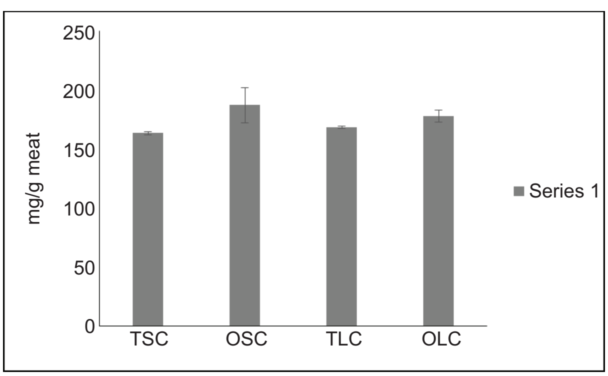

Figure 3. Protein content in the bovine diaphragm. TSSC, traditional short supply chain; OSSC, organic short supply chain; TLSC, traditional long supply chain; OLSC, organic long supply chain. Data are expressed as mean ± SD. * significance of OSSC versus TSSC and OLSC versus TLSC. *p* < 0.05.

involves animals living and eating directly on the fields and/or eating products composed of natural substances. The orientation toward more agroecological and low input farming systems can therefore present benefits for the nutritional properties of meat. Furthermore, the properties of the meat also depend on the treatments after slaughter and whether the meat is sold via the SSC or LSC.

The relationship between breed and beef quality has long been discussed. Many different breed-dependent factors have been positively or negatively associated with beef quality, such as age at physiological maturity, growth path, muscle structure, amount, composition, and distribution of intramuscular fat and content of connective tissue (Bonny *et al.*, 2017).

Meat color greatly influences the visual appearance of beef as well as the consumer's choice, as visual appearance is the first level of beef quality perceived by the consumer. Bright red beef is associated with freshness and higher quality, while paler or darker beef is often perceived as near spoilage or lower quality (Brewer *et al.*, 2001). The red color of meat is due to the conversion of deoxymyoglobin into oxymyoglobin, which has a red color, after exposure to oxygen. A prolonged exposure to oxygen leads to the activation of oxidative metabolism and the accumulation of free radical by-products, which are responsible for the oxidation of myoglobin into metmyoglobin and the consequent brown coloration of meat (Corlett *et al.*, 2021). Studies have reported that the majority of the variability observed in beef color is related to the a* and L* coordinates in conjunction, with the a* coordinate accounting for oxidation state

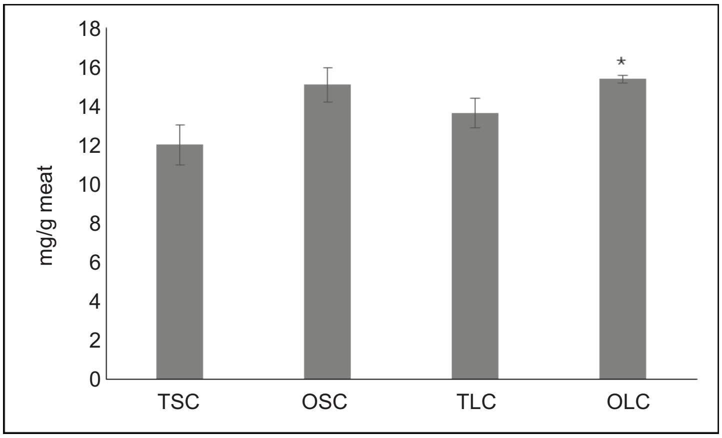

Figure 4. Total phospholipid content in the bovine diaphragm. TSSC, traditional short supply chain; OSSC, organic short supply chain; TLSC, traditional long supply chain; OLSC, organic long supply chain. Data are expressed as mean ± SD. *, significance of OSSC versus TSSC and OLSC versus TLSC. *p* < 0.05.

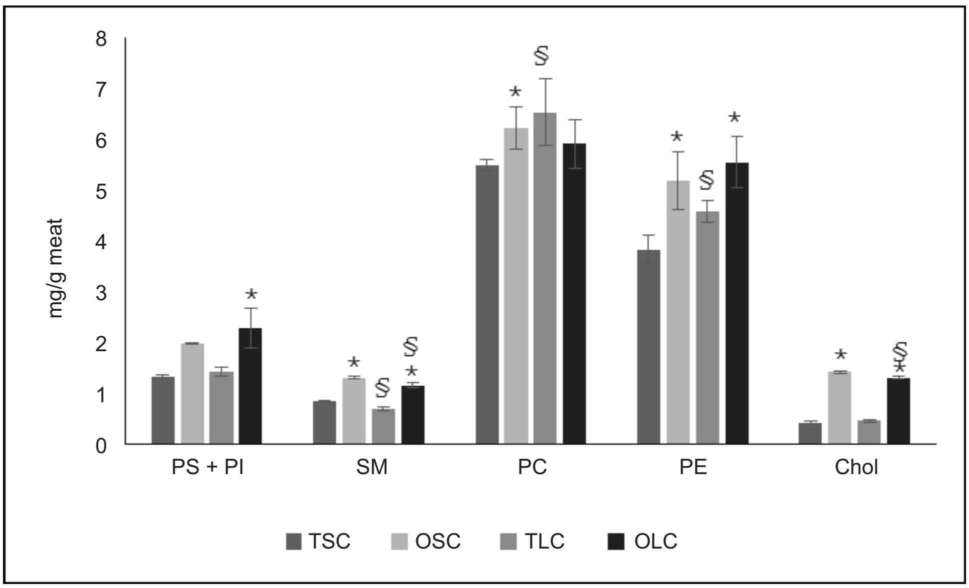

Figure 5. Lipid composition in the bovine diaphragm. TSSC, traditional short supply chain; OSSC, organic short supply chain; TLSC, traditional long supply chain; OLSC, organic long supply chain; PS, phosphatidylserine; PI, phosphatidylinositol; SM, sphingomyelin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; Chol, cholesterol. Data are expressed as mean ± SD. *significance of OSSC versus TSSC and OLSC versus TLSC; §, TLSC versus TSSC and OLSC versus OSSC. *p* < 0.05.

and pigmentation; however, the level of intramuscular fat content and the redox state is more related to the b* coordinate (Xie *et al.*, 2012).

Tenderness is considered the single most important factor for the consumer's perception of beef quality. It is widely accepted that beef tenderness is a rather inconsistent characteristic, with huge variation between breeds, animals, meat cuts, and many other variables, which is recognized as one of the main problems for the beef industry around the world (Špehar *et al.*, 2008). The variability of beef tenderness evaluation is also linked to the difficulty in the development of a standardized, repeatable, and reliable method to objectively assess this characteristic at the laboratory level (Warner *et al.*, 2022). Researchers have systematically attempted to determine the most repeatable and accurate method to assess beef tenderness. To date, the Warner-Bratzler method and its

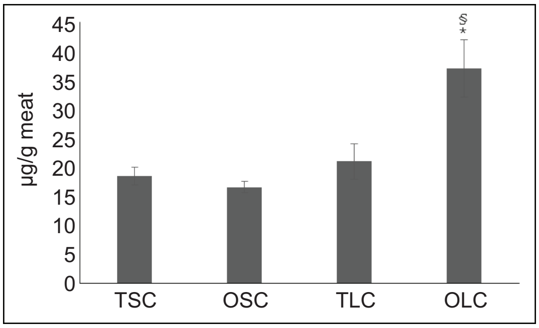

Figure 6. Advanced glycation end products in the bovine diaphragm. TSSC, traditional short supply chain; OSSC, organic short supply chain; TLSC, traditional long supply chain; OLSC, organic long supply chain. Data are expressed as mean ± SD. *, significance OLSC versus TLSC; §, OLSC versus OSSC. *p* < 0.05.

variations are recognized as the best approach to measure beef tenderness (Silva *et al.*, 2017); for the purpose of this study, a modified version of the Warner-Bratzler method was used.

In this study, we sought to determine the effect of TSSC, OSSC, TLSC, and OLSC on the bovine meat properties by analyzing the diaphragm. We demonstrated that meat derived from organic farming (OSSC) has significantly greater antioxidant properties than those from traditional farming (TSSC). The fact that the meat comes from a SSC (OSSC, TSSC) or LSC (OLSC, TLSC) does not influence the result. Furthermore, we demonstrated that the total content of PLs in samples derived from both SSC and LSC organic farming is also higher than that of samples derived from traditional farming. Importantly, by analyzing each class of PLs, we observed a high level of PS + PI, SM, PE, and Chol in organic samples. This data is relevant, considering the role that these PLs have on human health. PS is involved in the repair of damaged membranes (Li *et al.*, 2024a) and protects against pathogens (Groß *et al.*, 2024); PI is essential in metabolic balance (Li *et al.*, 2024b); SM facilitates brain development (Albi *et al.*, 2022), regulates lipid metabolism, and prevents metabolic syndrome (Li *et al.*, 2024); PE is a regulator of metabolic energy and acts on skeletal muscle insulin sensitivity (Grapentine *et al.*, 2019); and Chol is involved in brain function (Paseban *et al.*, 2023).

Additionally, our study demonstrated the lower level of AGEPs in TSSC and OSSC meats compared with TLSC and OLSC samples. The long chain was responsible for the increase in AGEP content, especially in organic samples. As a consequence, it is possible to assume that organic samples are more susceptible to the formation of AGEPs, which are known to be involved in the onset and exacerbation of many diseases, especially in the cardiovascular and nervous systems (Reddy *et al.*, 2022).

In conclusion, our work demonstrates that meat from organic animals has better antioxidant power and a better PL and cholesterol composition than meat derived from traditional farming, and that the LSC does not significantly change these properties. Instead, the LSC strongly influences the AGEP content in meat, especially of organic origin.

Authors Contributions

The conception and design of the study B..C. and E.A.; experiments A.V., F.F., E.C., S.C.; analysis and interpretation of data G.S., B.C.C., E.A.; drafting the article or revising it critically for important intellectual content L.P., B.C.C., G.S., E.A.; final approval of the version to be submitted B.C.C., E.A. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

Authors declare no conflicts of interests.

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