



Chemical-physical characteristics of buffalo (*Bubalus bubalis*) meat subjected to different aging times

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ABSTRACT. The aim of this study was to evaluate the effects of different aging times on the meat characteristics from young Murrah buffaloes slaughtered at 20–24 months of age (experiment I; n=10) and Murrah buffalo heifers slaughtered at 32–36 months of age (experiment II; n=10), with the purpose of determining the best aging time to tenderize meat from both experiments. Samples of the *longissimus thoracis* muscle from buffaloes slaughtered at each age were aged for 7, 14, and 21 days ($0 \pm 1^\circ\text{C}$). After this period, analysis of cholesterol, pH, cooking loss, shear force, myofibrillar fragmentation index, meat color, and fatty acid profile was performed. Aging greatly improved the tenderness ($p < 0.05$) but caused a change in color ($p < 0.05$), which, even without difference between the treatments for subjective perception of the color (ΔE ; $p > 0.05$), was considered very noticeable to the human eye. Furthermore, aging reduced the polyunsaturated fatty acids ($p < 0.05$) and did not affect the levels of cholesterol ($p > 0.05$) in the meat of young buffalo and buffalo heifers of advanced age. Based on the evaluated meat characteristics, concludes that seven days of aging are sufficient for older buffalo heifers, but 21 days of aging are required for the younger male group.

Keywords: cholesterol, fatty acids, *longissimus thoracis*, Murrah, tenderness.

Características químico-físicas da carne bubalina (*Bubalus bubalis*) submetida a diferentes tempos de maturação

RESUMO. Objetivou-se com o estudo avaliar os efeitos de diferentes tempos de maturação sobre as características da carne de búfalos Murrah jovens abatidos com 20-24 meses de idade (experimento I; n=10) e novilhas bubalinas Murrah abatidas com 32-36 meses de idade (experimento II; n=10), com a finalidade de determinar o melhor tempo de maturação para ambos os experimentos. As amostras do músculo *longissimus thoracis* foram maturadas durante 7, 14 e 21 dias ($0 \pm 1^\circ\text{C}$). Após esse período, foi realizada análise de colesterol, pH, perdas de peso por cocção, força de cisalhamento, índice de fragmentação miofibrilar, cor da carne e perfil de ácidos graxos. A maturação melhorou a maciez ($p < 0,05$), no entanto, modificou a cor da carne ($p < 0,05$), a qual, mesmo sem diferença entre os tratamentos para percepção subjetiva da cor (ΔE ; $p > 0,05$), foi considerada como muito perceptível ao olho humano. Além disso, a maturação reduziu os ácidos graxos polinsaturados ($p < 0,05$) e não afetou os níveis de colesterol ($p > 0,05$) na carne de búfalos jovens e novilhas bubalinas com idade avançada. Com base nas características avaliadas, conclui-se que sete dias de maturação são suficientes para as novilhas bubalinas mais velhas, mas 21 dias de maturação são necessários para o grupo de búfalos mais jovens.

Palavras-chave: colesterol, ácidos graxos, *longissimus thoracis*, Murrah, maciez.

Introduction

The world population of buffaloes is ~198.9 million head, with most being located in developing countries, especially India, Pakistan and China. Brazil is number one buffalo producer in the Western hemisphere, with a herd of approximately 1.2 million heads (Ministério da Agricultura, Pecuária

e Abastecimento [MAPA], 2016). Their carcass provide a higher yield of round cuts than cattle carcass, thereby supplying the industry with a higher production volume of primal cuts with high commercial value (Mello et al., 2017). Buffalo meat production may be a promising market, with potentially be preferred by consumers due to its

lipid composition (e.g. lower cholesterol and saturated fatty acids), nutritive properties (e.g. iron) and excellent palatability attributes (e.g. more tender, flavorful and juicy) (Giordano et al., 2010; Giuffrida-Mendoza et al., 2015; Huerta-Leidenz, Rodas-González, Vidal, Lopez-Nuñez, & Colina, 2016). Furthermore, buffalo meat is a rich source of hypocholesterolemic fatty acids, which help prevent cardiovascular diseases (Mello et al., 2017). However, the trade of buffalo meat is little disseminated and very restricted in Brazil, being ~90% of buffalo meat is marketed as beef (Corrêa & Tramoso 2002; Andrighetto et al., 2008). Moreover, because of their great milk aptitude is common the late slaughter of cows or heifers this species, compromising the sale of their meat due to low quality (Mello et al., 2017).

Aging is presented as an alternative to better the quality of meat from buffalo (Irueta, Cadoppi, Langman, Grigioni, & Carduza, 2008). However, few studies have evaluated this technique for this species and, for the most part, have focused instead on the processes of protein degradation (Naveena, Mendiratta, & Anjaneyulu, 2004; Neath et al., 2007), and an ideal point has not been established for young and old animals. Thus, the aim of this study was to evaluate the effects of different aging times on the characteristics of meat from young Murrah buffaloes (20 and 24 months of age), and Murrah buffalo heifers (32 and 36 months of age), with the main purpose of determine the best aging time to tenderize meat from both buffalo ages.

Material and methods

These experiments (experiment I and II) were carried out in accordance with the ethical principles of animal experimentation (Protocol 27/2012) determined by the institution's Ethics Committee on Animal Use (CEUA).

Animals, diets and slaughtering

Experiment I: Ten non-castrated water buffaloes Murrah male from a commercial farm located at Bocaina, São Paulo, Brazil (22°8'4"S, 48°30'58"W, 564 m altitude) were used. Young buffaloes were confined (initial live weight equal to 360 ± 9.05 kg) during 100 days. The diet offered during the finishing had a roughage to concentrate ratio at 50:50 on a dry matter basis, with sugar cane as the roughage. The concentrate supplement provided to the animals contained 30% citrus pulp, 25% mineral salt, 18% soy hulls, 15% corn and 12% mineral mixture, on a dry matter basis. At the end of

the finishing period, the animals were weighed (482 ± 9.01 kg) and slaughtered (20 to 24 months of age, on average) in a commercial slaughterhouse.

Experiment II: Ten water buffalo Murrah heifers, at 32 to 36 months of age, on average, from a commercial farm, located at Pompéia, São Paulo, Brazil (22°06'23"S, 50°10'29"W, 583 m altitude) were used. Murrah buffalo heifers were purchased from a dairy herd, wherein were raised exclusively on pasture (*Urochloa brizantha*, cv. marandu) and transported to a commercial slaughterhouse. These animals were not weighed, thus, no carcass yield was obtained.

Sample collection

The animals (young buffaloes and buffalo heifers) were slaughtered and the half-carcasses remained in the cold storage chamber (0 ± 2°C) for 24 hours for the establishment of *rigor mortis*. After 24 hours in the cold storage chamber, the lefts half-carcasses were sectioned between the 9th and 13th thoracic vertebra of the *longissimus thoracis* (LT) muscle. Samples were transported to the Laboratory of Bromatology of the College of Animal Science at UNESP, Dracena Campus, Sao Paulo, Brazil (21°29'0"S, 51°32'1"W, 419 m altitude).

Subsamples of the LT muscle with approximately 2.5 cm in thick were obtained of each animal using a band saw (model 255, Beccaro, Toronto, ON, Canada) as follows : the section between the 9th and 10th thoracic vertebrae was used to fatty acid profiles not aged (0 days) and aged (7, 14 and 21 days); 10th and 11th thoracic vertebrae was used to myofibrillar fragmentation index (MFI) not aged (0 days) and aged (7, 14 and 21 days); the 11th and 12th thoracic vertebrae section was used to cholesterol analysis not aged (0 days) and aged (7, 14 and 21 days); the 12th and 13th thoracic vertebrae were used to hydrogen potential (pH), cooking loss, Warner-Bratzler shear force (WBSF) and muscle color not aged (0 days) and aged (7, 14 and 21 days).

Each subsamples was individually vacuum-packaged in plastic package (18 µ) using a JETVAC® sealer (200-B, Selovac, SP, Brazil) and subsequently aged in an incubator (TE-371, Tecnal, SP, Brazil) at 0°C ± 1°C for 7, 14, and 21 days of aging and the subsamples not aged were frozen (-20°C). After aging, subsamples from each sampling time were frozen (-20°C) for 30 days for further analysis.

Chemical analysis

Analysis of fatty acid profiles was conducted using the method described by Bligh and Dyer (1959) for total lipids, using chloroform, methanol

and water (2:2:1.8). Conversion to fatty acid methyl esters was performed according to the methodology used by Bannon et al. (1982). On a threaded cap tube with approximately 150 mg of lipid 0.25 mol.L^{-1} ; 5.0 mL of sodium methoxide solution in methanol/diethyl ether (1:1) was added. The tube was shaken vigorously for about 3 min. Then, 3.0 mL of isooctane and 15 mL of saturated sodium chloride were added. The tube was shaken vigorously again and rested for phase separation, then the supernatant was collected in Eppendorf tubes labeled for chromatographic analysis. For further chromatographic analysis, according to the method described by Visentainer (2012), the fatty acid methyl esters (FAME) were separated in a gas chromatograph (Model 3300, Thermo Scientific, DE, USA), equipped with a flame ionization detector, automatic injector and a fused silica capillary column CP- 7420 SELECT FAME (100 m; 0.25 mm and $0.39 \mu\text{m}$). The column injector and temperatures were 230°C and 240°C , respectively. Initially, the column temperature was maintained at 165°C for 18 min. Then, it was raised to 235°C at a rate of 4°C min^{-1} . The total chromatographic run time was 32.5 min. The flow rates for the carrier (H_2), auxiliary (N_2) and flame detector (H_2 and synthetic air) gases were 1.2, 30, 30 and 300 mL min^{-1} , respectively the splitting ratio was 1/80. For identification, the FAME retention times were compared to standards from Sigma - Aldrich (Sigma, St. Louis, MO). Retention times and peak area percentages were processed automatically using Chromquest 5.0 software.

Cholesterol analysis was performed according to the enzymatic method described by Saldanha, Mazalli and Bragagnolo (2004). The isolation of lipids was conducted using 50% potassium hydroxide, ethanol and hexane. An aliquot of 3 mL of the hexane extract was dried under N_2 , then isopropanol was added until complete solubilization. To measure cholesterol quantities, a laboratory kit Laborlab S/A was used, consisting of two color reagents (1) containing 0.025 mol L^{-1} of 4-aminophenazone; 2) containing 0.055 mol/L of phenol), plus the enzyme reagent (3 U mol^{-1} cholesterol-oxidase, 20 U mL^{-1} POD and 300 U mol^{-1} lipase). The working reagent was prepared by combining 0.5 mL of each of the two color reagents, 19 mL of distilled water and 0.4 mL of the enzyme reagent. Thereafter, 3mL was added to the samples of the working reagent and they were heated for 10 minutes at 37°C in a water bath. After 90 min rest, their absorbance was determined on a spectrophotometer at 499 nm. The calibration curve was constructed from a cholesterol standard

solution, with concentrations that varied from 0.01 to 0.05 mg mL^{-1} .

Physical analyses

The hydrogenation potential (pH) was measured in each subsample using not aged (24h *post mortem*) and aged (7, 14 and 21 days) using a pH meter (HI 99163, Hanna Instruments, Woonsocket, RI, USA) with a penetration electrode (Béltran et al., 1997).

For determination of the cooking loss, each subsample ($12.24 \pm 0.89 \text{ cm}$ width, $4.74 \pm 0.89 \text{ cm}$ length, 2.54 cm of thick and weighing $112.25 \pm 30.83 \text{ g}$), was weighed and placed on an electric hot plate that had been preheated to 170°C , until the temperature of the geometric center of each sample reached 72°C , which was verified using a digital thermometer (Skewer type model, Incoterm, RS, Brazil). The sample was then removed from the electric hot plate (Grill Mega 2, Britania, SC, Brazil) and cooled in room environmental temperature at 25°C , which was verified using an infrared thermometer (Mult Temp, Incoterm, RS, Brazil). The subsample was then reweighed to determine the cooking loss, which was calculated from the variation in subsample weight before and after being subjected to heat treatment, expressed as percentage. Then, from these subsamples was performed the Warner-Bratzler shear force (American Meat Science Association [AMSA], 1995).

Tenderness was determined by Warner-Bratzler shear force (WBSF) and the myofibrillar fragmentation index (MFI). Ten core with a diameter of 1.27 cm were obtained from each roasted sample ($12.24 \pm 0.89 \text{ cm}$ width, $4.74 \pm 0.89 \text{ cm}$ length, 2.54 cm of thick and weighing $112.25 \pm 30.83 \text{ g}$) of the LT muscle. They were placed with the fibers oriented perpendicular to a Warner-Bratzler shear force device coupled with a texture analyzer (TA-SBA CT3, Brookfield, USA) to determine WBSF (AMSA, 1995).

Myofibrillar fragmentation index was carried out according to the procedures by Culler, Parrish, Smith, Smith and Cross (1978). Samples used were free of fat and excess connective tissue. The protein concentration of the myofibril solution was determined by the biuret method (Gornall, Bardawill, & Maxima, 1949). Dilution (0.5 mg mL^{-1}) in a final volume of 8 mL was used for determine the MIF. Absorbance at 540 nm was measured using a digital spectrophotometer.

Meat color was determined by reading at three different points in the LT muscle, using a Minolta CR-400 colourimeter (Konica Minolta Sensing, Inc., Osaka, Japan). The CIELAB system considers reflectance readings of light in three dimensions: L^*

(lightness), a^* (redness) and b^* (yellowness), according to the methodology described by Honikel (1998). To complement the color parameters of the meat using the coordinates L , a^* , and b^* , the was determined the perception of color (ΔE) according to MacDougal (1994), using the following formula: $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{0.5}$. The ΔE index allows to evaluate the differences in color over time, that is, between the initial evaluation time and the measurement times after opening the package, identifying if the changes were perceptible to the human eye.

Statistical analysis

Experiment I: Ten samples of *longissimus thoracis* muscle from young buffaloes slaughtered at 20-24 months were analyzed using a completely randomized design, with four treatments and 10 replicates per treatment, defined in a split-plot design, with plots assigned according to animals and subplots according to aging time.

Experiment II: Ten samples of *longissimus thoracis* muscle from buffalo heifers slaughtered at 32-36 months of age were analyzed using a completely randomized design, with four treatments and 10 replicates per treatment, defined in a split-plot design, with plots assigned according to animals and subplots according to aging time.

The data were submitted to the Univariate Normal procedure (Statistical Analysis System [SAS], 2010), and the normality of the data was confirmed by the Shapiro-Wilk test ($W \geq 0.90$). Orthogonal polynomial contrast statements were used to test linear and quadratic effects of different aging times in experiments I and II separately using the statistical program SAS 9.3 (SAS, 2010). Differences were considered significant when $p < 0.05$.

Results and discussion

Experiment I

Aging does not affect cholesterol levels in the meat of young buffalo ($p > 0.05$; Table 1). This is in contrast to other studies that have found a reduction in cholesterol levels due to aging of the meat (Souza, Arthur, & Canniatti-Brazaca, 2007; Mello et al., 2016). This decrease was probably caused by cholesterol oxidation in other types of fat.

The average pH values of LT muscles subjected to different aging times fit a quadratic regression ($p = 0.03$; Table 1). Previous studies explain this relationship by enzymatic attack during aging (Oliveira, Soares, & Antunes, 1998). This is a process that increases the osmotic pressure of the medium as a result of protein degradation to smaller molecules, and intramolecular reorganization of

these proteins, which determines changes in their electrical charges (Lawrie, 1977), therefore increasing the pH (Oliveira et al., 1998). In the present study, when the enzyme activity decreased (between 14 and 21 days of aging), evidenced by the reduction of myofibrillar degradation in this period (MFI), the pH also decreased (Table 1).

Furthermore, studies with beef cattle, a species which most resembles the buffalo, show that values of pH were higher than observed for meats aged of Nellore and Red Norte (Andrade et al., 2010). We suggest that the higher pH values found in our study reflect better proteolytic enzyme activity and, ultimately, a greater reduction in WBSF (Béltran et al., 1997). The values calculated in our analyses can also explain the lack of differences ($p > 0.05$; Table 1) in cooking loss between the different aging times, since, when the opposite occurs (i.e., the pH is low at less than 5.4, according to Terlouw et al., 2008), the meat suffers high weight losses from cooking because the pH is far from the isoelectric point of muscle proteins (Huff-Lonergan & Lonergan, 2005).

We observed a linear reduction in WBSF with aging time ($p < 0.01$; Table 1). Thus, between 0 and 7 days, 7 and 14 days, and between 14 and 21 days, the WBSF fall by 11.28, 15.50, and 10.49 N , respectively. Previous work with beef cattle showed that meat reached an optimal WBSF of 29.81 N within 21 days (Andrade et al., 2010). Similarly, another previous study with buffalo meat indicated that at 0, 15, and 25 days of aging resulted in WBSF of 33.45, 24.91, and 22.95 N , respectively (Irueta et al., 2008). Although with similar behavior, buffalo meat in the present study is classified as less soft compared to those mentioned above, and only 21 days of aging approaches Latin American customer satisfaction, which is obtained when the WBSF is around 40.13 N (Rodas-González, Huerta-Leidenz, Jerez-Timaure, & Miller, 2009). Also, average values of the myofibrillar fragmentation index increased linearly with aging time ($p < 0.01$; Table 1). These results are consistent with those previously reported showing that 21-day aged meat of Race Red Norte and Nellore animals had a higher myofibrillar fragmentation index of 84.01% in comparison to an index of 60.18% of meat that did not aged (Andrade et al., 2010).

In relation to the color of the aged meat, our analyses revealed that aging affected average values of L^* and a^* ($p < 0.01$), but did not alter average values of b^* ($p > 0.05$; Table 1). The L^* values increased linearly with aging. These data corroborate those found by Andrade et al. (2010), who explained the increase in lightness as an effect of vacuum packaging, which draws water containing myoglobin from intra- and extracellular spaces in the muscle fibers. The a^* values

correlates with the muscle myoglobin content (Sañudo et al., 1997). The instability of redness can be attributed to the greater amount of polyunsaturated fatty acids (PUFAs) present in buffalo meat, which may be more oxidizing, especially when these PUFAs are not derived from pastures, since they are not associated with more antioxidants in the form of alpha-tocopherol, carotenoids, and flavonoids, which stabilize the fatty acid (Scollan et al., 2006).

We also observed changes in color (ΔE) in the first seven days of aging that would be perceptible to the human eye, and after seven days, considered very evident (Prändl, Fischer, Schmidhofer, & Sinell, 1994). However, longer aging periods did not induce further significant changes to ΔE ($p = 0.11$; Fig. 1). In contrast, previous work with beef found that the greatest change in ΔE (an almost 4-fold increase) occurred between days 7 and 14 of aging (Andrade et al., 2010). This difference may indicate that buffalo meat can be subjected to aging with less noticeable changes to appearance than those observed for beef.

The linoleic fatty acid (C18:2 ω 6) content of meat decreased linearly with aging time. Moreover, because this fatty acid accounts for the majority of omega-6 fatty acids, the category also followed a linear decrease ($p = 0.04$). We also observed a tendency of linear reduction ($p = 0.07$) of the alpha-linolenic acid (C18:3 ω 3). Since both are the most representative of the PUFAs, this also presented the same variation ($p < 0.01$; Table 2). This fact can be explained by lipid oxidation during the aging period. While saturated fatty acids (SFAs) are more resistant to the oxidation, the PUFAs are more sensitive and may oxidize at the temperature of the aging meat (Geay, Bauchart, Hocquette, & Culioli, 2001).

Importantly, the biological effect of essential fatty acids depends on the ratio between acids of the families' ω 6 and ω 3 present in the phospholipids that form the membranes of the meat. The Japan Society of Lipid Nutrition recommends that the

ratio of ω 6: ω 3 should be 4:1 for healthy adults and 2:1 for the prevention of chronic diseases in the elderly (Uauy, Mena, & Valenzuela, 1999). In the present study, the proportion of polyunsaturated fatty acids of the ω 6 and ω 3 families was found to be below the recommended values in both experiments ($p > 0.05$; Table 2).

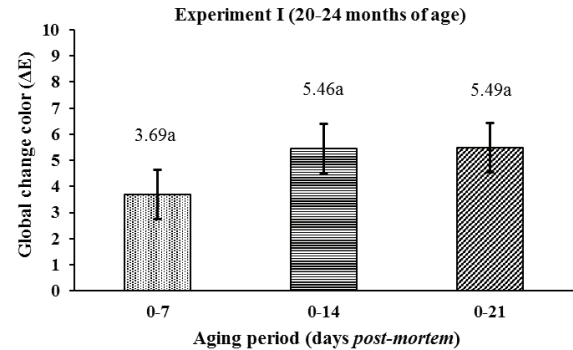


Figure 1. Subjective perception of color (ΔE_{0-7} , ΔE_{0-14} and ΔE_{0-21}) in aged samples of *longissimus thoracis* muscle from young buffaloes slaughtered at 20-24 months of age ($p = 0.11$, SE = 0.95).

Experiment II

Aging also does not affect cholesterol levels in the meat of the buffalo heifers with advanced age ($p > 0.05$; Table 3). It is important to note that the amount of cholesterol found in heifer meat (experiment II) is higher compared to non-castrated male meat (experiment I) and, this may be related to the age of the animals, because younger animals would have lower cholesterol content in their meat (Madruga et al., 2002).

The average pH values of LT muscles subjected to different aging times also presented a quadratic regression ($p < 0.01$; Table 3). We suggest the same justification of the experiment I. However, the lower enzymatic activity in the present experiment is reported between 7 and 14 days of aging, evidenced by the reduction of myofibrillar degradation in this period (MFI), the pH also decreased (Table 2).

Table 1. The effects of different aging times on the cholesterol levels (mg 100g⁻¹), pH, cooking loss (CL) (%), shear force (WBSF) (N), myofibrillar fragmentation index (MFI) (%) and color (L^* , a^* , b^*) of *longissimus thoracis* muscle from young buffaloes slaughtered at 20-24 months, (LSM \pm SE^a).

Trait	Experiment I (20-24 months of age)				P-value for regression ^c	
	Aging period (days post-mortem)				Linear	Quadratic
	0	7	14	21		
Cholesterol ^c	48.50 \pm 2.13	57.42 \pm 1.20	43.71 \pm 3.83	49.42 \pm 2.74	0.399	0.640
pH	5.64 \pm 0.03	5.66 \pm 0.02	5.77 \pm 0.03	5.66 \pm 0.03	0.256	0.030 ¹
CL (%)	27.57 \pm 1.19	26.44 \pm 1.01	26.58 \pm 1.21	25.19 \pm 1.55	0.173	0.923
WBSF (N)	85.91 \pm 0.42	74.63 \pm 0.44	59.13 \pm 0.37	48.64 \pm 0.38	0.001 ²	0.450
MFI (%)	62.93 \pm 6.16	81.92 \pm 5.00	100.04 \pm 3.41	117.82 \pm 3.15	0.001 ³	0.303
L^*	32.08 \pm 0.64	34.31 \pm 0.48	35.40 \pm 0.53	36.72 \pm 0.54	0.001 ⁴	0.149
a^*	16.35 \pm 0.34	15.24 \pm 0.51	12.97 \pm 0.68	15.61 \pm 0.70	0.165	0.003 ⁵
b^*	7.28 \pm 0.24	7.40 \pm 0.27	6.86 \pm 0.27	7.93 \pm 0.31	0.265	0.091

L^* = Lightness; a^* = Redness; b^* = Yellowness. ^aLeast square means \pm Standard error considered significant when $p < 0.05$. ^cFitting a simple linear or logarithmic regression to aging times. Experiment I: $Y^1 = -0.0006x^2 + 0.0153x + 5,6299$ ($R^2 = 0.55$); $Y^2 = -0.1854x + 8.7893$ ($R^2 = 0.99$); $Y^3 = 2.468x + 62.25$ ($R^2 = 0.96$); $Y^4 = 0.2147x + 32.372$ ($R^2 = 0.97$); $Y^5 = 0.0192x^2 - 0.4664x + 16.654$ ($R^2 = 0.71$).

Table 2. The effects of different aging times on the fatty acid profile (%) of *longissimus thoracis* muscle from young buffaloes slaughtered at 20-24 months of age, (LSM \pm SE^A).

Fatty acid (%)	Experiment I (20-24 months of age)				P-value for regression ^B	
	Aging period (days <i>post-mortem</i>)				Linear	Quadratic
	0	7	14	21		
C14:0	0.05 \pm 0.02	0.03 \pm 0.01	0.03 \pm 0.01	0.03 \pm 0.01	0.251	0.279
C14:1	1.78 \pm 0.15	1.65 \pm 0.07	1.72 \pm 0.01	1.69 \pm 0.04	0.647	0.580
C15:0	0.19 \pm 0.01	0.16 \pm 0.01	0.17 \pm 0.01	0.17 \pm 0.01	0.320	0.353
C15:1	0.23 \pm 0.03	0.20 \pm 0.02	0.22 \pm 0.01	0.22 \pm 0.02	0.928	0.501
C16:0	27.23 \pm 1.07	28.28 \pm 0.53	28.08 \pm 0.49	28.59 \pm 0.40	0.205	0.685
C16:1	0.21 \pm 0.03	0.21 \pm 0.01	0.21 \pm 0.02	0.19 \pm 0.01	0.468	0.669
C17:0	0.49 \pm 0.04	0.45 \pm 0.02	0.47 \pm 0.01	0.43 \pm 0.02	0.133	0.993
C17:1 ω 9	1.03 \pm 0.03	1.02 \pm 0.04	1.01 \pm 0.01	0.97 \pm 0.03	0.114	0.616
C18:0	33.68 \pm 1.29	33.01 \pm 0.41	31.93 \pm 0.76	32.53 \pm 0.72	0.246	0.420
C18:1 ω 9c9	30.71 \pm 2.12	30.15 \pm 0.60	31.17 \pm 1.00	30.32 \pm 1.19	0.986	0.891
C18:1 ω 7	0.41 \pm 0.01	0.44 \pm 0.02	0.43 \pm 0.01	0.40 \pm 0.01	0.460	0.055
C18:2 ω 6	0.57 \pm 0.28	0.06 \pm 0.01	0.06 \pm 0.01	0.05 \pm 0.01	0.044 ¹	0.093
C18:3 ω 6	0.10 \pm 0.01	0.08 \pm 0.01	0.08 \pm 0.01	0.08 \pm 0.01	0.132	0.336
C18:3 ω 3	2.37 \pm 0.29	2.24 \pm 0.25	2.20 \pm 0.09	2.02 \pm 0.10	0.078 ²	0.417
C20:0	0.10 \pm 0.01	0.08 \pm 0.01	0.08 \pm 0.01	0.09 \pm 0.02	0.709	0.055
C20:1 ω 9	0.10 \pm 0.01	0.09 \pm 0.01	0.12 \pm 0.01	0.12 \pm 0.01	0.120	0.973
C20:4 ω 6	0.07 \pm 0.01	0.07 \pm 0.02	0.05 \pm 0.01	0.05 \pm 0.01	0.140	0.919
C22:0	0.18 \pm 0.02	0.24 \pm 0.06	0.10 \pm 0.02	0.15 \pm 0.03	0.205	0.927
C22:6 ω 3	0.04 \pm 0.01	0.03 \pm 0.01	0.04 \pm 0.01	0.03 \pm 0.01	0.703	0.709
C24:1 ω 9	0.04 \pm 0.01	0.05 \pm 0.02	0.05 \pm 0.01	0.05 \pm 0.01	0.577	0.315
ω 3	2.41 \pm 0.29	2.27 \pm 0.25	2.24 \pm 0.09	2.05 \pm 0.10	0.101	0.399
ω 6	0.73 \pm 0.30	0.21 \pm 0.02	0.19 \pm 0.01	0.19 \pm 0.02	0.049 ³	0.112
MUFA	34.51 \pm 2.03	33.81 \pm 0.51	34.93 \pm 1.03	33.96 \pm 1.10	0.935	0.885
PUFA	3.15 \pm 0.42	2.48 \pm 0.26	2.42 \pm 0.10	1.75 \pm 0.29	0.002 ⁴	0.964
SFA	61.92 \pm 2.18	62.25 \pm 0.47	60.86 \pm 1.24	61.99 \pm 0.84	0.830	0.731
Trans	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.000	0.000
ω 6/ ω 3	0.28:1	0.09:1	0.08:1	0.08:1	0.625	0.134

^ALeast square means \pm Standard error considered significant when $p < 0.05$. ^BFitting a simple linear or logarithmic regression to ageing times. Experiment I: $Y^1 = -0.0227x + 0.4257$ ($R^2 = 0.58$); $Y^2 = -0.0162x + 2.3811$ ($R^2 = 0.94$); $Y^3 = -0.1015x + 0.5267$ ($R^2 = 0.65$); $Y^4 = -0.0632x + 3.1293$ ($R^2 = 0.91$).

Warner-Bratzler shear force (WBSF) presented a linear reduction along the aging periods evaluated ($p < 0.01$; Table 3). Thus, the fall WBSF between 0 and 7 days was 15.20 N; between 7 and 14 days 11.08 N and between 14 and 21 days 6.96 N. This fact can also be observed in the mean values of the myofibrillar fragmentation index (MFI), which, generally, increased during aging ($p < 0.01$; Table 3), classifying the meat of these animals at 7, 14 and 21 days of aging as very soft (Culler et al., 1978). Regarding myofibrillar degradation with different aging times, 54.61% was obtained between 0 and 7 days, -0.11% between 7 and 14 days and 5.06% between 14 and 21 days. It is noteworthy that in spite of the similar patterns with experiment I, we obtained in experiment II, a higher final index, indicating that the aging process greatly improves the quality of older buffalo meat and may help demystify this type of meat, often seen as tough by consumers.

The lower tenderness of the non-castrated male meat (experiment I) may be related to the slower *post mortem* degradation in this category (Huff-Lonergan, Parrish, & Robson, 1995). According to Morgan, Wheeler, Koohmaraie, Crouse and Savell (1993), the skeletal muscle protein mass of non-castrated animals is higher, indicating a lower degradation index of this protein. On the other hand, Huff-Lonergan et al. (1995) observed that the meat of

older animals also showed a lower rate of *post mortem* degradation; however, this did not occur in the present study, possibly due to the different origins of the experimental groups and the sex.

In relation to the origin, because the buffaloes presented milk aptitude, the slaughter of males, considered disposable in a dairy herd, may be seen as an alternative for meat production (Mello et al., 2017). However, these animals generally do not receive adequate management, leaving the best conditions, mainly nutritional, to the females that will be the future breeding cow and income generators for the rural property. Thus, dietary restrictions normally imposed on males of dairy origin in the initial phase may have repercussions on their later performance (Rocha, Fontes, Paulino, & Ladeira, 1999). As for sex, in addition to the fact that the males presented less fat in the meat than the females (marbling = 2.60 \pm 0.52 vs 3.67 \pm 0.57, respectively, in the present study), according to Bonagurio, Pérez, Garcia, Bressan and Lemos (2003), they usually present a more dense muscular constitution, which results in less soft meat, requiring a longer aging time.

As in experiment I, both the WBSF and the MFI demonstrated the effectiveness of using the aging process to improve the quality of buffalo heifers' meat with advanced age, which appeared softer at day 21 of aging. However, seven days of aging were

enough to ensure the satisfaction of Latin-American consumers, which is obtained when the shear force is around 40.13 N (Rodas-González et al., 2009).

Regarding the color, the aging times did not affect the average values of lightness (L^*) and redness (a^*) ($p > 0.05$; Table 3), however, the average values of yellowness changed (b^*) by increasing linearly with aging time ($p < 0.01$, Table 3). This result differs from those found in aged meat from crossbred buffaloes (Murray x Mediterranean) after 0, 15 and 25 days, as no differences in b^* values were observed between the aging times (Irurueta et al., 2008). On the other hand, these results are similar to those found in beef aged for 1, 7, 14 or 21 days, and it is explained by the fact that the carotenoid pigments providing yellowness are antioxidants, therefore occurred an increase in the average values of b^* and not a decrease (Andrade et al., 2010).

The color data shows that perceptions of the difference in color of the buffalo heifer meat were within the range of perceptions evident to the human eye ($p = 0.13$; Fig. 2) (Prändl et al., 1994), but were still less noticeable than those observed in aged beef, which is consumed more often than buffalo meat. Despite the changes observed in the present study, average values in both experiments for buffalo meat color were within the proposed standards at all aging times (Muchenje et al., 2009).

In relation to fatty acids, the palmitic (C16:0), adenic (22:4 ω 6) and docosahexaenoic (22:6 ω 3) fatty acids, including omega-3 (ω 3) fatty acids, since the 22:6 ω 3 represent a large proportion of the ω 3 category, as well as the PUFAs (22:4 ω 6, 22:6 ω 3) present in the meat of the buffalo heifers aged 32-26 months, showed a linear decrease in average value, when the aging periods increased ($P < 0.05$; Table 4). Despite a reduction in PUFAs, should note that, of all the fatty acids presents this category, aging promoted a reduction in only docosahexaenoic and adenic acids.

Table 3. The effects of different aging times on the cholesterol levels (mg 100 g⁻¹), pH, cooking loss (CL) (%), shear force (WBSF) (N), myofibrillar fragmentation index (MFI) (%) and color (L^* , a^* , b^*) of *longissimus thoracis* muscle from buffalo heifers slaughtered at 32-36 months of age, (LSM \pm SE^A).

Trait	Experiment II (32-36 months of age)				P-value for regression ^C	
	Aging period (days post-mortem)				Linear	Quadratic
	0	7	14	21		
Cholesterol ^C	123.79 \pm 4.26	110.23 \pm 2.98	121.67 \pm 3.74	109.13 \pm 4.22	0.864	0.384
pH	5.69 \pm 0.04	5.88 \pm 0.06	5.86 \pm 0.03	5.77 \pm 0.03	0.323	0.002 ¹
CL (%)	19.11 \pm 1.37	19.47 \pm 1.98	19.69 \pm 1.55	19.96 \pm 0.82	0.713	0.979
WBSF (N)	59.62 \pm 0.41	44.42 \pm 0.44	33.34 \pm 0.18	26.38 \pm 0.09	0.001 ²	0.191
MFI (%)	92.68 \pm 6.19	147.29 \pm 8.55	147.18 \pm 6.72	152.24 \pm 2.33	0.001 ³	0.003 ⁴
L^*	35.96 \pm 0.56	37.26 \pm 0.61	36.73 \pm 0.33	36.90 \pm 0.57	0.363	0.311
a^*	18.34 \pm 0.41	17.85 \pm 0.43	18.37 \pm 0.21	18.79 \pm 0.71	0.203	0.206
b^*	4.27 \pm 0.23	5.45 \pm 0.27	5.48 \pm 0.25	5.74 \pm 0.23	0.003 ⁵	0.063

^ALSM = Least square means; ^BLeast square means \pm Standard error considered significant when $p < 0.05$. ^CFitting a simple linear or logarithmic regression to aging times. Experiment II: $Y^1 = -0.0014x^2 + 0.0331x + 5.697$ ($R^2 = 0.96$); $Y^2 = -0.1614x + 5.87$ ($R^2 = 0.97$); $Y^3 = 2.551x + 108.0$ ($R^2 = 0.67$); $Y^4 = -0.252x^2 + 7.860x + 95.66$ ($R^2 = 0.92$); $Y^5 = 0.0634x + 4.569$ ($R^2 = 0.76$).

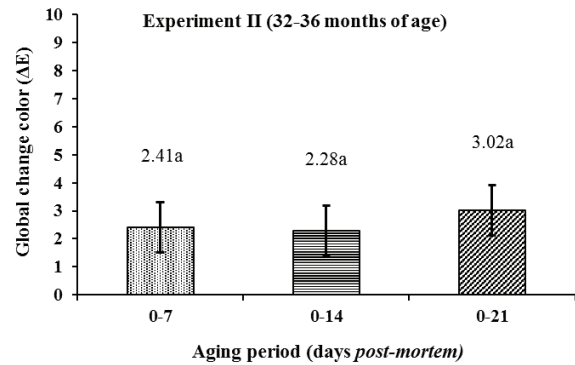


Figure 2. Subjective perception of color (ΔE_{0-7} , ΔE_{0-14} and ΔE_{0-21}) in aged samples of *longissimus thoracis* muscle from buffalo heifers ($p = 0.13$, SE = 0.89) slaughtered at 32-36 months of age.

Regarding SFAs, though presented a significant reduction in palmitic fatty acid, these are considered more resistant to oxidation (Geay et al. 2001). This result should be considered positive, since palmitic acid influence on blood cholesterol levels, raising the low density lipoproteins (LDL) and promoting hypercholesterolemia (Fuentes, 1998).

Like the young buffalo meat (experiment I), the buffalo heifer meat also presented a ratio between acids of the families' ω 6 and ω 3, according to the standards proposed by the Japan Society of Lipid Nutrition, which recommends that the ratio of ω 6: ω 3 should be 4:1 for healthy adults and 2:1 for the prevention of chronic diseases in the elderly (Uauy et al., 1999). However, it is important to note that young buffalo meat is healthier for this parameter, since the ratio is lower than that found in buffalo heifer meat of advanced age. Thus, although heifer meat is softer, it is because of the lower ratio of ω 6: ω 3 and, especially, because of the lower amount of cholesterol in the meat of the animals of experiment I, which is necessary to encourage the slaughter of young animals.

Table 4. The effects of different aging times on the fatty acid profile (%) of *longissimus thoracis* muscle from buffalo heifers slaughtered at 32-36 months of age, (LSM \pm SE^A).

Fatty acid (%)	Experiment II (32-36 months of age)				P-value for regression ^B	
	Aging period (days post-mortem)				Linear	Quadratic
	0	7	14	21		
C12:0	1.98 \pm 0.11	1.88 \pm 0.12	1.96 \pm 0.10	1.83 \pm 0.07	0.429	0.875
C14:0	0.45 \pm 0.04	0.41 \pm 0.05	0.45 \pm 0.04	0.40 \pm 0.02	0.537	0.964
C15:0	0.26 \pm 0.02	0.23 \pm 0.02	0.26 \pm 0.01	0.26 \pm 0.01	0.628	0.487
C16:0	24.60 \pm 0.60	24.23 \pm 0.25	24.21 \pm 0.33	23.08 \pm 0.56	0.035 ¹	0.432
C16:1	0.30 \pm 0.01	0.28 \pm 0.01	0.32 \pm 0.01	0.56 \pm 0.22	0.099	0.271
C16:1 ω 9	1.15 \pm 0.06	1.12 \pm 0.11	1.14 \pm 0.06	1.08 \pm 0.08	0.602	0.902
C16:1 ω 7	0.50 \pm 0.03	0.47 \pm 0.01	0.47 \pm 0.01	0.46 \pm 0.02	0.098	0.608
C16:1 ω 5	1.96 \pm 0.11	1.84 \pm 0.08	1.87 \pm 0.05	1.92 \pm 0.03	0.818	0.259
C17:0	0.30 \pm 0.01	0.28 \pm 0.01	0.32 \pm 0.01	0.56 \pm 0.22	0.099	0.271
C18:0	32.0 \pm 2.10	30.17 \pm 1.67	29.75 \pm 0.51	31.13 \pm 1.32	0.677	0.283
C18:1 ω 9	4.02 \pm 0.26	3.69 \pm 0.39	4.06 \pm 0.17	3.84 \pm 0.07	0.889	0.881
C18:1 ω 9c9	30.53 \pm 0.45	31.23 \pm 1.59	31.08 \pm 0.57	31.74 \pm 1.19	0.446	0.982
C18:1 ω 7	0.81 \pm 0.03	0.84 \pm 0.04	0.78 \pm 0.02	0.75 \pm 0.03	0.087	0.387
C18:2 ω 6	1.38 \pm 0.14	1.29 \pm 0.26	1.24 \pm 0.09	1.02 \pm 0.08	0.103	0.690
C18:3 ω 3	0.49 \pm 0.02	0.45 \pm 0.03	0.42 \pm 0.07	0.38 \pm 0.04	0.211	0.290
C18:4 ω 3	0.49 \pm 0.03	0.45 \pm 0.04	0.54 \pm 0.01	0.48 \pm 0.03	0.731	0.610
C20:0	0.31 \pm 0.02	0.28 \pm 0.02	0.31 \pm 0.01	0.31 \pm 0.01	0.996	0.479
C22:0	0.13 \pm 0.03	0.13 \pm 0.06	0.09 \pm 0.01	0.04 \pm 0.01	0.073	0.558
C22:6 ω 3	0.38 \pm 0.04	0.34 \pm 0.07	0.25 \pm 0.01	0.20 \pm 0.02	0.006 ²	0.574
ω 3	1.36 \pm 0.05	1.24 \pm 0.08	1.21 \pm 0.07	1.06 \pm 0.07	0.038 ³	0.263
ω 6	1.73 \pm 0.22	1.62 \pm 0.40	1.47 \pm 0.10	1.13 \pm 0.09	0.070	0.676
MUFA	33.29 \pm 0.38	33.94 \pm 1.71	33.79 \pm 0.56	34.59 \pm 1.37	0.447	0.950
PUFA	3.29 \pm 0.26	3.04 \pm 0.47	3.02 \pm 0.15	2.38 \pm 0.15	0.042 ⁴	0.526
SFA	61.70 \pm 2.81	59.17 \pm 1.78	58.89 \pm 0.41	58.97 \pm 1.30	0.299	0.455
Trans	4.22 \pm 0.27	3.85 \pm 0.41	4.30 \pm 0.18	4.06 \pm 0.07	0.985	0.869
ω 6/ ω 3	1.26:1	1.26:1	1.21:1	1.07:1	0.315	0.633

^ALeast square means \pm Standard error considered significant when $p < 0.05$. ^BFitting a simple linear or logarithmic regression to ageing times. Experiment II: $Y^1 = -0.065x + 24.71$ ($R^2 = 0.81$); $Y^2 = -0.008x + 0.396$ ($R^2 = 0.95$); $Y^3 = -0.011x + 1.379$ ($R^2 = 0.53$); $Y^4 = -0.039x + 3.344$ ($R^2 = 0.84$).

Conclusion

Aging process greatly improves the tenderness, however, aging causes changes in color perceptible to the human eye, reduces polyunsaturated fatty acids and does not affect the levels of cholesterol in the meat of young buffalo and buffalo heifers with advanced age. Based on the evaluated meat characteristics, concludes that seven days of aging are sufficient for older buffalo heifers, but 21 days of aging is required for the younger male group.

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