Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.00424

Nutritional composition and physicochemical changes in Maracaju sausage during freezing storage

Renata Ribeiro Campagnoli ANDRADE[1], Angela Dulce Cavenaghi ALTEMIO[1], Maria Ildilene da SILVA[2], Valfredo Fiqueira da SILVA[2], Milton Teixeira de MORAES JÚNIOR[2], Cláudia Andréa Lima CARDOSO[3], Elenice Souza dos Reis GOES[2]*

Abstract

This study characterized Maracaju sausages and evaluated their physicochemical changes during storage for six months under freezing conditions, with a focus on lipid stability. The sausages were purchased immediately after production from three producers affiliated with the Association of Producers of Traditional Maracaju Sausage (APTRALMAR, Mato Grosso do Sul), in the municipality of Maracaju, Mato Grosso do Sul (MS). The centesimal composition and texture profile were consistent across brands, indicating standardization. All the brands exhibited viable aerobic mesophilic microorganism counts above those permitted by law. In terms of color, the a*, b*, and saturation chromas increased up to 90 days, with a subsequent reduction. pH and shear force remained unchanged throughout storage. The highest cooking loss occurred at 120 days. Water retention capacity was lower at 30 and 90 days. The peroxide index was only detected in the first month, while the thiobarbituric acid reactive substance values increased with extended storage. Fatty acid composition remained stable for up to 90 days, with significant reductions in all fatty acids after 120 days. These findings suggest that oxidation processes remain stable over a period of up to 90 days of freezing storage.

Practical Application: Maracaju sausage kept frozen has an estimated oxidative stability of 90 days.

Keywords: fatty acids; sausages; fresh sausage; lipid oxidation; TBARs.

1 INTRODUCTION

Gastronomic identity is an important element for tourist destinations, contributing to the creation of a place's brand (Suna & Alvarez, 2021). In Mato Grosso do Sul, the local cuisine stands out for its use of fresh and authentic ingredients, such as fish from crystal-clear rivers, high-quality beef, exotic fruits, and aromatic herbs (Arguelho et al., 2023). The region's gastronomic diversity includes not only typical dishes but also festivals and fairs that provide opportunities to experience local flavors (Arguelho et al., 2023).

Among the traditional dishes, Maracaju sausage is a highlight and celebrated at the Sausage Festival, an annual event that attracts visitors, generates temporary employment opportunities, and promotes the visibility of the municipality (Arguelho et al., 2023). The differential of the sausage lies in its composition, using only beef and natural ingredients (Arguelho et al., 2023). Its production follows a standardized formulation established for producers belonging to the Association of Producers of Traditional Maracaju Sausage (APTRALMAR).

Studies characterizing regional products essential to local gastronomy can provide valuable data for both consumers and producers regarding the microbiological safety and storage stability of these products.

Maracaju sausages are classified as fresh sausages. Sausages are among the most popular and widely consumed food products in the world (Kavuşan et al., 2020). However, they are highly perishable products, as they are made from fresh ground meat, which favors the growth of pathogenic and spoilage microorganisms (Hugo & Hugo, 2015). Although freezing slows down the deterioration of meat products, changes in proteins and lipid oxidation continue to occur at low temperatures (Lu et al., 2022).

Oxidation is the primary non-microbial phenomenon affecting meat and meat products, leading to undesirable changes in product quality, reduced shelf life, and adverse health effects due to toxic secondary oxidation products (Nacak et al., 2021). These reactions not only reduce the nutritional value of meats by depleting essential fatty acids and vitamins, but also lead to sensory quality deterioration. Initial changes include alterations in color, texture, and the development of rancid odor and taste, which influences consumer acceptance (Domínguez et al., 2019).

Received: Oct. 31, 2024.
Accepted: Dec. 10, 2024.
[1]*Universidade Federal da Grande Dourados, Faculty of Engineering, Postgraduate Program in Food Science and Technology, Dourados, MS, Brazil.*
[2]*Universidade Federal da Grande Dourados, Faculty of Agrarian Sciences, Dourados, MS, Brazil.*
[3]*Universidade Estadual do Mato Grosso do Sul, Course of Chemistry, Dourados, MS, Brazil.*
*Corresponding author: elenicegoes@ufgd.edu.br
Conflict of interest: nothing to declare.
Funding: none.

The high lipid content of fresh sausages make the product more susceptible to lipid oxidation, with subsequent changes to the product's organoleptic properties. The oxidative deterioration of polyunsaturated fatty acids in food leads to the formation of hydroperoxides, along with short-chain aldehydes, ketones, and other oxidized compounds, which are key contributors to flavor, texture, color, and nutritional deterioration in meat and meat products (Ali et al., 2018).

Lipid oxidation is a complex process involving multiple interactive mechanisms, with the detection of primary and secondary oxidation products being the most common method for assessing the degree of lipid oxidation (Domínguez et al., 2019; Zhao et al., 2020).

Unsaturated fatty acids react with molecular oxygen via free radicals, forming hydroperoxides as the initial oxidation products (Domínguez et al., 2019). These are generally evaluated by the peroxide value and the iodine method (Domínguez et al., 2019; Zhao et al., 2020). Although odorless, hydroperoxides are unstable and decompose quickly, generating secondary compounds such as aldehydes, ketones, and acids, which are responsible for unpleasant tastes and odors in meat, with aldehydes being the main contributors to volatile flavors due to their low odor threshold and high concentration, in addition to interacting with proteins, altering nutritional and organoleptic properties (Domínguez et al., 2019). The thiobarbituric acid reaction substance (TBARS) assay is commonly used to assess the degree of secondary lipid oxidation. This method mainly measures malondialdehyde compounds formed during lipid oxidation, providing and assessment of the degree of oxidation and deterioration of foods (Zhao et al., 2020).

In this context, the aim of this study was to characterize the centesimal composition, microbiological, and texture of Maracaju sausages, as well as to evaluate their physicochemical changes over six months of storage under freezing, with a focus on lipid stability.

2 MATERIAL AND METHODS

2.1 Obtaining the sausages

The Maracaju sausage samples were purchased from three different producers belonging to APTRALMAR, in Maracaju, Mato Grosso do Sul (MS). The municipality is located in the southern part of Brazil's Midwest at an altitude of 384 meters, with geographical coordinates of 21°36'52" south latitude and 55°10'06" west longitude. It is approximately 160 km from the state capital, Campo Grande.

The sausages are packaged in polyethylene or nylon-poly bags, weighing approximately one kilogram, and are sold either refrigerated (between 2 and 7°C) or frozen (below -18°C) at an average price of R$ 60. The samples used in this study were purchased on the same day they were produced and frozen. They were transported in a thermal box to the laboratory at Universidade Federal de Grande Dourados (UFGD). At the laboratory, the samples were fractionated, separating the portions for zero-time tests (T_0) while the remaining portions were stored in a horizontal freezer at -18 ± 2°C.

For characterization purposes, the centesimal composition and caloric value (n = 3 for each brand) and microbiological analyses (n = 2 for each brand) were carried out using raw samples and the analysis of the texture profile (n = 10 for each brand) was conducted on samples baked at 220°C for 20 minutes, reaching a minimum internal temperature of 72°C.

The sausages were stored frozen (-18°C) for six months. Every 30 days (0, 30, 60, 90, 120, 150, and 180 days), an intact package of sausage from each brand was opened (three packages total), and each package was partitioned for analysis of pH (n = 5), color (n = 10), weight loss by cooking (WLC; n = 5), water retention capacity (n = 5), peroxide index (n = 2), TBARS (n = 3), and fatty acid profile (n = 2) using the raw samples, and shear force analysis (n = 5) with the roasted samples.

2.2 Centesimal composition, caloric value, and texture profile analysis

The analyses of centesimal composition were carried out in triplicate for each brand of raw sausage. Moisture and ash contents were determined according to the AOAC methodology (2006). Crude protein content was determined using the semi-micro Kjeldahl method described by Silva and Queiroz (2002). Lipid analysis was carried out according to the methodology of Bligh and Dyer (1959). Carbohydrate content was determined by difference, based on the remaining constituents (Instituto Adolfo Lutz, 2008). The caloric value was calculated according to Atwater and Woods (1896), where the conversion factors are considered to be 4 kcal.g^{-1} for proteins and carbohydrates, 2 kcal.g^{-1} for total dietary fiber, and 9 kcal.g^{-1} for lipids. Results were expressed in kcal/100 g.

Texture profile analysis (TPA) was carried out (n = 10) using cylindrical slices 25 mm thick, previously baked at 220°C for 20 minutes, using a TA-XT plus texturometer, Stable Micro Systems Ltd. (Surrey, England), equipped with a 50 kg load cell and a 36 mm cylindrical probe (P/36). TPA was performed with two 60% compression cycles with a 5-second interval between compressions. Pre-test, test, and post-test speeds were set at 1, 5, and 5 mm/s, respectively. Hardness, fracturability, elasticity, cohesiveness, chewability, and resilience parameters were calculated using the Exponent software package, version 6.1.9.1 (Stable Micro Systems, Surrey, England).

2.3 Microbiological analysis

The count of viable aerobic mesophilic microorganisms (according to ABNT, 2015), total *Escherichia coli* count (AOAC, 2019), and *Salmonella* spp. (AOAC, 2016) were determined at time zero (T_0), as recommended by ANVISA (2022). These tests were repeated on different packages for each brand.

2.4 Physico-chemical parameters

The pH of the Maracaju sausage samples was determined using a digital pH meter (Testo 205, Testo Limited, Hampshire, UK) equipped with a meat insertion electrode.

Instrumental color analysis was carried out on the inside of the sausages using a Konica Minolta Chroma Meter CR

410 portable colorimeter (Konica Minolta Optics Inc., Japan). The parameters included L* (luminosity), chroma a* (red intensity), chroma b* (yellow intensity), and C* (saturation). The hue angle (h°) was calculated according to Equation 1.

$$h^\circ = arctang\ \frac{b}{a} \quad (1)$$

The analysis of shear force was carried out on 2.5 cm high slices, previously baked at 220°C for 20 minutes, using a TA-XT plus texturometer, Stable Micro Systems Ltd. (Surrey, England), equipped with a Warner-Bratzler-type shear cell, with pre-test speed: 20 mm/s, post-test: 10 mm/s, test: 20 mm/s, and a distance of 20 mm.

The analysis of WLC was carried out as described by You et al. (2022), with modifications. Sausage samples were cut into 1 cm slices, weighed on a precision scale, and packed in plastic bags. They were placed in a water bath at 90°C for 20 minutes and then cooled to room temperature. After removing the packets and eliminating any surface water, each sample was weighed. WLC was calculated using Equation 2:

$$WLC(\%) = \frac{(M1 - M2)}{M1} \times 100 \quad (2)$$

Where M1 is the mass of the sausage before cooking (g) and M2 is the mass of the sausage after cooking (g).

To determine the water retention capacity (WRC) (You et al., 2022), thawed sausage samples (~2 g) were accurately weighed, wrapped with filter paper (3 layers), placed in a centrifuge, and then centrifuged at 10,000×g for 10 minutes. The samples were removed from the tube and filter paper and then weighed again. WRC was calculated according to Equation 3.

$$WRC\ (\%) = 100 - \frac{M2}{M1} \times 100 \quad (3)$$

Where M1 is the weight of the sample before centrifugation (g), and M2 is the weight of the sample after centrifugation (g).

The peroxide index was determined according to the ISO 3960 methodology (2017).

TBARS were analyzed according to the Cd 19-90 method, described by the American Oil Chemists Society (1993), with modifications. 7 g of sample were weighed and 0.015 g of ethylenediamine tetraacetic acid (EDTA), 0.015 g of propyl gallate, and 30 mL of 7.5% trichloroacetic acid were added. After homogenization, the material was filtered and 2.5 ml of the filtrate and 2.5 ml of 46 mM thiobarbituric acid were added to test tubes, which were heated in a water bath at 95°C for 35 minutes. After cooling, the samples were analyzed in a spectrophotometer at a wavelength of 532 nm. The results were calculated from a standard curve of 1,1,3,3-tetraethoxypropane and expressed as mg of malonaldehyde per kg of sample.

2.5 Fatty acid profile

A test was carried out to identify the fatty acid profile ($n = 2$) of raw Maracaju sausage samples from each brand evaluated throughout the storage period (0, 30, 60, 90, 120, 150, and 180 days).

The esterification of total lipids was carried out, and the fatty acid methyl esters (FAMEs) were separated using a gas chromatograph (SHIMADZU), model CG2010 PLUS, equipped with a flame ionization detector (FID), a split injector, and a sample split ratio of 1:50. A 100 m long capillary column with an internal diameter of 0.25 mm and a film thickness of 0.25 µm was used. The chromatographic conditions were programmed as follows: column temperature starting at 60°C for 2 minutes, rising to 160°C in a ramp of 3°C per minute, and remaining at this temperature for 20 minutes, and then increased to 240°C from 31 to 70 minutes. Helium 6.0 was used as the carrier gas at a flow rate of 2 mL/min, while nitrogen served as the make-up gas at 25 mL/min, with an injector temperature of 270°C, detector temperature of 300°C, and an injection volume of 1 µL (AOAC, 2006). Fatty acids were identified by comparing the retention times of sample fatty acids with those of standards. A total of 37 fatty acid methyl standards from the FAME Mix (Sigma-Aldrich) were used to identify the fatty acids, and their quantification was carried out by area normalization. The results were expressed as relative percentage area.

2.6 Statistical procedure

The characterization results, considering three different sausage brands, were evaluated using analysis of variance (ANOVA) at a 5% significance level. In the event of significant differences ($P < 0.05$), the means were compared using the Tukey test, with Statistica 7.0 software (StatSoft, St Tulsa, OK, USA). For the results of the shelf-life analysis, the averages across the brands were calculated, and ANOVA was carried out to assess differentiation in the results over the storage periods (0, 30, 60, 90, 120, 150, and 180 days). Additionally, the means obtained across all analysis times per brand were evaluated using ANOVA at a 5% significance level and Tukey's test was applied to identify significant differences ($P < 0.05$) when applicable. The results were expressed as mean ± standard error of the mean.

3 RESULTS AND DISCUSSION

Table 1 shows the results obtained for the centesimal composition and calorific value of Maracaju sausages. No significant differences ($P > 0.05$) were observed among the brands in terms of moisture, proteins, lipids, carbohydrates, ash content, or caloric value, which demonstrates the standardization of this product across different producers.

Normative Instruction No. 4 of March 31, 2000, which approves the Technical Regulation for the Identity and Quality of Sausage, establishes quality parameters for physical-chemical characteristics, such as humidity (maximum 70%), protein (minimum 12%), and lipids (maximum 30%) (Brasil, 2000). Thus, the samples analyzed were consistent with the Sausage Identity and Quality Standard. Fresh bovine sausages of Australian origin have been reported to contain 62.2% moisture, 15.6% protein, and 14.4% lipids (Cunningham et al., 2015).

In the texture profile analysis (Table 2), no significant differences were observed ($P > 0.05$) among the brands, a result that may be attributed to the use of only prime meats in the preparation of this type of product, which contributes to its juiciness and standard. This analysis simulates the chewing process similar to that in the human mouth, providing parameters of hardness (force required to compress food between the molars), elasticity (extent to which a deformed material returns to its initial state), cohesiveness (degree to which a food can be deformed before breaking), fracturability (force required to fracture a material), and chewability (energy required to chew solid food to a state suitable for swallowing) (Novaković & Tomašević, 2017). Thus, the absence of differences in texture profiles among the different brands demonstrates standardization, which is an important factor for a traditional product.

Table 3 shows the results obtained from the microbiological analyses conducted during two sampling periods. As observed in the first sampling, two of the brands analyzed showed results above those allowed by current Brazilian legislation (ANVISA, 2022), necessitating a follow-up sampling. In the second sampling, all brands had viable aerobic mesophilic microorganism counts above the values allowed by legislation for food consumption (ANVISA, 2022). This outcome precluded the sensory analyses, which would have been essential to define the shelf life based on the sensory profile of the products.

The count of aerobic mesophilic microorganisms in food reflects its hygienic and sanitary quality. Several factors contribute to food contamination, with the primary ones being poor hygiene conditions at the production site, lack of suitable equipment and utensils, and lack of personal hygiene, especially among handlers (Silva et al., 2023). A recent study evaluating the microbiological quality of meat and contact surfaces in butcher stores and traditional retail establishments showed that most of the facilities visited presented a moderate hygiene and health risk, highlighting the greater need for government surveillance (Serhan et al., 2024).

The most common genera of meat spoilage bacteria are *Staphylococcus, Bacillus, Campylobacter, Clostridium, Listeria, Salmonella*, lactic acid bacteria, *Pseudomonas* spp., *Acinetobacter* spp., and *Moraxella* spp., which can cause discoloration, unpleasant odors, and slime on the surface of meat (Atlabachew & Mamo, 2021). Of these, *Salmonella* spp., which is included in the Brazilian legislation for meat products (ANVISA, 2022), is one of the main triggers of foodborne illnesses, causing salmonellosis, a disease with a high morbidity and mortality rate in industrialized and developing countries (Rincon-Gamboa et al., 2021).

The results of the microbiological analysis of the Maracaju sausage samples highlight the need to adopt strict sanitary control measures during product processing. In addition, they

Table 1. Centesimal composition and caloric value of Maracaju sausages.

Parameters (%)	Maracaju sausages			Mean
	Brand 1	Brand 2	Brand 3	
Humidity^{NS}	64.18 ± 2.56	63.57 ± 0.48	65.95 ± 0.53	64.57 ± 0.84
Proteins^{NS}	17.40 ± 0.41	18.20 ± 0.38	17.45 ± 0.68	17.68 ± 0.28
Lipids^{NS}	8.47 ± 0.38	10.31 ± 0.72	10.57 ± 1.00	9.79 ± 0.50
Carbohydrates^{NS}	7.98 ± 2.76	5.98 ± 0.49	4.18 ± 1.66	6.05 ± 1.09
Ashes^{NS}	1.96 ± 0.15	1.95 ± 0.08	1.85 ± 0.21	1.92 ± 0.08
Caloric value (kcal/100 g) ^{NS}	177.79 ± 11.89	189.49 ± 5.79	181.70 ± 4.77	182.99 ± 4.41

^{NS}Not significant ($P > 0.05$).

Table 2. Texture profile analysis of Maracaju sausages.

	Maracaju sausages			Mean
	Brand 1	Brand 2	Brand 3	
Hardness^{NS}	6.11 ± 1.00	6.83 ± 0.52	6.90 ± 1.10	6.61 ± 0.49
Elasticity^{NS}	0.77 ± 0.04	0.71 ± 0.03	0.71 ± 0.02	0.73 ± 0.02
Cohesiveness^{NS}	0.55 ± 0.04	0.56 ± 0.03	0.55 ± 0.02	0.55 ± 0.02
Fracturability^{NS}	3.48 ± 0.84	2.57 ± 0.30	3.77 ± 1.21	3.27 ± 0.48
Chewability^{NS}	2.76 ± 0.68	2.72 ± 0.33	2.68 ± 0.42	2.72 ± 0.27

^{NS}Not significant ($P > 0.05$).

Table 3. Microbiological analysis of Maracaju sausages.

	Maracaju sausages			Reference values*
	Brand 1	Brand 2	Brand 3	
Sampling 1				
Count of viable aerobic mesophilic microorganisms (CFU/g)	5.30×10^2	3.20×10^3	1.40×10^6	Max: 1.00×10^6
Total *Escherichia coli* count (CFU/g)	2.70×10^1	$< 1.00 \times 10^1$	$< 1.00 \times 10^1$	Max: 1.00×10^2
Testing for *Salmonella* spp (in 25 g)	Absence	Presence	Absence	Absent in 25g
Sampling 2				
Count of viable aerobic mesophilic microorganisms (CFU/g)	5.40×10^6	1.60×10^6	4.20×10^5	Max: 1.00×10^6
Total *Escherichia coli* count (CFU/g)	9.1	9.1	$< 1.00 \times 10^1$	Max: 1.00×10^2
Detection *of Salmonella* spp (CFU/g)	Absence	Absence	Absence	Absent in 25 g

*ANVISA (2022); CFU: Colony Forming Unit.

reinforce the importance of complying with Good Manufacturing Practices (GMP), which are essential for guaranteeing food safety and microbiological quality.

Only the *Escherichia coli* counts complied with the legislation in all samples. Among the foodborne pathogens associated with meat, *E. coli* is a producer of Shiga toxin (STEC), whose poisoning is considered a serious public health issue (Assis et al., 2021).

Color is one of the most important sensory attributes of frozen foods, and its vividness directly affects consumer choice (Lu et al., 2022). Frozen storage influences color stability (Leygonie et al., 2012). Changes in color parameters can also be related to the amount of water, lipid oxidation, and protein denaturation (Coombs et al., 2017). In terms of the color of the Maracaju sausages (Table 4), brand 2 showed the lowest brightness ($P < 0.05$). However, no significant differences ($P > 0.05$) were observed

Table 4. Instrumental color of Maracaju sausages stored under freezing conditions for 180 days.

	Maracaju sausages			
	Brand 1	**Brand 2**	**Brand 3**	
Luminosity (L)*				Mean$^{NS:}$
0	53.40 ± 2.73	46.12 ± 1.02	47.55 ± 2.10	49.02 ± 1.29
30	48.34 ± 2.36	46.31 ± 0.73	54.92 ± 2.77	49.86 ± 1.38
60	59.26 ± 4.33	45.05 ± 3.78	46.59 ± 3.04	50.30 ± 2.40
90	52.57 ± 2.93	43.72 ± 4.24	55.79 ± 4.71	50.69 ± 2.44
120	56.16 ± 3.60	46.17 ± 2.51	47.16 ± 2.13	49.83 ± 1.78
150	40.50 ± 2.82	43.47 ± 3.12	55.39 ± 3.41	46.45 ± 2.11
180	40.82 ± 1.74	49.85 ± 3.54	41.10 ± 1.68	43.92 ± 1.58
Mean*	50.15 ± 1.36 a	45.81 ± 1.10 b	49.78 ± 1.25 a	
Red intensity (a)*				Mean*
0	6.34 ± 0.52	10.65 ± 0.51	10.90 ± 0.61	9.30 ± 0.49 d
30	9.18 ± 0.81	13.45 ± 0.68	11.89 ± 0.86	11.50 ± 0.55 bcd
60	12.16 ± 0.88	15.54 ± 1.02	12.79 ± 0.73	13.50 ± 0.56 ab
90	12.00 ± 0.86	16.35 ± 0.73	18.28 ± 1.07	15.54 ± 0.70 a
120	10.41 ± 0.70	10.50 ± 1.07	11.18 ± 0.99	10.70 ± 0.52 cd
150	14.34 ± 1.69	10.40 ± 1.05	12.73 ± 1.63	12.49 ± 0.88 bc
180	11.39 ± 0.91	11.22 ± 1.08	6.76 ± 0.67	9.79 ± 0.64 d
Mean*	10.83 ± 0.45 b	12.59 ± 0.43 a	12.08 ± 0.52 a	
Yellow intensity (b)*				Mean*
0	9.93 ± 0.75	7.52 ± 0.58	7.96 ± 0.94	8.47 ± 0.47 of
30	9.78 ± 0.63	9.95 ± 0.49	10.68 ± 0.92	10.14 ± 0.40 bcd
60	12.16 ± 0.89	11.41 ± 0.58	10.87 ± 0.81	11.48 ± 0.44 bc
90	16.34 ± 1.34	12.46 ± 0.78	15.49 ± 1.16	14.76 ± 0.70 a
120	10.42 ± 1.13	9.10 ± 1.05	9.35 ± 0.71	9.62 ± 0.55 cd
150	11.90 ± 1.09	9.84 ± 0.65	13.60 ± 0.92	11.78 ± 0.58 b
180	6.49 ± 0.62	8.91 ± 0.99	4.80 ± 0.78	6.73 ± 0.55 e
Mean^{NS}	11.00 ± 0.48	9.88 ± 0.33	10.39 ± 0.51	
Saturation (C)*				Mean*
0	11.95 ± 0.62	13.19 ± 0.40	13.77 ± 0.66	12.97 ± 0.35 of
30	13.59 ± 0.72	16.75 ± 0.79	16.19 ± 0.91	15.51 ± 0.52 c
60	17.36 ± 0.98	19.47 ± 0.77	16.93 ± 0.80	17.92 ± 0.52 b
90	20.49 ± 1.26	20.67 ± 0.79	24.13 ± 1.25	21.76 ± 0.70 a
120	14.93 ± 1.05	14.30 ± 0.98	14.91 ± 0.61	14.71 ± 0.51 cd
150	19.22 ± 1.24	14.62 ± 0.75	19.23 ± 0.98	17.69 ± 0.69 b
180	13.29 ± 0.83	14.73 ± 0.93	8.63 ± 0.65	12.22 ± 0.66 e
Mean^{NS}	15.83 ± 0.51	16.24 ± 0.43	16.26 ± 0.62	
h°				Mean*
0	56.58 ± 3.24	35.14 ± 2.86	35.36 ± 3.81	42.36 ± 2.63 ab
30	47.10 ± 3.07	36.57 ± 0.93	41.66 ± 2.97	41.78 ± 1.62 ab
60	45.08 ± 2.63	36.81 ± 2.72	40.19 ± 2.54	40.69 ± 1.60 ab
90	53.00 ± 2.79	37.26 ± 1.93	40.15 ± 2.28	43.47 ± 1.83 a
120	44.15 ± 3.36	40.82 ± 4.46	40.46 ± 4.07	41.81 ± 2.24 ab
150	41.65 ± 5.37	43.99 ± 3.95	47.85 ± 5.02	44.50 ± 2.72 a
180	30.01 ± 3.27	39.14 ± 4.55	34.53 ± 5.39	34.56 ± 2.59 b
Mean	45.37 ± 1.58 a	38.53 ± 1.24 b	40.03 ± 1.49 b	

^{NS}Not significant ($P > 0.05$); *Different lowercase letters in the same row or column differ according to Tukey's test ($P < 0.05$).

in luminosity (L*) as storage time increased. The evolution of meat color is largely explained by oxidative processes. During oxidation, the increasing content of metmyoglobin affects the color and appearance of the meat, ultimately determining its shelf life (Salueña et al., 2019). However, brightness does not usually change significantly during oxygenation and oxidation of the meat, which explains its stability over time (Salueña et al., 2019). Studies on color changes in beef during aging have also shown no significant differences in brightness (Cierach & Niedźwiedź, 2014; King et al., 2012).

For the Maracaju sausages, the average red intensity increased up to 90 days of storage, followed by a decline after 120 days (Table 4 and Figure 1A). The highest average yellow intensity was observed at 90 days (14.76), with the lowest at 180 days (6.73) ($P < 0.05$). Throughout frozen storage, changes in meat color may result from the reduction in the chemical form of myoglobin. Free radicals produced during lipid oxidation can alter the chemistry of the heme group and initiate myoglobin oxidation, resulting in the loss of product color, showing a brown color and reducing the red intensity (Pinheiro et al., 2019). Thus, both a* and b* decrease during oxidation, with more pronounced changes observed in a* (Salueña et al., 2019). Given the values obtained for a* and b*, it can be inferred that oxidative effects were less pronounced until 90 days of storage, with a subsequent decrease in red intensity.

A similar effect to that observed for red intensity was also seen in the evolution of saturation (C*) in the samples (Table 4 and Figure 1B), with an increase up to 90 days of storage, followed by a subsequent decrease ($P < 0.05$). For the hue angle (h°), the lowest average was observed at 180 days of storage, with higher averages at 90 and 150 days ($P < 0.05$). Chroma C* and hue angle are obtained directly from a* and b* and are better correlated with the human visual perception of color (Salueña et al., 2019). The increase in metamyoglobin levels during oxidation leads to a marked decrease in C* values and only a slight increase in h° (Salueña et al., 2019), which is in line with the results obtained in this study.

Table 5 shows the results for the parameters pH, cooking loss, water retention capacity, and shear force of the Maracaju sausage samples stored under freezing for 180 days.

pH is an important characteristic of sausages, as it determines the quality and shelf life of the product and even influences microbiological safety (Ebied et al., 2017). In this study, no differences in pH were observed during the storage period, nor between the brands ($P > 0.05$). As an indicator of food stability, pH often correlates with microbial and chemical reactions (Lu et al., 2022). Various factors can be attributed to changes in muscle pH; and an increase in pH during storage may be due to the accumulation of amines and ammonia by psychrophilic bacteria (Koziol et al., 2015). Despite this, some authors report no significant changes in pH during storage, or even a decrease, which may also be related to the metabolism of lactic acid bacteria producing compounds such as lactic acid (Zhai et al., 2018).

In the analysis of WLC, the lowest average was observed at 60 days of storage (29.98%), while the highest WLC (33.87%) was found at 120 days of storage ($P < 0.05$). Brand 2 had the highest average WLC among those evaluated ($P < 0.05$). WLC during heat treatment is caused by the contraction of muscle fibers and intramuscular connective tissue, with the intensity depending on temperature and cooking method (Ježek et al., 2020). Considering that meat products are a rich source of proteins, essential minerals, and vitamins, an increased loss of these nutrients decreases their nutritional quality (Akhter et al., 2022).

For WRC, lower averages were observed at 30 and 90 days of storage ($P < 0.05$). No significant differences in WRC were found between the different brands ($P > 0.05$). WRC in meat products influences visual acceptability, weight loss, cooking yield, as well as sensory characteristics at the time of consumption (Warner, 2023).

Water retention usually affects the textural properties of meat products (Pematilleke et al., 2021). However, no changes were observed in the shear force of the sausages according to storage time ($P > 0.05$). When freezing is done slowly (in a regular freezer, for example), intracellular water migrates to intermolecular water, forming large ice crystals that can cause mechanical damage to muscle fibers and deterioration in texture (Xie et al., 2021). A study on beef shelf life found that *drip loss* increased with storage time, reflecting an increase in shear force as well (Qian et al., 2018). Thus, an increase in the shear force with extended freezing time was expected. However, due to the

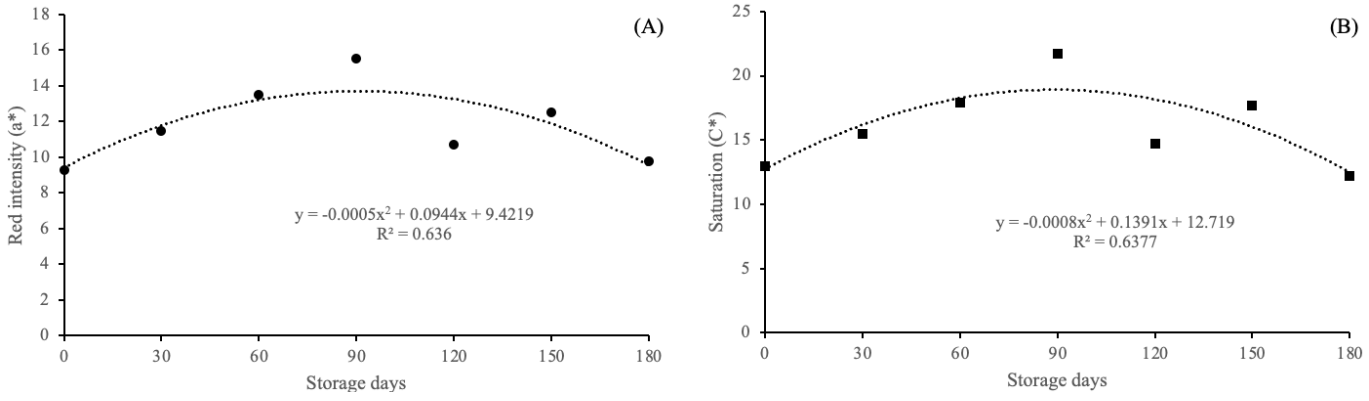

Figure 1. (A) Red intensity and (B) saturation of Maracaju sausages stored under freezing conditions for 180 days.

Table 5. Results of pH, cooking loss, water retention capacity, and shear force of Maracaju sausages stored under freezing for 180 days.

	Maracaju sausages			
	Brand 1	**Brand 2**	**Brand 3**	
pH				Mean $^{NS:}$
0	5.48 ± 0.02	5.49 ± 0.01	5.57 ± 0.02	5.51 ± 0.01
30	5.56 ± 0.01	5.28 ± 0.19	5.65 ± 0.06	5.50 ± 0.07
60	5.52 ± 0.02	5.36 ± 0.01	5.53 ± 0.04	5.47 ± 0.03
90	5.63 ± 0.01	5.50 ± 0.01	5.84 ± 0.20	5.66 ± 0.07
120	5.70 ± 0.04	5.54 ± 0.02	5.68 ± 0.02	5.64 ± 0.02
150	5.64 ± 0.03	5.50 ± 0.04	5.61 ± 0.01	5.58 ± 0.02
180	5.52 ± 0.22	5.50 ± 0.02	5.62 ± 0.01	5.55 ± 0.07
Mean^{NS}	5.58 ± 0.03	5.45 ± 0.03	5.64 ± 0.03	
Cooking loss (%)				Mean*
0	28.46 ± 0.69	33.93 ± 1.35	31.18 ± 0.39	31.19 ± 0.77 ab
30	30.38 ± 1.25	33.13 ± 0.88	29.03 ± 1.43	30.85 ± 0.79 ab
60	27.03 ± 1.41	32.38 ± 0.86	30.54 ± 0.71	29.98 ± 0.81 b
90	27.79 ± 1.87	33.37 ± 0.95	29.64 ± 1.19	30.27 ± 0.97 ab
120	33.39 ± 0.71	36.19 ± 0.47	32.04 ± 2.80	33.87 ± 1.02 a
150	27.62 ± 2.04	34.15 ± 0.65	32.85 ± 0.69	31.54 ± 1.03 ab
180	31.28 ± 1.03	29.93 ± 1.44	31.59 ± 0.82	30.93 ± 0.63 ab
Mean*	29.42 ± 0.60 b	33.30 ± 0.46 a	30.98 ± 0.52 b	
Water retention capacity (%)				Mean*
0	84.49 ± 1.41	85.63 ± 1.44	85.78 ± 1.28	85.30 ± 0.75 a
30	80.04 ± 1.26	75.82 ± 3.65	82.12 ± 1.48	79.33 ± 1.46 b
60	87.67 ± 0.22	86.30 ± 0.76	77.43 ± 5.39	83.80 ± 2.07 ab
90	82.86 ± 0.97	77.43 ± 0.83	77.67 ± 2.29	79.32 ± 1.05 b
120	83.73 ± 0.61	80.22 ± 0.62	82.19 ± 1.57	82.05 ± 0.67 ab
150	86.28 ± 0.63	81.29 ± 0.98	83.75 ± 1.82	83.77 ± 0.86 ab
180	83.00 ± 1.85	80.85 ± 1.64	85.34 ± 1.06	83.06 ± 0.96 ab
Mean^{NS}	84.01 ± 0.55	81.08 ± 0.85	82.04 ± 1.02	
Shear force (N)				Mean$^{NS:}$
0	59.85 ± 5.77	59.72 ± 8.05	83.81 ± 14.29	67.79 ± 6.16
30	39.13 ± 6.60	63.88 ± 7.64	60.98 ± 12.44	54.66 ± 5.76
60	67.39 ± 6.07	95.93 ± 11.77	92.13 ± 5.99	85.15 ± 5.62
90	57.86 ± 5.21	51.49 ± 11.34	90.86 ± 4.51	66.74 ± 6.17
120	42.05 ± 5.53	58.24 ± 11.44	43.28 ± 10.86	47.86 ± 5.52
150	35.64 ± 9.42	67.95 ± 7.64	47.43 ± 7.27	50.34 ± 5.64
180	77.89 ± 13.53	68.46 ± 7.26	46.94 ± 3.65	64.43 ± 5.98
Mean ^{NS}	54.26 ± 3.71	66.53 ± 3.97	66.49 ± 4.70	

^{NS}Not significant ($P > 0.05$); *Different lowercase letters in the same row or column differ according to Tukey's test ($P < 0.05$).

high variability of the data, no significant changes in the hardness of the sausages were observed as storage time increased.

Table 6 shows the lipid oxidation parameters assessed. The peroxide index was observed only during the first month of storage, with the highest average for brand 1 ($P < 0.05$). However, at subsequent analysis times, no peroxides were detected in the samples. This is possible because the peroxide index is more commonly used as an indicator of the initial stages of oxidation (Mehta et al., 2015). The first compounds formed during oxidation are peroxides, especially hydroperoxides, also referred to as primary oxidation products (Mehta et al., 2015). In contrast to other lipid-derived products, hydroperoxides are odorless and do not contribute to aroma (Domínguez et al., 2019).

In this study, the analysis of TBARS (Table 6) showed the lowest average at time 0, followed by a subsequent increase, mainly after 90 days of storage ($P < 0.05$). The data also showed a linear increase (Figure 2). These results are in line with other studies that show an increase in the lipid oxidation of meats as storage time increases (Qian et al., 2018).

Although sub-zero temperatures in freezer storage can slow down most reactions in the food system, some biochemical reactions can still occur, including auto-oxidation and lipid hydrolysis (Lu et al., 2022). This is because the ice crystals formed during freezing can damage cells, inducing the release of oxidants, especially non-heme iron, which can accelerate lipid oxidation (Sun et al., 2019). Lipid oxidation causes rancidity in both cooked and raw meat, diminishing consumer acceptability (Shahidi, 2016). In addition, lipid oxidation is known to promote the occurrence of protein dysregulation, resulting in the loss of essential amino acids and detrimental effects on meat quality (Ali et al., 2018).

Table 6. Peroxide index and thiobarbituric acid reactive substances of Maracaju sausages stored under freezing conditions.

	Maracaju sausages			Mean
	Brand 1	Brand 2	Brand 3	
Peroxide value (meq/kg)				
0	0.99 ± 0.00 a	0.55 ± 0.00 b	0.57 ± 0.00 b	0.70 ± 0.00
30	ND	ND	ND	ND
60	ND	ND	ND	ND
TBARs (mg of malonaldehyde /kg)				Mean
0	0.14 ± 0.01	0.12 ± 0.05	0.15 ± 0.00	0.13 ± 0.02 d
30	0.15 ± 0.02	0.12 ± 0.01	0.20 ± 0.01	0.16 ± 0.01cd
60	0.15 ± 0.00	0.14 ± 0.00	0.20 ± 0.01	0.16 ± 0.01 bcd
90	0.19 ± 0.03	0.14 ± 0.01	0.22 ± 0.01	0.18 ± 0.01 bcd
120	0.24 ± 0.01	0.14 ± 0.01	0.23 ± 0.02	0.20 ± 0.02 bc
150	0.26 ± 0.02	0.15 ± 0.04	0.25 ± 0.05	0.22 ± 0.03 ab
180	0.28 ± 0.03	0.21 ± 0.01	0.30 ± 0.01	0.27 ± 0.02 a
Mean	0.20 ± 0.01 a	0.15 ± 0.01 b	0.22 ± 0.01 a	

[ND]Not detected.

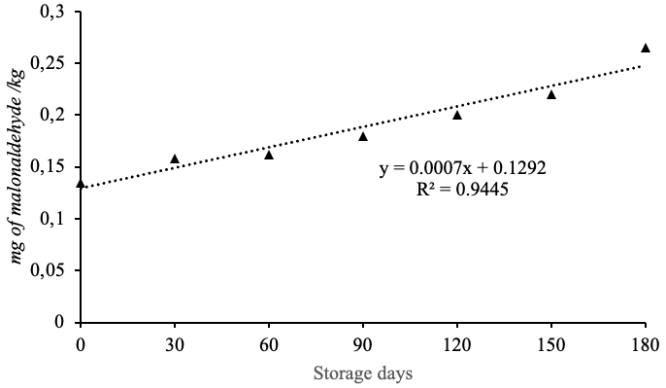

Figure 2. Thiobarbituric acid reactive substances of Maracaju sausages stored under freezing conditions for 180 days.

The main factors influencing lipid oxidation in meat are fat content and fatty acid composition, as fatty acids serve as substrates for oxidation processes (Domínguez et al., 2019). Regarding the fatty acid composition of the different brands (Table 7), no significant differences were observed between the brands for the fatty acids evaluated ($P > 0.05$).

However, when the fatty acid profiles were compared according to storage time (Table 8), significant differences ($P < 0.05$) could be observed for all the fatty acids analyzed. In general, the results showed that the various fatty acids remained stable up to 90 days of storage. However, after 120 days, a decrease was observed in most of the fatty acids in the sausages. All saturated fatty acids decreased after 120 days of storage. Only C16:1, C20:1, and C20:2 exhibited an increase in their averages after 120 days of freezing. Some fatty acids (C14:1, C20:3 n6, C20:5 n3, and C22:6 n3) were only detected up to 90 days of storage. In addition, unidentified peaks appeared in the analysis after 120 days of freezing.

Lipid oxidation is a complex phenomenon induced by oxygen in the presence of initiators such as heat, free radicals, light, photosensitizing pigments, and metals, and occurs through different reaction pathways:

- free radical-mediated auto-oxidation;
- photo-oxidation;
- enzymatic oxidation;
- thermal oxidation (Shahidi, 2016).

The first two types of oxidations consist of a combination of reactions involving triplet oxygen ($3O_2$), which can be considered a fundamental state, and singlet oxygen ($1O_2$) (Shahidi, 2016).

Polyunsaturated fatty acids are more susceptible to triplet oxygen oxidation initiated by free radicals than monounsaturated fatty acids (Ahmed et al., 2016). Singlet oxygen can also react with electron-rich double bonds of unsaturated molecules, but the reaction rate of triplet oxygen increases with the degree of unsaturation (Ahmed et al., 2016). This may explain why the main polyunsaturated fatty acids (C20:3 n6, C20:5 n3, and C22:6 n3) were only observed up to 90 days of storage, indicating an increase in oxidative processes after this period.

The oxidative deterioration of polyunsaturated lipids in food leads to the formation of hydroperoxides, short-chain aldehydes, ketones, and other oxidized compounds, which are considered responsible for the deterioration in taste, texture, color, and nutritional quality of meat and meat products (Ali et al., 2018).

4 CONCLUSION

The Maracaju sausages from the different brands exhibited similar nutritional and textural characteristics, indicating product standardization. However, the microbiological profile (mesophile and Salmonella counts) did not comply with Brazilian legislation, suggesting the need for greater sanitary supervision. During 180 days of storage, both pH and shear force remained stable, and brightness showed no significant changes. However, a reduction in red intensity and saturation was observed after 120 days. The peroxide, TBAR, and fatty acid profile results showed that lipid oxidation was observed over time, becoming more pronounced after 120 days, indicating stability up to 90 days of freezing storage.

Table 7. Fatty acid profile of Maracaju sausages from three different brands.

Fatty acid	Brands			Mean[NS]
	Brand 1	Brand 2	Brand 3	
C10:0	0.10 ± 0.02	0.09 ± 0.02	0.09 ± 0.02	0.09 ± 0.01
C12:0	0.10 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
C14:0	0.23 ± 0.03	0.21 ± 0.03	0.21 ± 0.03	0.22 ± 0.02
C16:0	23.69 ± 0.84	24.10 ± 1.04	23.07 ± 1.00	23.62 ± 0.54
C17:0	0.47 ± 0.02	0.46 ± 0.02	0.46 ± 0.03	0.47 ± 0.01
C18:0	12.05 ± 0.20	11.94 ± 0.23	11.68 ± 0.33	11.89 ± 0.15
C20:0	0.23 ± 0.05	0.23 ± 0.05	0.22 ± 0.05	0.23 ± 0.03
C16:1	2.18 ± 0.23	2.31 ± 0.29	2.23 ± 0.25	2.24 ± 0.14
C17:1	0.15 ± 0.00	0.14 ± 0.00	0.15 ± 0.00	0.14 ± 0.00
C18:1n9	32.05 ± 0.86	31.59 ± 0.85	32.32 ± 0.89	31.99 ± 0.48
C18:2 n6	20.36 ± 0.41	20.04 ± 0.45	20.66 ± 0.28	20.35 ± 0.22
C18:3 n3	1.42 ± 0.07	1.46 ± 0.05	1.49 ± 0.04	1.46 ± 0.03
C20:2	1.35 ± 0.23	1.25 ± 0.18	1.51 ± 0.32	1.37 ± 0.14
C20:1	1.20 ± 0.34	1.12 ± 0.30	1.18 ± 0.33	1.16 ± 0.18
*c*20:3 3n	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
C20:4 6n	0.32 ± 0.02	0.32 ± 0.02	0.32 ± 0.02	0.32 ± 0.01
Others	8.80 ± 0.41	10.07 ± 0.34	9.26 ± 0.46	9.38 ± 0.34

[NS]Not significant ($P > 0.05$).

Table 8. Fatty acid profile of Maracaju sausages stored under freezing conditions for 180 days.

Fatty acid	Time (days)						
	0	30	60	90	120	150	180
C10:0	0.12 ± 0.00 a	0.12 ± 0.00 a	0.13 ± 0.01 a	0.13 ± 0.00 a	0.07 ± 0.00 b	0.04 ± 0.00 c	0.02 ± 0.00 d
C12:0	0.10 ± 0.00 abc	0.11 ± 0.01 ab	0.12 ± 0.00 ab	0.13 ± 0.00 a	0.09 ± 0.01 bc	0.08 ± 0.01 cd	0.05 ± 0.01 d
C14:0	0.25 ± 0.00 c	0.26 ± 0.00 bc	0.28 ± 0.01 ab	0.31 ± 0.00 a	0.16 ± 0.01 d	0.14 ± 0.01 of	0.12 ± 0.00 e
C16:0	25.85 ± 0.42 a	25.79 ± 0.39 a	25.57 ± 0.27 a	25.32 ± 0.27 a	21.47 ± 0.34 b	20.75 ± 0.36 b	20.59 ± 0.33 b
C17:0	0.48 ± 0.02 ab	0.48 ± 0.01 ab	0.52 ± 0.01 a	0.54 ± 0.01 a	0.43 ± 0.02 bc	0.42 ± 0.01 bc	0.39 ± 0.01 c
C18:0	12.55 ± 0.05 a	12.42 ± 0.03 a	12.32 ± 0.04 a	12.30 ± 0.05 a	11.54 ± 0.29 b	11.22 ± 0.24 b	10.89 ± 0.18 b
C20:0	0.32 ± 0.01 a	0.33 ± 0.01 a	0.32 ± 0.01 a	0.33 ± 0.00 a	0.11 ± 0.00 b	0.09 ± 0.00 b	0.09 ± 0.00 b
C14:1	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	ND	ND	ND
C16:1	1.68 ± 0.01 c	1.70 ± 0.00 c	1.71 ± 0.01 c	1.72 ± 0.01 c	2.68 ± 0.06 b	2.98 ± 0.08 a	3.21 ± 0.11 a
C17:1	0.15 ± 0.00 a	0.15 ± 0.00 a	0.15 ± 0.00 a	0.15 ± 0.00 a	0.15 ± 0.00 a	0.14 ± 0.00 ab	0.13 ± 0.00 b
C18:1n9	33.65 ± 0.25 a	33.76 ± 0.28 a	33.89 ± 0.23 a	33.94 ± 0.20 a	29.91 ± 0.13 b	29.53 ± 0.23 b	29.22 ± 0.18 b
C20:1	0.50 ± 0.00 c	0.49 ± 0.00 c	0.50 ± 0.00 c	0.50 ± 0.00 c	1.73 ± 0.03 b	2.11 ± 0.04 a	2.32 ± 0.12 a
C18:2 n6	21.07 ± 0.20 a	21.06 ± 0.09 a	21.15 ± 0.08 a	21.30 ± 0.02 a	19.47 ± 0.34 b	19.34 ± 0.35 b	19.07 ± 0.28 b
C18:3 n3	1.56 ± 0.03 a	1.56 ± 0.01 a	1.58 ± 0.03 a	1.57 ± 0.01 a	1.32 ± 0.06 b	1.31 ± 0.05 b	1.29 ± 0.04 b
C20:2	0.86 ± 0.02 b	0.88 ± 0.01 b	0.88 ± 0.01 b	0.87 ± 0.00 b	1.78 ± 0.13 b	1.97 ± 0.16 b	2.37 ± 0.26 b
*c*20:3 3n	0.10 ± 0.00 a	0.11 ± 0.00 a	0.10 ± 0.00 a	0.11 ± 0.00 a	0.09 ± 0.00 b	0.09 ± 0.00 b	0.08 ± 0.00 b
C20:3 n6	0.11 ± 0.00	0.11 ± 0.00	0.10 ± 0.00	0.11 ± 0.00	ND	ND	ND
C20:4 6n	0.34 ± 0.00 a	0.35 ± 0.00 a	0.37 ± 0.00 a	0.36 ± 0.00 a	0.28 ± 0.01 b	0.27 ± 0.00 b	0.27 ± 0.00 b
C20:5 3n	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	ND	ND	ND
C22:6 3n	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	ND	ND	ND
Others	ND	ND	ND	ND	8.73 ± 0.38	9.52 ± 0.44	9.88 ± 0.31

Different lowercase letters in the same row or column differ according to Tukey's test ($P < 0.05$).

REFERENCES

Agência Nacional de Vigilância Sanitária (ANVISA) (2022). Normative Instruction No. 161, of July 1, 2022. Establishes the lists of microbiological standards for foods. *Federal Official Gazette*.

Ahmed, M. J. P. T., Pickova, J., Ahmad, T., Liaquat, M., Farid, A., & Jahangir, M. (2016). Oxidation of lipids in foods. *Sarhad Journal of Agriculture*, *32*(3), 230-238. https://doi.org/10.17582/journal.sja/2016.32.3.230.238

Ali, F., Abdel-Atty, N., & Helmy, E. (2018). Improving the quality and extending the shelf life of chilled fresh sausages using natural additives and their extracts. *Journal of Microbiology, Biotechnology and Food Sciences*, *7*(6), 580-585. https://doi.org/10.15414/jmbfs.2018.7.6.580-585

Akhter, M. S., Khatun, H., Hashem, M. A., Rahman, M. M., & Khan, M. (2022). Effect of storage periods on the quality and shelf life of beef liver at refrigerated temperature. *Meat Research*, *2*(2), 1-5. https://doi.org/10.55002/mr.2.2.17

American Oil Chemistry Society (1993). *Determination of 2-Thiobarbituric acid value*. Cd 19-90. American Oil Chemistry Society.

Arguelho, J. F., Ponticelli, L. S., Lima, C. S., & Castilho, M. A. D. (2023). The Gastronomic Tourism present in the Bioceanic Route in Mato Grosso do Sul. *Interações*, *24*(4), e2444211. https://doi.org/10.20435/inter.v24i4.4211

Assis, D. C. S., da Silva, T. M. L., Brito, R. F., da Silva, L. C. G., Lima, W. G., & Brito, J. C. M. (2021). Shiga toxin-producing *Escherichia coli* (STEC) in bovine meat and meat products over the last 15 years in Brazil: A systematic review and meta-analysis. *Meat Science*, *173*, 108394. https://doi.org/10.1016/j.meatsci.2020.108394

Associação Brasileira de Normas Técnicas (ABNT) (2015). *ABNT NBR ISO 4833-1:2015*. Microbiology of the food production chain - Horizontal method for the enumeration of microorganisms. Part 1: Colony counts at 30°C by the pour plate technique. ABNT.

Association of Official Analytical Chemists (AOAC) (2006). *Official Methods of Analysis* (18th Ed.). AOAC International.

Association of Official Analytical Chemists (AOAC) (2016). *Official methods of analysis* (20th ed.). AOAC International.

Association of Official Analytical Chemists (AOAC) (2019). *Official methods of analysis*. AOAC International.

Atlabachew, T., & Mamo, J. (2021). Microbiological quality of meat and swabs from contact surface in butcher shops in Debre Berhan, Ethiopia. *Journal of Food Quality*, *2021*(1), 7520882. https://doi.org/10.1155/2021/7520882

Atwater, W. O., & Woods, C. D. (1896). The chemical composition of American Food Materials. Department of Agriculture; Office of Experiment Stations. Bulletin No. 28.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911-917. https://doi.org/10.1139/o59-099

Brasil (2000). Ministry of Agriculture and Supply. Secretariat of Agricultural Defense. Department for the Inspection of Products of Animal Origin. *Technical regulation on the identity and quality of sausages*. Ministry of Agriculture and Supply.

Cierach, M., & Niedźwiedź, J. (2014). Effects of three lighting intensities during display on discoloration of beef semitendinosus muscle. *European Food Research and Technology*, *239*, 377-383. https://doi.org/10.1007/s00217-014-2231-y

Coombs, C. E., Holman, B. W., Friend, M. A., & Hopkins, D. L. (2017). Long-term red meat preservation using chilled and frozen storage combinations: A review. *Meat Science*, *125*, 84-94. https://doi.org/10.1016/j.meatsci.2016.11.025

Cunningham, J., Nguyen, V., Adorno, P., & Droulez, V. (2015). Nutrient composition of retail samples of Australian beef sausages. *Nutrients*, *7*(11), 9602-9617. https://doi.org/10.3390/nu7115491

Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants*, *8*(10), 429. https://doi.org/10.3390/antiox8100429

Ebied, A. S., Morshdy, A. E. M., Abd-El-Salam, E., Hussein, M. A., & Elewa, E. S. (2017). Effect of pomegranate peel powder on the hygienic quality of beef sausage. *Journal of Microbiology, Biotechnology and Food Sciences*, *6*(6), 1300-1304. https://doi.org/10.15414/jmbfs.2017.6.6.1300-1304

Hugo, C. J., & Hugo, A. (2015). Current trends in natural preservatives for fresh sausage products. *Trends in Food Science & Technology*, *45*(1), 12-23. https://doi.org/10.1016/j.tifs.2015.05.003

Instituto Adolfo Lutz (2008). Chemical and physical methods for food analysis. Instituto Adolfo Lutz. Retrieved from http://www.ial.sp.gov.br/resources/editorinplace/ial/2016_3_19/analisedealimentosial_2008.pdf

ISO (2017). *ISO 3960*. Animal and vegetable fats and oils - Determination of peroxide value - Iodometric (visual) endpoint determination. ISO.

Ježek, F., Kameník, J., Macharáčková, B., Bogdanovičová, K., & Bednář, J. (2020). Cooking of meat: effect on texture, cooking loss and microbiological quality—a review. *Acta Veterinaria Brno*, *88*(4), 487-496. https://doi.org/10.2754/avb201988040487

Kavuşan, H. S., Serdaroğlu, M., Nacak, B., & İpek, G. (2020). An approach to manufacture of fresh chicken sausages incorporated with black cumin and flaxseed oil in water gelled emulsion. *Food Science of Animal Resources*, *40*(3), 426-443. https://doi.org/10.5851/kosfa.2020.e23

King, D. A., Shackelford, S. D., Kalchayanand, N., & Wheeler, T. L. (2012). Sampling and aging effects on beef longissimus color stability measurements. *Journal of Animal Science*, *90*(10), 3596-3605. https://doi.org/10.2527/jas.2011-4871

Koziol, K., Maj, D., & Bieniek, J. (2015). Changes in the color and pH of rabbit meat in the aging process. *Medycyna Weterynaryjna/Veterinary Medicine-Science and Practice*, *71*(2), 104-108.

Leygonie, C., Britz, T. J., & Hoffman, L. C. (2012). Impact of freezing and thawing on the quality of meat: Review. *Meat Science*, *91*(2), 93-98. https://doi.org/10.1016/j.meatsci.2012.01.013

Lu, N., Ma, J., & Sun, D. W. (2022). Enhancing physical and chemical quality attributes of frozen meat and meat products: Mechanisms, techniques and applications. *Trends in Food Science & Technology*, *124*, 63-85. https://doi.org/10.1016/j.tifs.2022.04.004

Mehta, B. M., Darji, V. B., & Aparnathi, K. D. (2015). Comparison of five analytical methods for the determination of peroxide value in oxidized ghee. *Food Chemistry*, *185*, 449-453. https://doi.org/10.1016/j.foodchem.2015.04.023

Nacak, B., Kavuşan, H. S., & Serdaroğlu, M. (2021). Effect of α-tocopherol, rosemary extract and their combination on lipid and protein oxidation in beef sausages. In *IOP Conference Series: Earth and Environmental Science* (Vol. 854, No. 1, p. 012062). IOP Publishing. https://doi.org/10.1088/1755-1315/854/1/012062

Novaković, S., & Tomašević, I. (2017). A comparison between Warner-Bratzler shear force measurement and texture profile analysis of meat and meat products: A review. In *IOP Conference Series: Earth and Environmental Science* (Vol. 85, No. 1, p. 012063). IOP Publishing. https://doi.org/10.1088/1755-1315/85/1/012063

Pematilleke, N., Kaur, M., Adhikari, B., & Torley, P. J. (2021). Relationship between masticatory variables and bolus characteristics of meat with different textures. *Journal of Texture Studies*, *52*(5-6), 552-560. https://doi.org/10.1111/jtxs.12629

Pinheiro, R. S., Francisco, C. L., Lino, D. M., & Borba, H. (2019). Meat quality of Santa Inês lamb chilled-then-frozen storage up to 12 months. *Meat Science*, *148*, 72-78. https://doi.org/10.1016/j.meatsci.2018.09.017

Qian, S., Li, X., Wang, H., Sun, Z., Zhang, C., Guan, W., & Blecker, C. (2018). Effect of sub-freezing storage (-6, -9 and -12°C) on quality and shelf life of beef. *International Journal of Food Science & Technology*, *53*(9), 2129-2140. https://doi.org/10.1111/ijfs.13800

Rincon-Gamboa, S. M., Poutou-Pinales, R. A., & Carrascal-Camacho, A. K. (2021). Antimicrobial resistance of Non-Typhoid Salmonella in meat and meat products. *Foods*, *10*(8), 1731. https://doi.org/10.3390/foods10081731

Salueña, B. H., Gamasa, C. S., Rubial, J. M. D., & Odriozola, C. A. (2019). CIELAB color paths during meat shelf life. *Meat Science*, *157*, 107889. https://doi.org/10.1016/j.meatsci.2019.107889

Serhan, M., Hourieh, H., El Deghel, M., & Serhan, C. (2024). Hygienic sanitary risk and microbiological quality of meat and meat-contact surfaces in traditional butcher shops and retail establishments-lessons from a developing country. *International Journal of Environmental Health Research*, *34*(1), 600-610. https://doi.org/10.1080/09603123.2022.2161487

Shahidi, F. (2016). Oxidative stability and shelf life of meat and meat products. In *Oxidative stability and shelf life of foods containing oils and fats* (pp. 373-389). AOCS Press. https://doi.org/10.1016/B978-1-63067-056-6.00010-0

Silva, D. J., & Queiroz, A. C. (2002). *Food analysis: chemical and biological methods*. UFV.

Silva, I. F., de Rezende-Lago, N. C. M., de Marchi, P. G. F., Messias, C. T., & Silva, L. A. (2023). *Microbiological quality of food*. Seven. Retrieved from https://sevenpublicacoes.com.br/editora/article/view/320

Sun, Q., Sun, F., Xia, X., Xu, H., & Kong, B. (2019). The comparison of ultrasound-assisted immersion freezing, air freezing and immersion freezing on the muscle quality and physicochemical properties of common carp (Cyprinus carpio) during freezing storage. *Ultrasonics Sonochemistry*, *51*, 281-291. https://doi.org/10.1016/j.ultsonch.2018.10.006

Suna, B., & Alvarez, M. D. (2021). The role of gastronomy in shaping the destination's brand identity: an empirical analysis based on stakeholders' opinions. *Journal of Hospitality Marketing & Management*, *30*(6), 738-758. https://doi.org/10.1080/19368623.2021.1877587

Warner, R. D. (2023). The eating quality of meat: IV-Water holding capacity and juiciness. In *Lawrie's meat science* (pp. 457-508). Woodhead Publishing. https://doi.org/10.1016/B978-0-323-85408-5.00008-X

Xie, Y., Zhou, K., Chen, B., Wang, Y., Nie, W., Wu, S., Wang, W., Li, P., & Xu, B. (2021). Applying low voltage electrostatic field in the freezing process of beef steak reduced the loss of juiciness and textural properties. *Innovative Food Science & Emerging Technologies*, *68*, 102600. https://doi.org/10.1016/j.ifset.2021.102600

You, S., Yang, S., Li, L., Zheng, B., Zhang, Y., & Zeng, H. (2022). Processing technology and quality change during storage of fish sausages with textured soy protein. *Foods*, *11*(22), 3546. https://doi.org/10.3390/foods11223546

Zhai, Y., Huang, J., Khan, I. A., Guo, Y., Huang, M., & Zhou, G. (2018). Shelf-life of boiled salted duck meat stored under normal and modified atmosphere. *Journal of Food Science*, *83*(1), 147-152. https://doi.org/10.1111/1750-3841.13947

Zhao, B., Zhou, H., Zhang, S., Pan, X., Li, S., Zhu, N., Wu, Q., Wang, S., Qiao, X., & Chen, W. (2020). Changes of protein oxidation, lipid oxidation and lipolysis in Chinese dry sausage with different sodium chloride curing salt content. *Food Science and Human Wellness*, *9*(4), 328-337. https://doi.org/10.1016/j.fshw.2020.04.013