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FOOD TECHNOLOGY

Effect of ultrasound on *Biceps femoris* muscle tenderization in Nellore cattle

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ABSTRACT: The effect of ultrasound on *Biceps femoris* muscle tenderness was investigated using a 2^2 Central Composite Rotatable Design (CCRD) with triplicates at the central point. We evaluated the following independent variables: ultrasound intensity ranging from 11.30 to 33.90 W cm^{-2} and exposure time between 35 and 205 s. The ultrasound bath's frequency (80 kHz) and temperature (10 ºC) were the fixed ones. To validate the model, the muscle was treated at the CCRD's optimized condition (80 kHz, 22.60 W cm^{-2}, 120 s, 10 ºC) evaluated, and compared with the muscle control sample (non-treated). A 22% shear force reduction was observed compared to the control sample (no ultrasound treatment) after 144 h, and stored at 5 ºC. Moreover, a sarcoplasmic calcium concentration increase was noted for ultrasound-treated muscle, probably activating the calpain enzyme system. In contrast, no significant influence ($P > 0.05$) was observed for pH, color index, lipid oxidation, water holding capacity, and drip loss by ultrasound treatment at the optimized conditions. Therefore, ultrasound application is promising and suitable for improving muscle tenderness without losing meat quality. This study highlighted the ultrasound effect on the tenderness of a less studied muscle (*Biceps femoris*) by combining short ultrasound exposure (120 s) and an 80 kHz frequency.
Key words: central composite rotatable design, shear force, emerging technology, lipid oxidation, sarcoplasmic calcium content.

Efeito do ultrassom no amaciamento do músculo *Biceps femoris* em bovinos Nelore

RESUMO: O efeito do ultrassom sob a maciez do músculo *Biceps femoris* foi avaliado usando um Delineamento Composto Central Rotacional (DCCR) 2^2 com triplicatas no ponto central. As variáveis independentes estudadas foram a intensidade do ultrassom que variou de 11,30 a 33,90 W cm^{-2} e o tempo de exposição de 35 e 205 s. A frequência do banho de ultrassom (80 kHz) e a temperatura (10 ºC) foram fixas. Para validar o modelo, a carne foi tratada na condição otimizada do DCCR (80 kHz, 22,60 W cm^{-2}, 120 s, 10 ºC), avaliada e comparada à amostra controle (não tratada). Uma redução de 22% na força de cisalhamento foi observada em comparação à amostra controle após 144 h e armazenada a 5 ºC. Além disso, um aumento da concentração de cálcio sarcoplasmático foi observado para o músculo tratado com ultrassom, o que provavelmente ativou o sistema enzimático da calpaína. Em contraste, o tratamento com ultrassom nas condições otimizadas não influenciou significativamente ($P > 0,05$) o pH, cor, oxidação lipídica, capacidade de retenção de água e perda de gotejamento. Portanto, a aplicação do ultrassom é promissora e adequada para melhorar a maciez do músculo sem perder a qualidade da carne. Este estudo destacou o efeito do ultrassom na maciez de um músculo pouco estudado (*Biceps femoris*) ao combinar exposição curta ao ultrassom (120 s) e uma frequência de 80 kHz.
Palavras-chave: projeto rotativo composto central, força de cisalhamento, tecnologia emergente, oxidação lipídica, conteúdo de cálcio sarcoplasmático.

INTRODUCTION

Ultrasound (US) is an emerging technology that analyses or changes food properties. It is the sound above the sound wave vibration frequency (ALARCÓN-ROJO & JANACUA-VIDALES, 2017). This technique is divided into (1) high frequency (from 2 to 20 MHz) and low intensity (<1 W cm^{-2}), with no destructive capacity, used in non-invasive image analysis, sensor and composition analyses, and dispersion or particle concentration in fluids (ALVES et al., 2013); (2) low frequency (from 20 to 100 kHz) and high intensity (10-1000 W cm^{-2}) that allows intermolecular bonds rupture and cell disruption due to the cavitation effect caused by high energy levels (PIYASENA et al., 2003).

Studies have highlighted the effect of ultrasound on improving fresh or processed meat, including tenderness improvement, water holding capacity (WHC) increase, maturation process acceleration (STADNIK & DOLATOWSKI, 2011; GONZALEZ-GONZALEZ et al., 2020),

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more stable emulsion formation (CICHOSKI et al., 2019), marinated product yield increase (KRASULYA et al., 2019), crystallization by controlling nucleation and crystal growth in frozen foods, thawing acceleration by sound energy heating effects (ALARCON-ROJO et al., 2015), microbial contamination reduction (CARAVEO et al., 2015), and recently, Firouz et al. (2022) in their review study confirmed that ultrasound may also improve quality and reduce the time and energy required for production of cooked, fried and fermented meat products.

Among these studies, improving tenderness is highlighted as one of the main attributes sought by consumers (HOPKINS & GEESINK, 2009). The effects caused by ultrasound are influenced by intrinsic (species, age, meat aging, muscle type, connective tissue and intramuscular fat content, myofibril protein compounds) and extrinsic (ultrasonic systems, time, intensity, frequency, and average temperature) factors (ALARCÓN-ROJO & JANACUA-VIDALES, 2017).

Ultrasound may contribute to meat tenderness by joint actions, such as tissue and collagen fiber physical disruption caused by cavitation or enzyme and calcium ion release that modify meat proteolytic activity (ALARCON-ROJO et al., 2019; GONZALEZ-GONZALEZ et al., 2020). Myofibrillar disruption (and possible tenderization) might be masked by the high collagen and elastin content in muscles with high connective tissue content. Therefore, evaluating the applied ultrasound parameters and their effect on the physical, chemical, and biochemical characteristics of meat is essential for its improvement and feasibility. The calpain complex enzymatic system is activated by calcium ions involved in *postmortem* proteolysis and; consequently, meat tenderizing (HOPKINS & GEESINK, 2009). The enzymes act directly on Line-Z proteins fragmentation, measured by the myofibril fragmentation index (ALARCON-ROJO et al., 2019).

Despite the many benefits of ultrasound, several studies reported possible meat quality degradation reactions, such as increased lipid oxidation (STADNIK & DOLATOWSKI; BARANOWSKA, 2008; FALLAVENA et al., 2020). Also, contradictory results have been reported for reduced meat's water holding capacity (FALLAVENA et al., 2020), and color (STADNIK & DOLATOWSKI, 2011). Although, the advantages and disadvantages of ultrasound treatment in meat products, as reported above, some conditions limit its application in industrial scale. Due to this, despite the various studies carried out, standardized ultrasound conditions have not yet been defined according to the specific characteristics for different meat cuts submitted to this technique. Therefore, studies contributing to standardization of conditions to the use of ultrasound in meat are essential to make such technology industrially feasible. This study aimed (1) to evaluate the effect of ultrasound on *Biceps femoris* muscle tenderization using an experimental design, varying the ultrasound intensity and ultrasound time, obtaining the optimized conditions, and (2) to determine the physico-chemical parameters of the ultrasound-treated muscle (in optimized conditions validated) and without ultrasound (control).

MATERIALS AND METHODS

The current study was conducted in two steps: (1) varying the ultrasound intensity and ultrasound time, obtaining the optimized conditions using an experimental design; and (2) determining the physico-chemical parameters of the ultrasound-treated muscle at optimized condition.

Sample preparation

Biceps femoris muscles from both sides of four electrically stimulated carcasses were obtained at 24 h *postmortem* from whole male Nellore cattle (*Bos taurus indicus*). Three of them were used to perform the Central Composite Rotatable Design (CCRD) and the fourth one was used to validate the CCRD in triplicate. The animals remained in pasture for 18 months, and they were slaughtered on different days as per official Brazilian legislation procedures (Brazil, 2021). The Animals` weight ranged from 500 to 550 kg, and carcass yield was approximately 55%. The pH was checked 24 h *postmortem* using a contact potentiometer (Hanna pH meter HI 99163, Woonsocket, RI, USA) equipped with a spear electrode (FC 232D, Woonsocket, RI, USA) penetrator. It was calibrated with standard buffers (pHs 4.01 and 7.02). Subcutaneous fat and connective tissue were removed from each muscle. Thirty six samples were cut from the *Biceps femoris* from each bovine in standardized height x width x length (20 x 50 x 60 mm), weighing approximately 60 g. The cuts were vacuum-packed (Selovac, Microvac CV8, Brazil) in polyethylene bags for further ultrasound application. The samples were stored at 5 °C during preparation.

Experimental design for ultrasound application on Biceps femoris muscle

Preliminary assays were randomly conducted on a multi-frequency ultrasound bath (37 kHz or 80 kHz) with a 330 W nominal power (Elmasonic P120H, Elma Ultrasonic, Germany) at 10 °C (close to a refrigerated temperature) during the whole process (FALLAVENA et al., 2020). The US frequency was tested at 37 and 80 kHz, using 22.6 W cm^{-2} ultrasound intensity for 120 s. The US effect was evaluated by shear force measurements after 24 and 72 h treatment and compared with a non-treated control sample. The 80 kHz US frequency was selected considering that the shear force reduced around 24% both for the 37 and 80 kHz after 24 h. However, shear force decreased by 26% and 13% for 80 kHz and 37 kHz, respectively (supplementary data), after 72 h. Previous studies estimated the delivered power into US bath by the calorimetry method (KODA et al., 2003).

The independent variables and levels of variation were previously selected based on the literature (CHANG et al., 2015) and preliminary tests. The CCRD included 2^2 assays plus three central points (11 assays) to evaluate two independent variables: (x_1) ultrasound intensity and (x_2) ultrasound exposure time. The experimental response (dependent variable) (y) was shear force (instrumental tenderness). The statistical design and the coded and real variables values are seen in table 1.

For samples' ultrasound treatment, the vacuum-packed ones were placed in the ultrasound bath with the largest surface area facing down. The duration of each assay was split evenly for each cut side, applying the ultrasound waves uniformly. For CCRD, a *Biceps femoris* muscle control sample (no ultrasound treatment) was used to compare the results. After the CCRD assay, the samples were stored at 5 ± 1 °C for 24, 72, and 144 h and shear force was determined.

Muscle evaluation at the optimized condition

After optimization, the *Biceps femoris* muscle of the fourth bovine was treated as per the optimized condition (80 kHz, 22.6 W cm^{-2}, 120 s, 10 ºC), in triplicate, and compared to the standard sample (no ultrasound treatment). The pH, color, lipid oxidation, WHC, Drip loss (weight loss by exudation), and sarcoplasmic calcium content were determined. The vacuum-packed samples were stored at 5 ± 1 ºC and analysed in triplicate, 24 h, 72 h, and 144 h after applying the ultrasound. A *Biceps femoris* muscle control sample (no ultrasound treatment) was used to compare the results.

Physico-chemical parameters determination

Warner Bratzler (WB) shear force

Meat shear force analysis was performed according to FALLAVENA et al. (2020), with modifications. Samples were removed from the polyethylene bags, wrapped in aluminum foil, cooked in a conventional electric oven at 220 °C until their geometrical center reached 70 ºC, and kept overnight at 5 °C. The samples were analyzed the following day. Afterward, ten cylindrical stripes of 20 ± 5×10 mm were taken off, parallel to the muscle fiber for each

Table 1 - CCRD 2^2 matrix with coded and real values for the independent variables and the responses of shear force (N) on storage times of 24, 72, and 144 h to evaluate the tenderness of the *Biceps femoris*.

Runs	x_1	x_2	Shear force (N)			
			24 h	72 h	144 h	Predicted values for 144 h
1	-1 (15.07)	-1 (60)	70.85 ± 5.88	55.43 ± 5.69	65.65 ± 3.04	54.42
2	-1 (15.07)	+1 (180)	78.36 ± 1.54	55.08 ± 2.87	52.10 ± 3.26	43.75
3	+1 (30.13)	-1 (60)	69.71 ± 1.30	68.29 ± 5.14	59.40 ± 4.68	62.72
4	+1 (30.13)	+1 (180)	57.36 ± 5.42	55.94 ± 3.19	49.82 ± 3.19	51.96
5	-α (11.30)	0(120)	63.61 ± 4.10	57.09 ± 0.39	52.61 ± 3.08	40.16
6	+α (33.90)	0(120)	69.47 ± 4.82	57.92 ± 2.70	56.71 ± 3.37	51.74
7	0 (22.60)	-α (35)	67.28 ± 2.18	56.70 ± 0.29	67.77 ± 1.39	67.86
8	0 (22.60)	+α (205)	59.38 ± 1.77	67.07 ± 4.14	54.00 ± 5.51	52.81
9	0 (22.60)	0(120)	62.17 ± 5.79	57.13 ± 1.81	47.98 ± 3.57	45.95
10	0 (22.60)	0(120)	64.59 ± 4.91	60.82 ± 5.75	45.04 ± 6.57	45.95
11	0 (22.60)	0(120)	63.35 ± 5.32	60.36 ± 4.16	44.82 ± 4.48	45.95

x_1: ultrasound intensity (W cm^{-2}); x_2: ultrasound time (s); results expressed as the mean ± standard error (n = 10).

CDDR assay. Instrumental cutting was performed in the transverse direction of the muscle fibers using a Texture Analyser (TA.XT2i, Stable Micro Systems, Godalming, UK) with a V-shaped blade (HDP/WBV). The following experimental conditions were applied: 100 kg load cell; 5.0 mm s^{-1} pretest speed; 2.0 mm s^{-1} test speed; 5.0 mm s^{-1} post-test velocity; 25 mm sample distance; 30 mm sample penetration distance; and 25 g applied force. Ten readings were taken for each sample, and the results were expressed in Newton (N) for the maximum force required to cut the samples.

pH and color

The pH was evaluated using a contact potentiometer and directly applied to the dorsal part of the *Biceps femoris*. The muscle surface color was measured using a Minolta® CR400 chromameter (Japan), with an integrating sphere and a 45º viewing angle, illuminat D, and illumination d/45, and L^* (lightness), a^* (red-green component) and b^* (yellow-blue component) were expressed in the CIELAB system dimensions.

Lipid oxidation

The TBARS (thiobarbituric acid reactive substances) index was used to evaluate lipid oxidation according to the TARLADGIS et al. (1964) method modified by CRACKEL et al. (1988). TBARS values were expressed in mg malonaldehyde (MDA) kg^{-1} sample. A 10 g sample was hydrolyzed in an acid medium and distilled. Thiobarbituric acid (TBA) (Sigma, T5500, MW 144.15) was added to the distillate and placed in a water bath at 85 ºC for 35 min. The absorption reading was taken at 530 nm using a Libra S22 spectrophotometer (Biochrom Ltd., England) after cooling to room temperature. A blank sample containing distilled water and TBA reagent was used. Calibration was done using a 1,1,3,3-tetra ethoxy propane (Sigma, T9889, MW 220.31) solution in deionized water (0.004 to 1.0 mol L^{-1}). The standard addition and recovery test showed a method accuracy at a 95% recovery level. The results were expressed as mg malondialdehyde (MDA) kg^{-1} sample.

Water holding capacity (WHC)

WHC was analyzed according to HAMM (1961) in triplicate, with modifications. The samples were weighed (2.00 g ± 0.01 g), placed between two filter papers, and left under a 10 kg weight for 5 min. The WHC was calculated as per Equation 1.

$$\%\,WHC = 100\% - \left[\frac{(initial\ weight - final\ weight)}{initial\ weight}\right] x\ 100 \quad (1)$$

Drip loss (DL)

DL was determined in ultra-sounded meat and the control samples after 24 h, 72 h, and 144 h. The vacuum bag containing the meat sample was opened, and the excess liquid poured off after weighing the meat sample. DL was expressed as the meat sample's weight percentage difference before and after US treatment:

$$DL\ (\%) = \frac{w(M1) - w(M2)}{w(M1)} \times 100$$

where w(M1) is meat sample weight before ultrasound treatment, and w(M2) is meat sample weight after ultrasound treatment at the respective storage time. Each determination was performed on three meat samples on the actual sampling day.

Sarcoplasmic calcium content

The bovine meat's sarcoplasmic Ca^{2+} levels were determined using 10 g samples in a 25 mL of 150 mM KCl solution, according to CHEAH et al. (1986). The samples were homogenized in a vortex mixer for 2 min and then centrifuged at 6,250 *g* for 4 min (3K30, Sigma, Germany). The supernatant was collected and centrifuged at 26,000 g for 4 min. The supernatant was collected and filtered. A Schinkel modifier (0.5% v v^{-1}) was added and Ca^{2+} was quantitatively determined by flame atomic absorption spectroscopy at 422.7 nm (Varian, AA240FS, USA).

Statistical analysis

The CCRD data were expressed as the mean ± standard deviation (n = 30). The CCRD assays were performed randomly, and the data were analyzed using the Statistica 8.0 software (Statsoft Inc., Tulsa, USA), also used to generate the response surface. The model adequacy was evaluated with the coefficient of determination (R^2) and the analysis of variance F-test (ANOVA) ($P < 0.05$). The optimized CCRD condition was validated in triplicate (n = 3). All physical and chemical parameters were determined in triplicate, except shear force, which was defined by 10 readings for each sample. Such data were statistically analyzed by ANOVA, followed by a mean difference comparison by Tukey's test using the same software ($P < 0.05$).

RESULTS AND DISCUSSION

Ultrasound effect on Biceps femoris muscle tenderness

The effect of ultrasound intensity (x_1) and ultrasound time (x_2) on *Biceps femoris* muscle's shear force (tenderness) was studied using CCRD and the results are presented in table 1. The shear force values

over storage time after ultrasound treatment ranged from 78.36 N (assay 2) to 44.82 N (assay 11), 24 h and 144 h storage and 15.07 W cm^{-2} and 180 s, and 22.60 W cm^{-2} and 120 s. Ultrasound intensity (x_1) and ultrasound exposure time (x_2) showed negative effects at 24 and 144 h after ultrasound treatment and positive ones at 72 h for both samples evaluated (Table 2). Variables' significant effects ($P < 0.05$) occurred on the *Biceps femoris* muscle with 144 h storage after ultrasound treatment, longer than that found by Stadnik and Dolatowski (2011), who applied ultrasound to the *Semimembranosus* muscle at 45 kHz, 2 W cm^{-2} and 120 s. The authors reported an increase in muscle tenderness only after 48 h of ultrasound exposure. However, in the study carried out by PEÑA-GONZÁLEZ et al. (2017) improvement in tenderness and juiciness of beef (*Longissimus dorsi*) treated with ultrasound (40 kHz, 11 W cm^{-2}, 60 min) was only observed after 14 days of storage at 4 ºC. These variations in results may be due to ultrasound treatment conditions, animal age, meat aging, muscle type, connective tissue, intramuscular fat content, and myofibril protein compounds (ALARCÓN-ROJO & JANACUA-VIDALES, 2017).

The significant parameters ($P < 0.05$) from the regression analysis allowed the development of a second-order model (Figure 1) for the *Biceps femoris* muscle's shear force as a function of ultrasound intensity and time. The model showed only the significant factors presented in table 2. Non-statistical parameters were incorporated into the model residues, and the mathematical model was re-parameterized. The F-test value (31.15) for the regression was significant ($P = 0.0001$; 7.16-fold higher than the critical value), and the variation percentage explained by the model was suitable ($R^2 = 93.03\%$); thus, the predictive model was used to generate a response surface (Figure 1). The statistical model fitting to the experimental data was confirmed by predicted values (Table 1).

Response surface analysis (Figure 1) supported that the optimal *Biceps femoris* shear force occurred near the central point for 22.60 W cm^{-2} ultrasound intensity (x_1) and 120 s exposure

Table 2 - Regression coefficient estimates for the responses of shear force (N) on storage times of 24, 72, and 144 h to evaluate the tenderness of the *Biceps femoris*.

Factors	Shear force (N) – 24 h			
	Regression coefficient	Standard error	t(5)	P-value
Mean	63.35*	3.24*	19.56*	0.0000*
x_1 (L)	-1.74	1.99	-0.88	0.4212
x_1 (Q)	2.64	2.37	1.11	0.3160
x_2 (L)	-2.00	1.99	-1.01	0.3595
x_2 (Q)	1.03	2.37	0.43	0.6833
x_1 . x_2	-4.96	2.80	-1.77	0.1370
	Shear force (N) – 72 h			
	Regression coefficient	Standard error	t(5)	P-value
Mean	59.44*	2.87*	20.73*	0.0000*
x_1 (L)	1.87	1.76	1.06	0.3370
x_1 (Q)	-1.23	2.10	-0.58	0.5838
x_2 (L)	0.24	1.76	0.14	0.8964
x_2 (Q)	0.97	2.10	0.46	0.6617
x_1 . x_2	-3.00	2.48	-1.21	0.2810
	Shear force (N) – 144 h			
	Regression coefficient	Standard error	t(5)	P-value
Mean	45.95*	1.55*	29.70*	0.0000*
x_1 (L)	-0.34	0.95	-0.36	0.7312
x_1 (Q)	4.10*	1.13*	3.62*	0.0151*
x_2 (L)	-5.33*	0.95*	-5.62*	0.0025*
x_2 (Q)	7.23*	1.13*	6.39*	0.0014*
x_1 . x_2	0.99	1.34	0.74	0.4921

x_1: ultrasound intensity (W cm^{-2}); x_2: ultrasound time (s); L: linear terms; Q: quadratic terms; * Significant factors ($P < 0.05$).

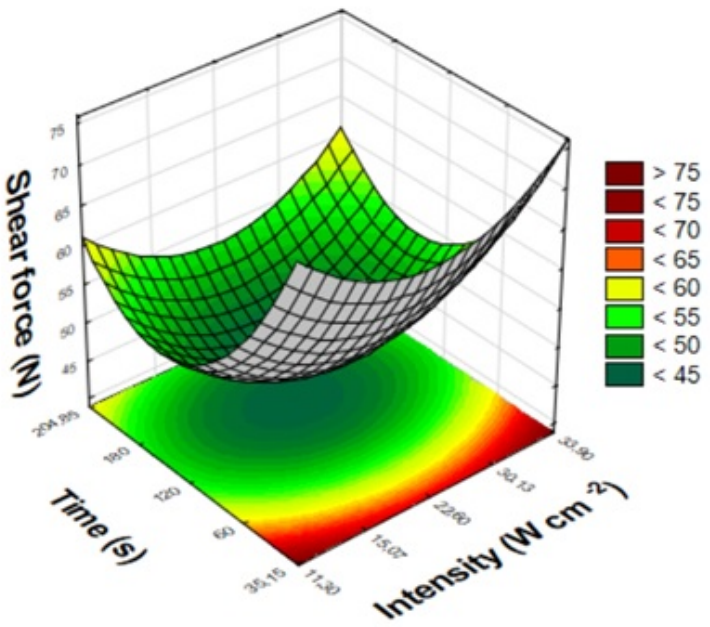

Figure 1 - Response surface for the shear force for the *Biceps femoris* muscle after 144 h of ultrasound application as a function of intensity (W cm^{-2}) and time (s).

time (x_2). Furthermore, according to response surface analysis, it was observed that prolonged *Biceps femoris* muscle's exposure to ultrasound increased the shear force, which may be explained by the collagen gelation (CHANG et al., 2015). FALLAVENA et al., (2020), who used 20 kHz and 22 - 84 W cm^{-2}, reported that high ultrasound intensity may decrease tenderness, and the treatment performed with 22 W cm^{-2} showed a decrease in shear force. In this study, the maximum intensity used was 33.90 W cm^{-2}, which improved tenderness.

The optimized conditions used in this study were 22.60 W cm^{-2} ultrasound intensity, 120 s exposure time, and 144 h storage after ultrasound treatment. The experimentally determined power and density power were respectively 384.6 W and 30.0 W L^{-1} at 80 kHz and 100% amplitude. Table 3 presents the shear force results for ultrasound-treated *Biceps femoris* muscle and the control samples (no ultrasound treatment).

The shear force for assays validation was statistically similar ($P > 0.05$). The shear force was 22% higher for the control sample, suggesting a less tender meat than the ultrasound-treated muscle.

Biceps femoris muscle evaluation at the optimized condition

After evaluating the effect of ultrasound on tenderness, *Biceps femoris* muscle under optimized conditions was analyzed for pH, color, lipid oxidation, WHC, drip loss, and sarcoplasmic calcium after 24, 72, and 144 h ultrasound treatment (22.60 W cm^{-2} and 120 s) (Table 4). The results were compared with the control sample.

pH

The pH values for *Biceps femoris* showed no significant difference ($P > 0.05$) between the control sample and the treated one. The same behavior was reported by FALLAVENA et al., (2020) and STADNIK & DOLATOWSKI (2011). However, after 144 h ultrasound exposure, the sample presented higher pH values and significantly differed from the 24 h ultrasound exposure samples. The pH values increase occurred due to the biochemical reactions caused by meat maturation (MOHAN et al., 2019). Studies have reported that prolonged ultrasound application may damage cells by releasing Ca^{2+} ions into the cytosol, changing

Table 3 - Validation of the study performed for shear force (N) from the *Biceps femoris* muscle treated by ultrasound (22.60 W cm^{-2} and 120 s) and stored for 144 h, and the control sample.

Run	----------*Biceps femoris* shear force (N)----------	
	Ultrasound-treated	Control sample
1	48.53[a] ± 3.55	59.27[a] ± 5.25
2	48.02[a] ± 5.37	63.02[a] ± 0.73
3	47.67[a] ± 0.97	63.45[a] ± 2.13

*Mean ± standard error (n = 30) followed by the same letters in the same column presented no differences by Tukey's test (P < 0.05).

protein structure, and neutralizing acid groups. It corroborates the hypothesis that ultrasound may accelerate meat maturation (JAYASOORIYA et al., 2007; STADNIK & DOLATOWSKI, 2011). Nevertheless, this was not observed under the present study conditions as both samples (treated and control) showed no significant difference (P > 0.05) for the times they were analyzed.

Color

Color is arguably the main meat quality attribute that influences consumers' purchase-decision (ALARCON-ROJO et al., 2019; DEY &NAGABABU, 2022); hence the relevance of ultrasound effect on this parameter. It was observed that L^* (luminosity) decreased (P<0.05) during storage for both ultrasound-treated *Biceps femoris* muscles and the control sample. Luminosity decreased due to meat's darkening caused by metmyoglobin formation, a brown pigment with Fe^{3+} resulting from myoglobin oxidation (MANCINI; HUNT, 2005). However, for each storage time evaluated, no significant difference (P > 0.05) between the ultrasound-treated samples and non-treated ones (control sample) was found, indicating that ultrasound did not influence meat color. Parameters a^* (red-green component) and b* (yellow-blue component) showed no difference (P > 0.05) with respect to storage time and when compared to the control sample. Moreover, Jayasooriya et al. (2007) and STADNIK & DOLATOWSKI (2011), also reported that meat color did not change with ultrasound treatment, which is a positive factor using ultrasound on meat.

However, prolonged ultrasound exposure may affect color, as CHANG et al. (2015) reported. They used a 45 kHz frequency for 30 min on *Semitendinosus* bovine muscle and observed that b^* parameter was significantly lower than that of control meat. CARAVEO et al. (2015), by applying ultrasound to *Semitendinosus* muscle using an

Table 4 - Results of pH, color index, water holding capacity (WHC), drip loss, and sarcoplasmic calcium results of *Biceps femoris* muscle after 24, 72, and 144 h of ultrasound treatment (22.60 W cm^{-2} and 120 s) and control samples (without ultrasound exposure).

Time	Sample	pH	----------Color index----------			WHC (%)	Drip Loss (%)	Sarcoplasmic calcium (μg g^{-1})
			L^*	a^*	b^*			
24 h	Ultrasound-treated	5.53[b] ± 0.02	45.73[a] ± 3.90	15.80[a] ± 1.73	10.16[a] ± 2.85	68.21[a] ± 1.78	1.99[bc] ± 0.93	6.86[c] ± 0.41
	Control	5.54[b] ± 0.05	44.56[ab] ± 2.86	16.29[a] ± 1.46	9.42[a] ± 1.21	69.50[a] ± 1.55	1.62[b] ± 0.29	5.49[d] ± 0.16
72 h	Ultrasound-treated	5.59 [ab] ± 0.05	41.39[bc] ± 2.06	14.57[a] ± 2.05	12.12[a] ± 1.96	69.19[a] ± 2.13	2.97[ac] ± 0.94	10.22[a] ± 0.26
	Control	5.59[ab] ± 0.02	40.51[c] ± 1.04	16.79[a] ± 1.61	10.75[a] ± 2.28	68.03[a] ± 1.84	2.85[abc] ± 0.93	7.66[bc] ± 0.20
144 h	Ultrasound-treated	5.62[a] ± 0.06	40.34[c] ± 2.31	15.91[a] ± 1.92	12.22[a] ± 1.75	68.07[a] ± 1.69	4.10[a] ± 0.73	9.97[a] ± 0.31
	Control	5.64[a] ± 0.03	39.65[bc] ± 1.09	15.07[a] ± 1.58	11.47[a] ± 2.72	68.40[a] ± 1.93	3.98[a] ± 1.37	7.83[b] ± 0.42

*Mean ± standard error (n = 9) followed by the same letters in the same column presented no differences by Tukey's test (P < 0.05).

ultrasound bath at 40 kHz frequency and 11 W cm^{-2} intensity for 60 and 90 min, found that in the first ultrasound application the L^* parameter increased and the a^* parameter decreased. However, no difference was observed between treated and control samples after 8 days of storage. *Biceps femoris* treated with 84 W cm^{-2} showed a significant difference for parameters a^* and b^* compared to the other treatments using lower intensity ultrasound. This treatment showed a less prominent red and more intense yellow, which led to a less intense overall color (FALLAVENA et al., 2020).

Lipid oxidation

The high temperatures and pressures occurring during the collapse of gas bubbles due to ultrasound application may form hydroxyl radicals ($OH^{\bullet}$) and hydrogen atoms generated from the water molecules dissociation in aqueous solutions (KANG et al., 2016). The TBARS values obtained in this study for ultrasound-treated *Biceps femoris* muscle and the control sample did not show any differences ($P > 0.05$) during storage (Figure 2). These results showed that using the ultrasound treatment did not cause any significant changes in the meat lipid oxidation under the proposed conditions. Both samples' lipid oxidation degree in this study went below the oxidation odor detection threshold (0.5 - 1 mg MDA kg^{-1}) (TARLADGIS et al., 1960). These results agree with those reported by Peña-González et al. (2017), who obtained TBARS values that indicated no compromise to the oxidative stability of ultrasound-treated meat samples stored under refrigeration. KANG et al. (2016) when evaluating the effect of ultrasound time (30 to 120 min) and ultrasound intensity (2.39 to 20.96 W cm^{-2}) on lipid oxidation found that the highest lipid oxidation occurred by increasing ultrasound intensity and time. The same was observed by FALLAVENA et al. (2020), who fixed 15 min for ultrasound treatment to *Biceps femoris* muscle after observing that 20.96 W cm^{-2} for 30 min generated an approximately 0.6 mg MDA kg^{-1} TBARS value, while using the same ultrasound intensity for 120 min resulted in a 1.25 mg kg^{-1} value. According to Faustman et al. (2010), lipid oxidation may also affect meat color. The primary and secondary products of lipid oxidation process may interfere with the myoglobin-oxygen binding, fostering color changes. As previously mentioned, there were no differences ($P > 0.05$) in the L^*, a^* and b^* of the ultrasound-treated *Biceps femoris* and the control sample (Table 4) in this study, indicating that shorter ultrasound treatment times are suitable.

Water holding capacity (WHC)

Ultrasound may change the spatial conformation and solubility of myofibrillar protein

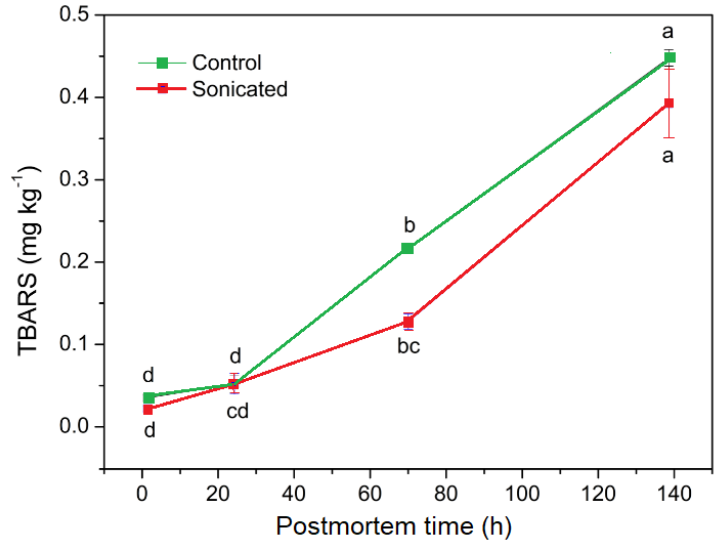

Figure 2 - Results of thiobarbituric acid reactive substances (TBARS) analysis as function of the storage time of samples from *Biceps femoris* at 5 ± 1 °C treated by ultrasound (80 kHz, 22.6 W cm^{-2}, 120 s), and control (without ultrasound treatment), and analyzed 24 h, 72 h, and 144 h after ultrasound application. *Equal letters do not differ statistically by Tukey's test ($P < 0.05$).

that binds and intercepts water, thus affecting WHC (CARAVEO-SUAREZ et al., 2022). However, this was not observed in the present study. The similar results obtained for WHC ($P > 0.05$) (Table 4) indicated that ultrasound and storage time after treatment had no effect on this property under the conditions studied. Contradictory results may be found in the literature depending on the used ultrasound conditions and the type of muscle. The *beef (musculus semimembranosus)* when treated with ultrasound (45 kHz, 2 W cm^{-2}, 120 s), showed a higher WHC than the untreated sample (STADNIK & DOLATOWSKI, 2011), and the beef *Longissimus dorsi* treated with ultrasound (37 kHz, 7 W cm^{-2}, 60 min) immediately after sonication presented a much lower WHC than that of non-sonicated samples; however, during storage at 4 ºC for 0 and 7 days the WHC of the sonicated samples increased showing higher values than the control (CARRILLO-LOPEZ et al., 2019). FALLAVENA et al., (2020), when studying the influence of ultrasound intensity and temperature on WHC, reported values of approximately 70%. The authors emphasized that WHC was directly affected by pH. Taking into account that in this study, the pH values were very similar for all samples, WHC presented the same performance. A pH decrease may shrink the polypeptide chain network, reducing WHC (ALARCON-ROJO et al., 2019), which did not occur in the present study.

Drip loss

DL values (Table 4) indicated a significant increase ($P < 0.05$) over storage time. However, the loss by exudation for the ultrasound-treated muscle was not higher than that of the control sample. DL also increased with aging when the *longissimus lumborum* bovine was treated with 24 kHz and 12 W cm^{-2} for up to 240 s (JAYASOORIYA et al., 2007) over an 8-day period. The weight loss increase by exudation during storage time was probably due to fiber disintegration, a natural process that occurs during chilled meat storage, ejecting water into the extracellular space (STRAADT et al., 2007).

CARAVEO-SUAREZ et al. (2022) evaluated the high-intensity ultrasound (HIU) effect on the physicochemical and textural properties of meat from Rararumi Criollo (an autochthonous Mexican bovine breed). The ultrasound conditions used were 11 W cm^{-2} intensity and 45 kHz frequency for 20 min on the *Triceps brachii* and *Longissimus dorsi*. The results showed a shear force decrease, higher for *Longissimus dorsi*, and significant differences ($P < 0.05$) in color were observed due to ultrasound treatment, muscle type, and days of storage. No significant differences ($P > 0.05$) were detected on meat pH, WHC, and DL despite the long ultrasound treatment, however, there were significant effects ($P < 0.05$) on muscle type, storage time, and ultrasound × storage and muscle × storage interactions.

Sarcoplasmic calcium content

The decrease in shear force is caused by proteolysis activated by Ca^{2+} ions. *Postmortem* calcium release increases due to energy-dependent regulatory systems inhibition. Ultrasonic disruption of cellular membranes and subcellular compartments increases Ca^{2+} availability to the calpain system. Also, ultrasound treatment is highly effective in releasing lysosome cathepsins (JAYASOORIYA et al., 2004, 2007). Table 4 shows that sarcoplasmic calcium values were higher in ultrasound-treated samples than in the control samples ($P < 0.05$). The sarcoplasmic calcium content in ultrasound-treated samples was more intense and significant ($P < 0.05$) after 72 h (4 days *postmortem*). These results proved that ultrasound treatment fostered early and intense physical tissue disruption caused by cavitation, contributing to increased calcium release, consequently, activating the calpain enzymatic system and improving meat tenderness (WARNER et al., 2017). DOLATOWSKI & STADNIK (2007) also observed a free calcium content increase in ultrasound-treated *Semimembranosus* muscle.

CONCLUSION

The combination of short ultrasound exposure (120 s) and 80 kHz frequency improved *Biceps femoris* tenderness by 22% shear force reduction after 144 h and stored at 5 ºC. Ultrasound treatment is a promising and suitable technique for improving muscle tenderness without losing any meat quality, with potential interest for meat industry applications.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the

collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

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