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High intensity ultrasound homogenizes and improves quality of beef *longissimus dorsi*

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Abstract

The present study aims to evaluate the uniformity of the high intensity ultrasound (US) effects on the quality of beef *longissimus dorsi*. For this purpose *L. dorsi* muscles from Hereford carcasses were cut into 2.54 cm thick slices. Each sample was marked into concentric areas of 2 cm wide. Ultrasound (37 kHz and 7 W/cm^2) treatment was performed for 60 min using an ultrasonic bath and treated meat was stored at 4 °C for 0 and 7 days. pH values decreased after 7 days of aging at 4 °C with and without ultrasound application ($P < 0.0001$). The color parameters a* and b* and WHC increased significantly in the sonicated samples after 7 d of storage at 4 °C ($P < 0.0001$). No differences by US ($P = 0.6711$) and storage time ($P = 0.4184$) were found. Therefore, ultrasonic intensity was homogeneously distributed in the samples and had no negative effects on the quality of the meat. A reduction ($P < 0.0001$) in psychrophilic and coliform ($P < 0.0001$) bacteria was observed by US, while mesophilic bacteria increased ($P < 0.0001$) by US. US could be considered as an efficient technology to be used in beef to improve meat quality and safety.

Keywords: emerging technologies; power ultrasound; high intensity ultrasound; bacterial loads; meat quality; shelf life.

Practical Application: The effects oh high intensity ultrasound on bovine *Longissimus dorsi* is homogeneously distributed in the whole meat sample. Ultrasonication appears to be a promising method among the recent techniques for bacterial reduction on meat without effect on pH, color and water holding capacity when applied to fresh meat. While conventional treatments for microbial inactivation include the use of high temperatures with the concomitant deterioration of the functional and sensorial properties of food, ultrasound treatment could be used as an assisted technology for the reduction of beef microbiota without affecting the quality of fresh and aged beef. High-power ultrasound offers an alternative to the traditional methods of food preservation and is considered a green, versatile, and emerging technology. Ultrasound produces cavitation in a liquid medium, contributing to the antimicrobial effect and increasing the shelf life of food without causing detrimental effects on functional properties of meat.

1 Introduction

The treatment of food with ultrasound (US) induces the phenomenon of acoustic cavitation, wherein microbubbles are generated in a liquid medium. Upon reaching their critical size, the bubbles implode, resulting in the release of accumulated energy that causes instantaneous and focal temperature increases. The local increase in temperature dissipates without causing any substantial increase in the overall temperature of the liquid being treated (Kudo et al., 2017). The energy released as well as the mechanical shock associated with the implosion affect the structure of the cells in the microenvironment. Low-frequency (18-100 kHz, $\lambda = 145$ mm) and high intensity (10-1000 W/cm^2) US waves exert physical, mechanical, and chemical effects, which are capable of permeating the cell membrane and inducing structural and physicochemical changes and accelerating chemical reactions (Alarcón-Rojo et al., 2015).

Very little is published on the use of US to improve technological and sensory qualities of beef. However, a few studies have highlighted its positive effects on the conservation of nutritional and organoleptic properties of meat products (Ünver, 2016) and microstructural changes to the myofibrils in beef (Stadnik et al., 2008; Ünver, 2016) that may have beneficial tenderizing actions (Alarcón-Rojo et al., 2015).

The controversy regarding the benefits of high-power US is associated with multiple factors influencing its applications. One of the most relevant factors is the amount of energy of the sound field generated, characterized by the power of sound (W), acoustic intensity (W/m^2), and acoustic energy density (Ws/m^3) (Knorr et al., 2004). The use of ultrasonic baths with different intensities and frequencies and diversity of results previously described make us question the homogeneous distribution of US in the product. Furthermore, it is unclear whether the effects of US are dependent on the area that experiences the highest levels of cavitation in the sample.

It is well known that ultrasound causes changes in physicial, chemical and functional properties of food (Terefe et al., 2016) and modifies its quality (Kentish & Feng, 2014). High intensity ultrasound tenderizes muscle by weakening muscular fibers and releasing proteases that denature meat proteins (Siró et al., 2009).

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It is not known if these effects takes place in the whole treated sample or only on those areas near the ultrasound transducers. Most researchers suppose that US exerts a homogeneous effect on the whole exposed area. To shed light on these uncertainties, we applied US to different areas of bovine longissimus dorsi and evaluated its effects on the physicochemical and microbiological variables. Observations were made immediately following US treatment and after 7 days of aging at 4 °C.

2 Materials and methods

2.1 Samples and treatments

Loin steak samples from 435 kg live weight Hereford carcasses were cut in 2.54 cm thick slices. Three concentric circles (of 2 wide each) were marked on the samples using plastic pins, without damaging the tissue (Figure 1).

Twelve treatments (Table 1), including two US (with and without US application), two storage periods at 4 °C (0 and 7 days), and three concentric areas (C1, C2, and C3), were applied. A three factor completely randomized (two ultrasound levels, two storage times and three concentric circles) was used. Three replicates per treatment were performed. All samples were individually vacuum packed; therefore, three slices were assigned for each treatment.

2.2 Application of ultrasonic treatment

Ultrasound treatment was performed on the vacuum-sealed samples in an ultrasonic bath (Elma®, Elmasonic S15H, Singen, Germany) with internal dimensions of 15.1 × 13.7 × 10 cm and a maximum capacity of 1.75 L (Figures 2 and 3). A volume of 500 mL distilled water was used as the acoustic transmission medium. The frequency and intensity of the equipment was 37 kHz and 14 W/cm^2, respectively. Samples were sonicated one at a time for 60 min (30 min per side). The bath temperature was maintained constant at 4 °C (measured with a thermocouple) during treatment using ice cubes. Distilled water was removed after each sample. At the end of the sonication time, the samples were opened either immediately or after 7 days for evaluation.

2.3 Determination of the optimal US power

The optimal US power level was determined using the calorimetric technique described by Margulis & Margulis (2003). US was applied to a set volume of distilled water and the temperature change of the fluid recorded at short time intervals for 180 s during sonication. The value of dT/dt was estimated from the graph of temperature as a function of time.

The power of US transmitted to the fluid was determined from the Equation 1 as follows:

$$P = m \times Cp \times \left(dT / dt \right) \quad (1)$$

where P is US power (W); m is the mass of the sonicated liquid (kg); and Cp is the specific heat at constant pressure (J/g) K. The effective power of US was expressed in watt per unit area of the emitting surface (W/cm^2) (Jambrak et al., 2014).

The US system had dT/dt value of 0.00668, which was used in the aforementioned power equation. Water was considered to have a heat capacity of 4.186 J/kg °C and solvent mass (m) of 500 g, resulting in a system output power of 14 W/cm^2.

2.4 Determination of pH

The pH of the meat was measured with a digital pH-meter (Sentron, Model 1001, The Netherlands). Measurements were taken directly in the meat following the method of Honikel (1998). The probe was inserted in the muscle perpendicularly to a depth of 2 cm, avoiding contact with fat and remnant connective tissue. Three measurements were obtained from each sample.

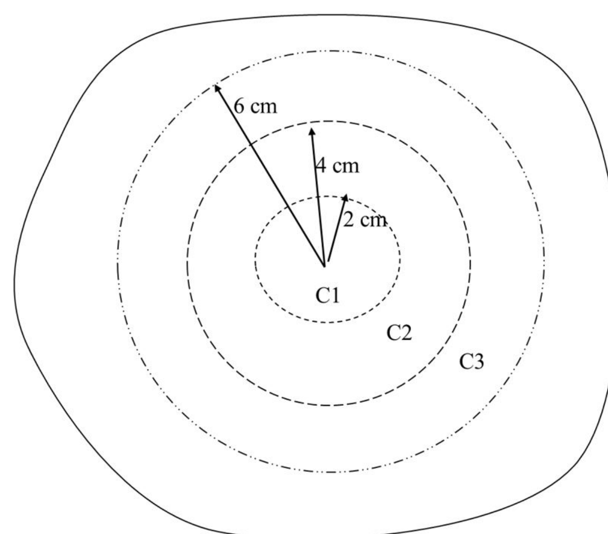

Figure 1. Location of the concentric circles within the sample (radii from the center of the sample: 2, 4 and 6 cm). C1, C2, C3 = concentric cirles of 2 cm wide.

Table 1. Treatment groups.

Treatment	Ultrasound	Concentric area	Storage 4 °C (day)
T1	With US	C1	0
T2	With US	C2	0
T3	With US	C3	0
T4	With US	C1	7
T5	With US	C2	7
T6	With US	C3	7
T7	Without US	C1	0
T8	Without US	C2	0
T9	Without US	C3	0
T10	Without US	C1	7
T11	Without US	C2	7
T12	Without US	C3	7

US = Ultrasound.

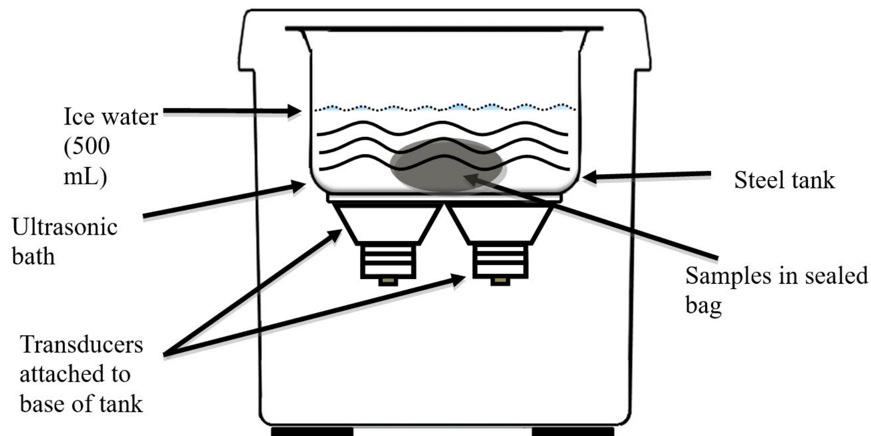

Figure 2. General characteristics of the ultrasonic bath.

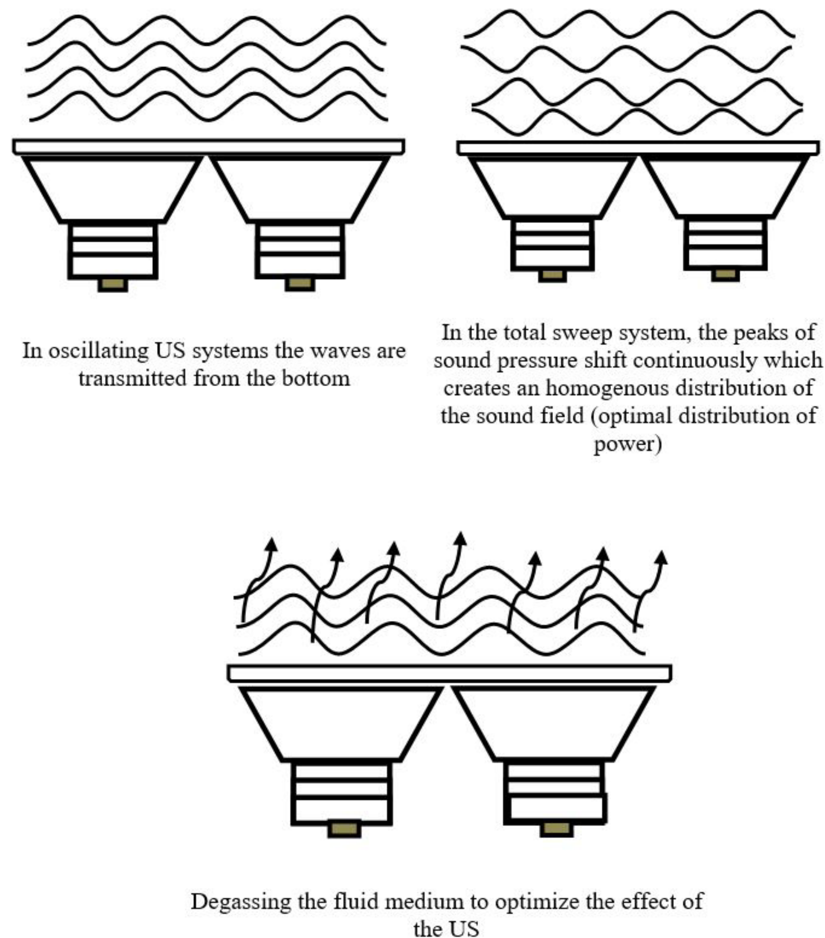

Figure 3. Distribution of US waves in Elmasonic S15H equipment.

2.5 Color measurement

The color space was determined by CIE L*a*b*, where L* is lightness, a* is redness, and b* is yellowness. The measurements were obtained with a colorimeter (Konica Minolta, CR 400, USA) and performed under Commission International Pour I Eclarige reference system as per AMSA methodology (American Meat Science Association, 2012). The connective tissue and visible fat were removed from the surface of the muscle and the surface was exposed to oxygen from the air. The sample was allowed to rest for at least 30 min to develop the blooming. Three measurements were obtained for each sample to register the values of L*, a*, b*, and c*.

2.6 Water-Holding Capacity (WHC)

WHC of meat was determined by the compression method proposed by Tsai & Ockerman (1981) using 0.3 g of sample. An analytical balance with a resolution of ± 0.05 g, filter paper number 54 (Whatman®), methacrylate plates, and 2.25 kg weights were used. The results were expressed as the percentage of exudate released according to the following Equation 2:

$$\%\ exudate = \left(\left(final - initial\ weight\ of\ the\ filter\ paper\right) / \ sample\ weight\right) \times 100 \quad (2)$$

2.7 Evaluation of shear force

Samples were prepared for the shear force analysis according to AMSA methodology (American Meat Science Association, 2015). Samples were cooked on electric plates (George Foreman Grilling Machine®, USA) to an internal temperature of 71 ± 0.1 °C and stored for 12 h at 4 °C. Following incubation, eight cylinders of 10 mm diameter were cut using a manual corer, taking care that the blocks were obtained parallel to the longitudinal orientation of the muscle fibers. Cylinders were cut using a Warner-Bratzler blade (triangular aperture of 60°) at a speed of 100 mm/min into 30 mm lengths. The peak force (expressed in kg-force) to cross-cut each cylinder was determined with TA-XT plus texture analyzer (Stable Micro Systems Ltd., Surrey, UK).

2.8 Microbiological analysis

Meat samples were vacuum sealed and stored under same atmosphere (4 °C). No microbiological counts were performed for each marked circle; instead, microbiological analyses were performed for each sample without separating the concentric circles. After disinfecting the outer part of the package meat sample was unpacked. Then, 1 mL of exudate was taken and placed in 10 mL of sterile maximum recovery diluent (MRD; saline peptone water made using 1.0 g/L peptone and 8.5 g/L sodium chloride, pH 7.0 ± 0.2). Exudates from the original sample were serially diluted from 1:10 up to 1:1,000,000, as described by Haughton et al. (2012). In the subsequent step, 1,000 μL of each dilution was inoculated into the specific medium described below by the extended plate technique.

For mesophilic and psychrophilic bacteria, the samples were inoculated onto plate count agar (CM0325, Oxoid, Basingstoke, UK) and incubated aerobically at 35 °C ± 2 °C for 48 ± 2 h or 5 °C ± 2 °C for 168 h. The evaluation of total coliform bacteria by plaque counts was performed on violet red bile glucose agar (Oxoid) covered with an overcoat once the plates were solidified to favor the conditions of micro-aerobiosis suitable for coliform bacteria following the methodology of Association of Official Analytical Chemists (2003). The plates were incubated at 35 °C ± 2 °C for 48 ± 2 h. To calculate colony-forming unit (CFU)/mL, the number of colonies was multiplied by the dilution factor used (1 for 1: 0, 10 for 1:10, and so on). The raw results measured as CFU/mL were transformed to logarithmic units ($\log_{10}$).

2.9 Statistical analysis

All data were analyzed through a completely randomized factorial design. Factors evaluated were US (with and without US), storage time (0 and 7 days), and area of concentric circles (C1, C2, and C3). The statistical model is described as follows (Equation 3):

$$Y_{ijkl} = \mu + A_i + B_j + C_k + AB_{ij} + AC_{ik} + BC_{jk} + ABC_{ijk} + E_{ijkl} \quad (3)$$

where Y_{ijkl} = dependent variable (pH, L*, a*, b*, chroma, WHC (%), shear force (kg_f) and mesophilic, psychrophilic and coliform bacteria ($\log_{10}$ CFU mL^{-1}); μ = Mean; A_i, B_j, C_k = Factor A effect (US), B (storage time: 0 y 7 days) and C (concentric circles: C1, C2 y C3); AB_{ij} + AC_{ik} + BC_{jk} + ABC_{ijk} = interaction effects A*B, A*C, B*C and A*B*C; E_{ijkl} = Experimental error.

Data were analyzed using SAS software (v. 9.00; SAS Institute, Cary, USA) with an α = 0.05.

3 Results and discussion

3.1 Measurement of pH

Differences in pH were observed in the interaction between US-treated and control samples and storage time ($P < 0.0001$). The results showed that high-power US decreased the pH of beef, and the pH value decreased after 7 d of aging (Figure 4).

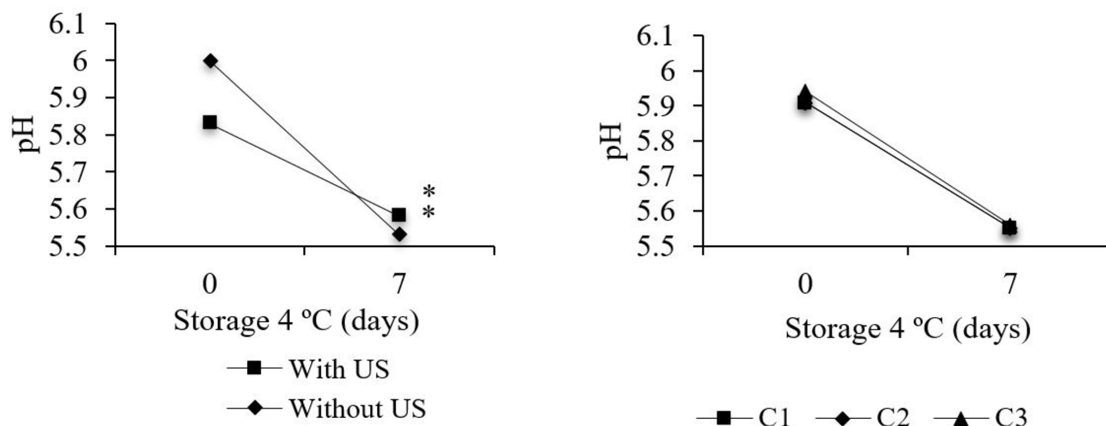

Figure 4. Effects of ultrasonic treatment, storage time, and concentric area on the pH of *longissimus dorsi*. Means with asterisk in each subfigure are statistically different (Tukey, $P \leq 0.05$).

Caraveo et al. (2015) reported similar results in semitendinosus bovine muscle (non-ultrasonicated, pH = 5.51 versus ultrasonicated at 40 kHz, 11 W/cm^2 for 60 min, pH = 5.35). In addition, pH reduction was observed in US-treated muscle (60 and 90 min) after day 10 of storage at 4 °C. In contrast, Stadnik & Dolatowski (2011) found no effect of US on aging of bovine semimembranosus muscle from 24-96 h. Other authors have revealed the absence of any influence of US on the meat pH (Jayasooriya et al., 2007; Stadnik et al., 2008; Stadnik & Dolatowski 2011). As shown in Figure 4, US intensity was homogeneously distributed in the samples, as demonstrated by the equal changes in pH throughout the samples. A no significant decrease in pH was observed for C1, C2 and C3 treated with US after 7 days of storage at 4 °C. The pH values observed are similar to those of a good quality meat (5.4 and 5.8) and are similar to other reports (Stenström et al., 2014) for aged chilled meat.

3.2 Color space CIE L*a*b*

Lightness (L*) of meat was unaffected by US (P = 0.3246) and storage time (P = 0.2406) (Table 2); however, L* of C2 was higher than other concentric areas (P = 0.0028). Storage time exerted effects on both red (a*) and yellow (b*) color of the meat (P < 0.0001), showing an increase on day 7 at 4 °C. The saturation value presented similar tendency and increased from 19.85 on day 0 to 24.1 on day 7. A significant interaction was observed between the parameters L*, a*, b*, and chroma (saturation) (Figure 5). Therefore, the observed changes may be a consequence of the natural phenomenon of muscle maturation. The stored muscles showed a greater "blooming" ability due to the low pH. Similar results have been reported (O'Keeffe & Hood, 1982), the difference is attributed to the capacity of "blooming" between stored and non-stored meat. The changes during maduration could be due to the loss of activity in enzymes that use oxygen. Low pH is the main factor in postmortem loss of mitochondrial structural integrity and functionality.

On the contrary, other studies have reported that CIE L*a*b* values are affected by US treatment (Stadnik & Dolatowski, 2011; Sikes et al., 2014), as the heat generated is sufficient to cause protein denaturation and oxidation of color pigments (Jayasooriya et al., 2007). Color measurements in US-treated pectoralis muscle (22 W/cm^2) reported by Pohlman et al. (1997) were different, as these author observed a change toward lighter color (lower luminosity), less red (low a* values), more yellow (high b* values), more orange (larger hue angle), and less brightness

Table 2. Effects of ultrasonic treatment, storage time, and concentric areas on CIE L*a*b of *longissimus dorsi*.

US	Luminosity	a*	b*	Chrome
With US	37.5 ± 2.19^a	19.01 ± 3.72^a	11.17 ± 3.08^a	21.75 ± 4.23^a
Without US	38.01 ± 2.32^a	19.12 ± 1.59^a	11.26 ± 1.15^a	22.19 ± 1.91^a
Storage time (days)				
0 d	37.45 ± 2.14^a	17.23 ± 2.36^b	9.82 ± 1.58^b	19.85 ± 2.71^b
7 d	38.06 ± 2.35^a	20.98 ± 1.93^a	12.61 ± 2.04^a	24.1 ± 2.19^a
Concentric areas				
C1	37.69 ± 1.6^b	18.88 ± 3.07^a	10.64 ± 2.37^a	21.69 ± 3.79^a
C2	38.99 ± 1.98^a	18.4 ± 2.96^a	11.8 ± 2.83^a	21.38 ± 3.15^a
C3	36.58 ± 2.51^b	19.91 ± 2.41^a	11.2 ± 1.55^a	22.85 ± 2.82^a

Means with different letters are statistically different (Tukey, $P \leq 0.05$).

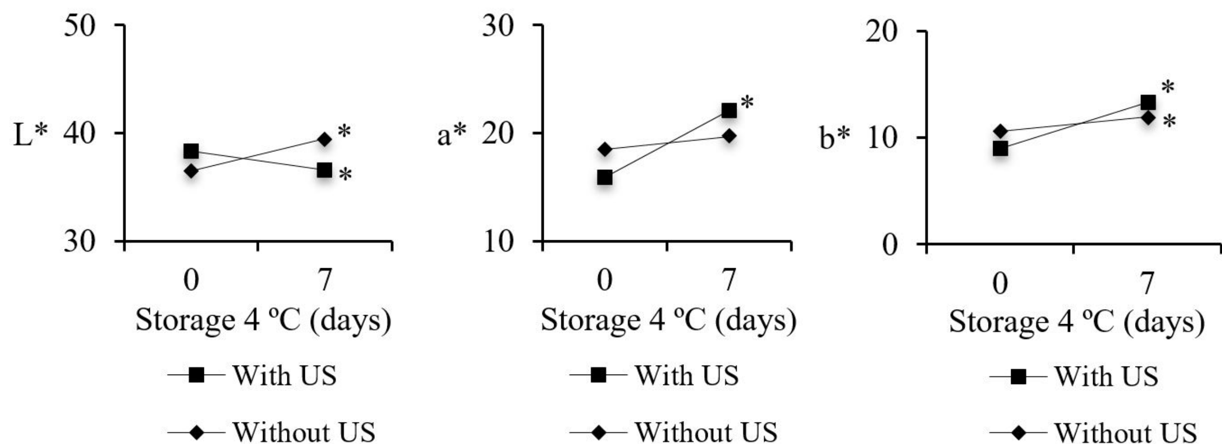

Figure 5. Effects of the interaction between ultrasound (US) and storage time (0 and 7 days) on the luminosity (L*), redness (a*) and yellowness (b*) of *longissimus dorsi*. Means with asterisk are statistically different (Tukey, $P \leq 0.05$).

with respect to the control. In addition, Stadnik & Dolatowski (2011) observed that US accelerates the change in total color, limits the formation of oxygenated myoglobin (MbO_2), and slows down the formation of metmyoglobin (MetMb).

3.3 Water-Holding Capacity (WHC)

No statistical differences were reported among US treatments (P = 0.1165), storage time (P = 0.9375) and concentric area (P = 0.9017). A significant interaction was observed between ultrasonic treatment and storage time (P = 0.0007). An increasing trend was observed in the muscle treated with US on day 7 at 4 °C (Figure 6). Immediately after sonication (0 d) WHC was much lower than that of no-sonicated samples. However, WHC of sonicated samples increased with storage showing higher values than the control. WHC is a vital variable in meat quality, mainly during aging, as the decrease in this parameter results in economic losses to the meat industry (Gambuteanu et al., 2013). WHC is obtained by subtracting the percentage of exudate released from 100%; hence, it refers to the water retained in the muscle.

In the non-sonicated muscles the WHC decreased after 7 days of storage at 4 °C, probably because during storage the pH decreased to values near 5.4, close to the isoelectric point of the proteins (when the net charge of the proteins is zero), particularly myosin, causing a lower capacity to attract water by reducing the space between the myofibrils (Kristensen & Purslow, 2001). We hypothesized that the samples treated with US had higher WHC because the pH was not as low as in the US-untreated muscle.

The results observed in the present study are in line with those reported by Chang et al. (2015), wherein US increased exudate and water loss rates in meat. However, Smith et al. (1991) found no effect of US on WHC and inferred that water immobilized in myofibrillar tissue remained fixed to proteins.

McDonnell et al. (2014) and Siró et al. (2009) revealed no effects of US on pork WHC during salting and considered US-assisted curing to be a surface-level phenomenon. In contrast, other authors showed that ultrasonicated meat displayed higher WHC than the untreated meat (Pohlman et al., 1997; Dolatowski et al., 2007; Stadnik et al., 2008). Kang et al. (2017) reported higher WHC during the curing of beef using US (150 and 300 W). These researchers found that the moderate oxidation of myosin causes polymerization, thereby contributing to the increase in WHC. WHC was similar between C1, C2, and C3, highlighting the homogeneity in the transfer of sound waves in samples through distilled water used as the propagation medium (Figure 6).

3.4 Shear force

Shear force of meat was shown to have no significant effect by US treatment (P = 0.6711), storage time (P = 0.4184), concentric area (P = 0.725), and interactions between factors. Similar to the natural maturation process, a decrease in muscle toughness was observed with storage time, but this effect was non-significant at day 7 of storage. A non-significant difference in toughness was found between different concentric areas of the muscle. Benefits with the use of US include the reduction in natural differences of the muscle texture, as observed with the lower variability in the shear force of US-treated meat samples (Table 3). Therefore, the use of ultrasonic baths may decrease the natural heterogeneity in the quality of bovine muscle.

Texture and tenderness are considered as the most important meat characteristics for the consumers. In general, the texture of the muscle in pre-rigor is tender, but the meat loses its tenderness during rigor-mortis owing to the effects of shortening of sarcomeres and loss of ATP. Meat texture also depends on the size of the fiber bundles within the perimysal connective tissue, i.e., on the fiber diameter and amount of connective tissue in the muscle (Lawrie & Ledward, 2006). It has been proposed that acoustic cavitation induces mechanical rupture of myofibrillar proteins (Stadnik et al., 2008), fragmentation of collagen macromolecules, and migration of proteins, minerals, and other compounds, thereby accelerating proteolysis or protein denaturation (Siró et al., 2009). Got et al. (1999) found that US application to pre-rigor muscle resulted in a slower

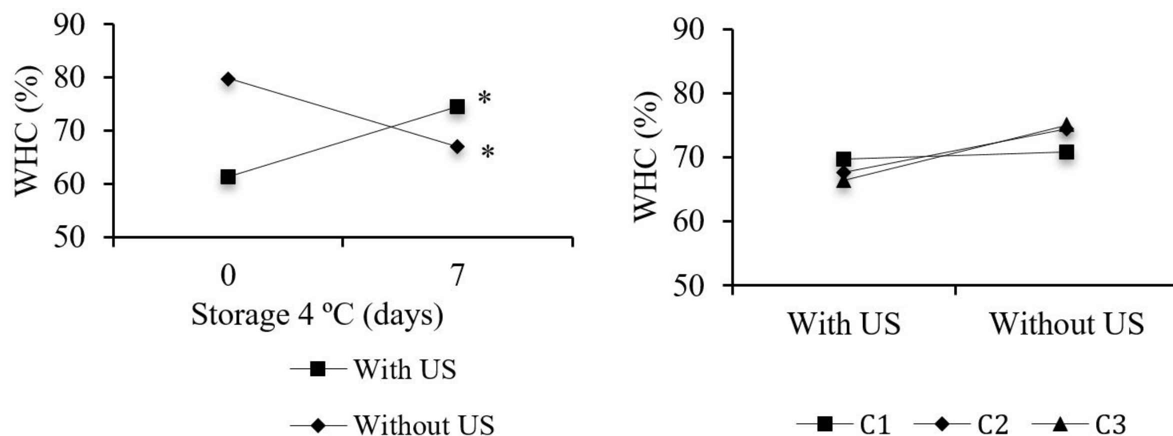

Figure 6. Effects of the interaction between ultrasound (US) and storage time (0 and 7 days) on the WHC of *longissimus dorsi*. Means with asterisk are statistically different (Tukey, *P≤0.05)*.

Table 3. Effects of ultrasonic treatment, storage time, and concentric areas on the WHC and shear force of *longissimus dorsi*.

US	WHC (%)	Shear force (kg)
With US	67.95 ± 3.08[a]	2.32 ± 3.69[a]
Without US	73.41 ± 1.8[a]	2.25 ± 0.80[a]
Storage time (days)		
0 d	70.55 ± 2.69[a]	2.36 ± 0.67[a]
7 d	70.81 ± 2.17[a]	2.21 ± 0.82[a]
Concentric areas		
C1	70.27 ± 3.17[a]	2.25 ± 0.83[a]
C2	71.05 ± 1.99[a]	2.39 ± 0.74[a]
C3	70.72 ± 2.36[a]	2.22 ± 0.68[a]

Means with different letters are statistically different (Tukey, $P \leq 0.05$).

rigor mortis and increased the length of sarcomeres by up to 15%. In addition, these authors observed an alteration in Z-line, due to a 30% increase in the release of calcium into the cytosol. However, these effects failed to affect the tenderness of the meat. The findings of the studies showing that US increases the tenderness of meat are contradictory. Jayasooriya et al. (2007) found that US (24 kHz, 12 W/cm^2) treatment of bovine muscle for 4 min increased the meat tenderness during storage. Stadnik & Dolatowski (2011) treated semimembranosus muscle with US (45 kHz and 2 W/cm^2 for 2 min) and observed a decrease in shear force. Similar results were observed by Chang et al. (2015) after treatment of semitendinosus muscle with US (40 kHz, 1500 W for 10, 20, 30, 40, 50, or 60 min) and Peña-González et al. (2017) after treatment of longissimus dorsi with US (40 kHz and 11 W/cm^2 for 60 min). On the other hand, Lyng et al. (1997) failed to observe any significant increase in meat tenderness after US treatment at 0.29-0.62 W/cm^2 intensity and 30-47 kHz frequency in *longissimus, semitendinosus*, or *biceps femoris* (McDonnell et al., 2014). Shear force may be expected to decrease after aging, as postmortem degradation of myofibrillar proteins is closely related to structural changes that result in greater tenderness (Lian et al., 2013). In this study, US-treated samples failed to follow this trend. The frequency, intensity, and time of application may be the contributing factors for our results. On the other hand, we observed homogeneity in the hardness of US-treated samples (C1, C2, and C3), indicating that the acoustic waves in US baths were uniformly transmitted through the samples. These results are consistent with those obtained for pH, color, and percentage exudate released. Hence, US baths for high-power sonication of food items of vegetable and/or animal origin may be useful in the food industry for the incorporation of additives into muscles during marination or ingredient addition in meat products (González-González et al., 2017).

3.5 Microbiological counts

The inactivation of microorganisms following exposure to US has been known for many years. The antimicrobial action is associated with the acoustic cavitation and its physical and chemical effects. The combination of US and other non-thermal methods is known to improve the effectiveness of this technology. The potential of US to damage and break biological cell walls may be useful to destroy living cells; however, very high intensities of US may be needed; Hence, US may be coupled with other methods such as bactericides and heat treatment (Jayasooriya et al., 2004). In this approach, US has been used in combination with other methods for the reduction of *Salmonella* in chicken (Lillard, 1993). US is used with marination in red wine against *Listeria monocytogenes* as well as *Brochothrix thermosphacta* and *Campylobacter jejuni* in pork muscles (Birk & Knochel, 2009).

In the present study, significant differences in mesophilic bacterial count was observed (Figure 7) owing to the effects of US ($P < 0.0001$), storage time ($P < 0.0001$), and interaction between US and storage time ($P = 0.0135$). Mesophilic bacteria significantly increased after day 7 of aging. The use of US decreased the mesophilic bacterial count. However, the bacterial count increased in both sonicated and control samples during storage. The results reported by several researchers on the effect of high-power US on the growth of mesophilic bacteria are variable. For instance, Dolatowski & Stasiak (2002) found that mesophilic and aerobic bacteria can be controlled using high-intensity US (25 kHz). However, other studies have shown that aerobic bacterial counts significantly increased after day 7 of aging, as US may increase the nutrient availability (Joyce et al., 2003), modify the structure of proteins, or alter microbial metabolism. Furthermore, Joyce et al. (2011) mentioned that sonication exerted the effect of disintegration or declumping in addition to bacterial inactivation. However, the scale of these effects depends on the intensity and frequency. We found a significant difference in psychrophilic bacteria (Figure 7) between US-treated and control samples ($P < 0.0001$), storage time ($P < 0.0001$), and interaction of US with storage time ($P < 0.0015$). High-power US decreased the count of psychrophilic bacteria. Thus, US is an effective method for controlling these microorganisms. Storage of up to 7 days decreased the count of psychrophilic bacteria in both control and sonicated samples. Sams & Feria (1991) reported that high-power US promoted the release of nutrients from food during refrigerated storage, resulting in a significant increase in the bacterial count. In the current study, refrigerated storage for 7 days increased the number of psychrophilic bacteria regardless of the use of US.

According to Figure 7 a significant decrease in the counts of coliform bacteria was observed with the use of US ($P < 0.0001$). Caraveo et al. (2015) observed a significant increase in total coliform counts during aging of semitendinosus bovine muscle at

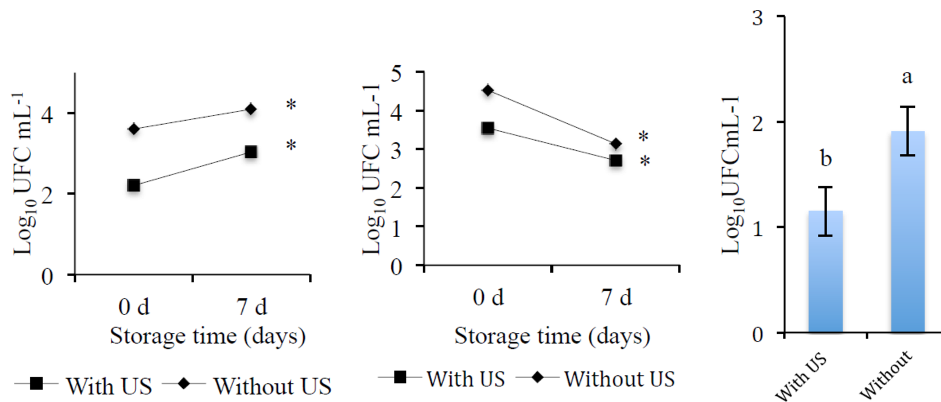

Figure 7. Effects of ultrasonic treatment and storage time on mesophilic bacteria (left), psychrophilic bacteria (centre) and coliform bacteria (right) in *longissimus dorsi*. Means with different letters or asterisk in each subfigure are statistically different (Tukey, $P \leq 0.05$).

4 °C following sonication for 60 or 90 min in an ultrasonic bath (40 kHz, 11 W/cm^2). On the other hand, studies with other food classes have reported the inhibition of *Escherichia coli* (Nazari & Jochen, 2010). The temperature of US treatment is known as a critical factor for the control of bacteria. Thermosonication of milk using an ultrasonic processor (20 kHz, 600 W, 120 µm) for 12 min at 20 °C and 60 °C was shown to induce a decrease of coliform from 3.07 to 2.49 log CFU/mL (Herceg et al., 2012). Other technologies paired with US include pressure (mansonication), osmosis (osmosonication), electrical pulses, ozone, ultraviolet irradiation, antimicrobial solutions, and enzyme solutions (Boziaris, 2014).

4 Conclusion

The application of US was shown to have no negative effects on the physicochemical properties of meat. US was demonstrated to preserve the safety of the beef. High-power US significantly lowered the counts of mesophilic, psychrophilic and coliform bacteria during storage. The combined results of luminosity, redness, saturation, pH and WHC indicate that the application of US is both feasible and effective, as the distribution of US waves decreases the natural heterogeneity in quality characteristics such as texture and WHC of bovine *longissimus dorsi*. This may be particularly beneficial in processes that include marination and ingredient addition. Furthermore, US is useful for the reduction of mesophiles, psycrophiles, and coliforms from meat.

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