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Ultrasound as a Potential Technology to Improve the Quality of Meat Produced from a Mexican Autochthonous Bovine Breed

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Abstract: The objective of this study was to evaluate the effect of high-intensity ultrasound (HIU) on the physicochemical and textural properties of meat from *Rararumi Criollo*, a Mexican autochthonous bovine breed. After slaughter, *Longissimus dorsi* and *Triceps brachii* muscles were separated from carcasses, cut into 2.5 cm slices and treated with HIU, except the control group, which was not sonicated. After treatment, samples were vacuum-sealed and stored at 4 °C for 0, 3, 6, 9, 12 and 15 d. HIU increased ($p < 0.05$) the luminosity and yellowness (b*) of meat. Higher b* ($p < 0.05$) was observed in *L. dorsi* than in *T. brachii* muscles. No effect ($p > 0.05$) of HIU was detected on drip loss, pH, the water holding capacity and the total collagen of meat. The shear force of HIU-treated meat was lower ($p < 0.05$) than control samples, indicating a tenderizing effect. There were differences between muscles. *L. dorsi* was more tender ($p < 0.05$), and it had higher pH and WHC values than *T. brachii.* Overall, HIU is a potential method for tenderizing *Raramuri Criollo* cattle meat without negative impacts on other quality characteristics. HIU is an emerging technology that could add value to indigenous breeds and provide a new opportunity for the growing meat market.

Keywords: native breeds; *Raramuri Criollo* cattle; beef; high-intensity ultrasound; meat quality

1. Introduction

Today, the global food industry aims to offer natural, healthy, safe food with high nutritional value and a longer shelf life [1]. In addition, with regard to food consumption, consumers are more health-conscious and aware of the quality, safety, and environmental friendliness of their food. For consumers, the most relevant aspects of food healthiness are the ingredients, nutrition facts, and additives, while packaging, food origin, and production type are associated with environmental impact [2]. In this regard, to extend the shelf life, physical and non-invasive methods have become important, as they can improve processing efficiency. One of the methods used for food processing is high-intensity ultrasound (HIU) [3]. In the last decade, HIU alone or in combination with other methods has been used to improve meat quality characteristics, such as its water-holding capacity, pH and colour. Previously, ultrasound has been used to modify the functional properties of proteins, lengthen shelf life, and inactivate microorganisms in meat and meat products [4]. High-intensity (>1 W/cm^2) and low-frequency ultrasound (20–100 kHz) generates cavitation bubbles that cause physical and chemical modifications in muscle

tissues [5]. Several studies have been carried out to determine the effect of HIU on beef quality, and they have reported favorable effects on meat tenderness and water-holding capacity [6,7]. Currently, there are many ultrasound studies on beef quality, and the vast majority have focused on cuts of high commercial value (*L. dorsi* and *Semitendinosus*); however, information on the effect of HIU on muscles with less commercial value (i.e., tougher, less juiciness, and redder) are scarce. Similarly, most of the scientific reports are based on European breeds' muscles, and at present, no study has been performed focused on the use of ultrasound on the properties of Mexican autochthonous bovine breeds.

Autochthonous or local cattle breeds have become increasingly important for sustainable meat production. Their natural evolution in particular regions/conditions has granted them advantages over conventional breeds such as Angus, as they can consume low-quality diets with higher efficiency, leading to the production of a protein with decreased environmental impacts on soil and vegetation [8–10]. However, meat produced from local breeds does not always satisfy the requirements of the meat industry, and hence, this meat is commercialized through niche markets [11,12].

The *Raramuri Criollo* cattle is an autochthonous breed from the Chihuahua Mountains of Mexico. Although these animals are mostly used for rodeo, they can also be used for meat production [13,14]. Producers who raise *Raramuri Criollo* cattle are interested in producing meat to meet the growing demand for grass-fed meat in the United States, as this breed is reared in the border between Mexico and the US [15]. In 2015, the organic and free-range meat market in the United States was valued between USD 1 billion and USD 3 billion [16]. However, anecdotal experiences have created an incorrect perception of the textural properties of *Criollo* meat in the market, characterizing it as tough and dry. To date, there is very little information on *Raramuri Criollo* meat's tenderness, colour and water-holding capacity. Furthermore, there are no reports on the effects of ultrasound on *Raramuri Criollo* meat's physicochemical properties. Therefore, the objective of this study was to determine the quality parameters of *L. dorsi* and *T. brachii* from *Raramuri Criollo* cattle and the effect of HIU on meat quality and microstructure. The hypothesis was that applying ultrasonication would improve *Raramuri Criollo* meat quality.

2. Materials and Methods

2.1. Animals

Meat from this study was obtained from *Raramuri Criollo* cattle fed on pasture until 17 months, finished for 3 months and fed concentrated fees (18% crude protein) [17] and grazed in meadows based on alfalfa and ryegrass. Animals were slaughtered (~30 m old) in the municipal slaughterhouse of the city according to the Official Mexican Regulations [18]. Whole *Longissimus dorsi* (n = 3) and *Triceps brachi* (n = 3) were separated from the carcasses and used for this study (Figure 1). These muscles were selected because they are from different anatomical regions and have different commercial values. *L. dorsi* is a soft muscle, and *T. brachii* is considered a tough muscle with low commercial value [19].

2.2. Meat Samples

Subcutaneous fat and connective tissue were removed from each muscle. Perpendicular to the muscle fiber direction, muscles were sliced into 144 samples with 2.5 cm thickness (n = 72 *L. dorsi* and n = 72 *T. brachii*). Each sample was randomly assigned to one of 24 treatments composed of 2 sonication times (time 0, which is the control, and 20 min), 6 storage times (0, 3, 6, 9, 12 and 15 days at 4 °C) and 2 muscles (*L. dorsi* and *T. brachii*), resulting in a completely random factorial experimental design with 3 factors (HIU time factor at 2 levels, storage time factor at 6 levels, and muscle type factor at 2 levels): 2 (HIU times) × 6 (storage periods) × 2 (muscle type) = 24. Therefore, the experiment had 24 treatments and 6 repetitions (n = 144). Except for the control, which was not sonicated, each sample was immediately individually vacuum-packed, sonicated, and stored at 4 °C for experimental analyses (Figure 1).

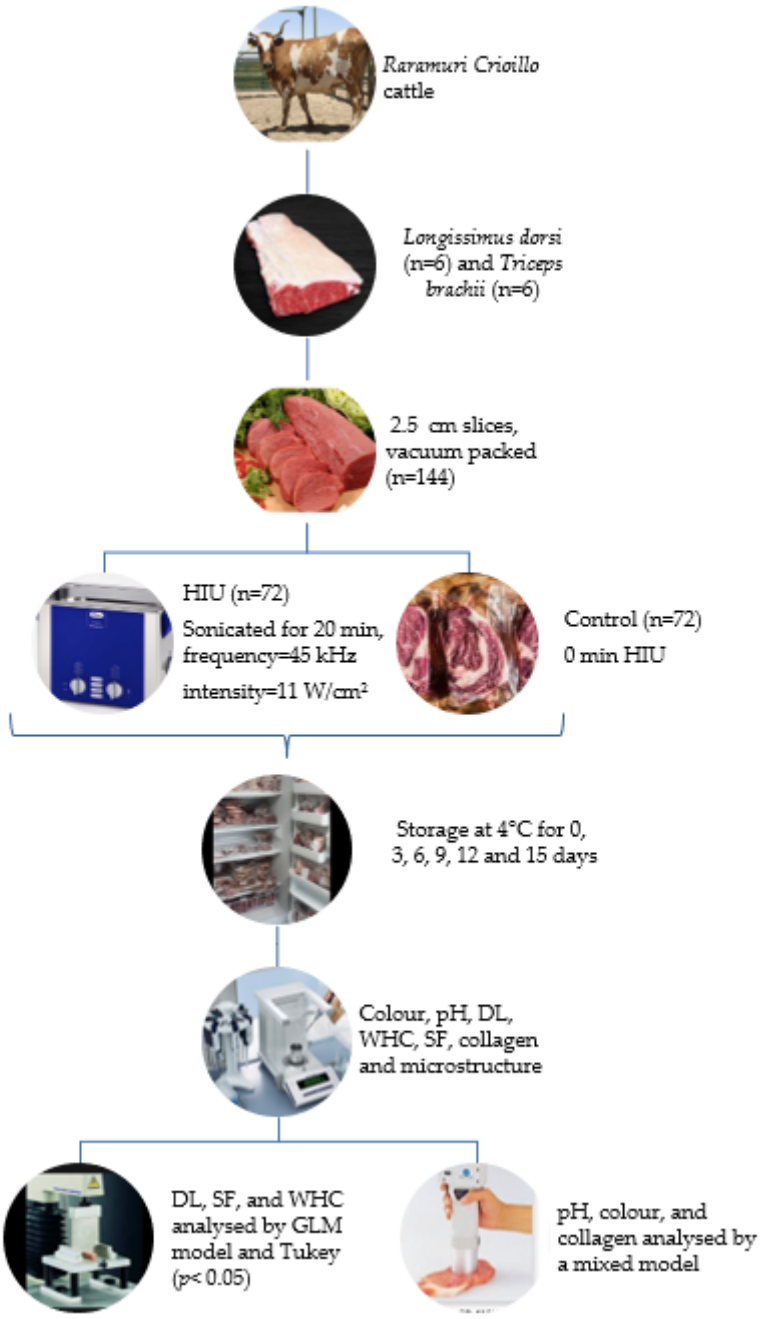

Figure 1. Diagram of the experiment. DL = drip loss; WHC = water-holding capacity; SF = shear force.

2.3. Ultrasound Treatment

Sample sonication was carried out in a bath (Elmasonic brand model Xtra ST, Singen, Germany) with a capacity of 30 L using distilled water as the diffusion medium. The water temperature was maintained in a range of 4.7–6 °C by using an immersion cooler (Julabo model FT402, Seelbach, Germany). The ultrasound treatment was performed at a frequency of 45 kHz with an intensity of 11 W/cm^2 for 20 min. Treatments were 0 min (control sample, not sonicated) and 20 min of ultrasound. After treatment, the samples were stored at 4 °C for 0, 3, 6, 9, 12 and 15 d. The effect of ultrasound and storage on meat tenderness and shear force was measured on day 0 immediately after sonication and was compared to the meat shear force from each storage period.

2.4. Colour Determination

Colour was determined using a colorimeter (PCE Instruments model PCE-XXM 20, Meschede, Germany). Before measurement, the equipment was calibrated following the manufacturer's instructions. Colour values were expressed as L* (light/dark), a* (red/green), b* (yellow/blue), C* (chroma) $(a^{*2} + b^{*2})^{1/2}$ and H° (hue angle) = arctan (b*/a*). Measurements were made after the oxygenation of samples on areas that were free of visible adipose and connective tissues. Three measurements were made per sample and the averages were recorded. The delta E (ΔE) value was also calculated to obtain the difference between the colours of the control and sonicated samples $(\Delta E^* = ((L^*_0 - L^*_t)^2 + (a^*_0 - a^*_t)^2 + (b^*_0 - b^*_t)^2)^{1/2}$, where the 0 subindexes correspond to non-sonicated meat samples, and the "t" subindexes correspond to meat samples treated for 20 min with ultrasound.

2.5. pH Determination

The pH was recorded using a potentiometer (Hanna Instruments; 99,163; Nusfalău, Romania) calibrated with buffer solutions of pH 4.0 and 7.0. Three readings were made at three different positions within the muscle sample, and means were recorded.

2.6. Drip Loss

The modified technique of Honikel and Hamm [20] was used. Briefly, 3 g of muscle was suspended in a plastic container and stored at 4 °C for 48 h. Then, the weight of the sample was recorded. Weight loss was expressed as a percentage.

2.7. Water-Holding Capacity

The water-holding capacity (WHC) was determined using the pressing method [21] modified by Tsai and Ockerman [22]. A sample of 0.5 g was placed between two filter papers (Number 1, 15 mm pore diam., Whatman, Sigma, Neustadt an der Weinstraße, Germany) and placed between two Plexiglas plates, on which a constant weight pressure of 10 kg was exerted for 15 min. Weight loss was expressed as a percentage.

2.8. Shear Force

Shear force was determined using the American Association for Meat Science method [23]. Briefly, samples were cooked on grills until the meat reached a temperature of 70 ± 1 °C at the geometric center. Subsequently, the samples were stored at 4 °C for 24 h. Then, 6 cylinders with a diameter of 12.7 mm were obtained per sample using an electric hole punch, taking care that these were parallel to the longitudinal orientation of the muscle fibers. The cylinders were cut using a Warner Bratzler blade in a "V" shape (60° triangular opening) at a speed of 2.0 mm/s. The maximum force (expressed as kg) to cut each cylinder transversely was recorded with a TA-TX-plus texture analyzer (Stable Micro Systems Ltd., Surrey, UK).

2.9. Collagen

The quantification of collagen was carried out using the methodology of the International Organization for Standardization [24]. This was based on the hydrolysis of 10 g of sample in 30 mL of sulfuric acid solution (3 M) at 105 °C for 16 h. Then, the hydrolyzate was filtered and diluted. The oxidation reaction was carried out with 4 mL of dilution and 2 mL of chloramine-T mixed in a shaker and was allowed to stand for 20 min at room temperature. Subsequently, 2 mL of p-dimethylamino-benzaldehyde solution was added, mixed in a shaker, and placed in a water bath at 60 °C for 20 min. The samples were cooled for 3 min in a water bath and left to stabilize for 30 min at room temperature. Finally, absorbance was measured (DLAB brand spectrophotometer model SP-UV1100, Beijing, China) at 558 nm. The standard curve was prepared with hydroxyproline at 0.5, 1, 1.5 and 2 µg/mL. The hydroxyproline content was calculated using the equation: Wn = 6.25 C/mV, where Wn is the hydroxyproline content expressed as a percentage by mass, C is the hydroxyproline concentration in µg/mL, m is the mass in grams of the

sample and V is the volume in ml of the hydrolysate, which was up to 250 mL. A conversion factor of 7.25 was used to calculate the collagen content [25].

2.10. Scanning Electron Microscopy (SEM)

A total of 3 treatments were used for SEM analysis: control and HIU treatment at 0, 6 and 15 d of storage. Cubic (0.5 cm^3) samples were cut under a stereoscope (Carl Zeiss, Oberkochen, Germany) from the surface of the sonicated and non-sonicated samples and fixed using a 2.5% glutaraldehyde solution diluted in a phosphate buffer solution (pH 7.2). Subsequently, cubes were dehydrated in ethanol at 30, 40, 50, 60, 70, 80, 90, 2 × 100% for 40 min. The samples were dried at a critical point using CO_2 as a transitional fluid dryer (Samdri-780 A Tousimis, Rockville, ML, USA) after which they were covered with gold-palladium (JEOL, fine coat sputter JFC-1100, Tokyo, Japan) to favor conduction for the scanning electron microscope (JEOL JSM 6390 SEM, Peabody, ML, USA). This was performed using an accelerating voltage of 5 kV and obtaining micrographs with magnifications of 200× to characterize fibres and spaces between muscle fibres.

2.11. Statistical Analysis

The data from drip loss, WHC and shear force were analyzed using the GLM general linear model procedure using a confidence level of 95%. A completely randomized experimental design that included three factors, ultrasound time (2), muscle type (2) and days of storage (6), as well as their possible interactions, resulting in a factorial design (2 × 2 × 6), was used. Tukey tests were used to determine significant differences within the means of ultrasound, storage time and muscle treatments. Variables measured over time (pH, colour coordinates, and types of collagen) were analyzed with a mixed model (MIXED) considering treatment, muscle, storage and their interactions as fixed effects, and treatment x muscle nested in storage day as a random effect. All analyses were performed using the statistical package SAS 9.4 [26].

3. Results and Discussion

The main effects of HIU, as well as the interaction between treatments and meat colour quality, are shown in Table 1. Main significant effects were observed on colour (HUE and ΔE) and shear force (SF). Storage had effects on pH, colour (b*, C*, HUE and ΔE), WHC, SF, and collagen (soluble, insoluble and total). Significant effects were also observed by muscle type on colour (b*, HUE and ΔE), pH, WHC, SF and collagen (soluble, insoluble and total) of meat, as presented below.

Table 1. Significant effects associated with the *F* statistic (*p*-values) by GLM or a mixed model analysis. The means represent 6 storage times (0, 3, 6, 9, 12 and 15 d) with 2 ultrasonication times (HIU, 0 and 20 min) from 2 muscles (*Longissimus dorsi* and *Triceps brachii*).

Variable [1]	Factor/Interaction						
	HIU	**Storage (S)**	**Muscle (M)**	**HIU × M**	**HIU × S**	**M × S**	**HIU × M × S**
pH	0.1991	<0.001	<0.0001	0.2505	0.0352	0.0015	0.4041
L*	0.0030	0.7416	0.1007	0.2187	0.4160	<0.0001	0.0667
a*	0.6512	0.0015	0.5546	0.2366	0.0828	<0.0001	0.0014
b*	0.0227	0.0475	<0.001	0.9913	0.0785	0.1346	0.3168
C*	0.7564	0.0031	0.3369	0.3834	0.1345	0.0002	0.0068
HUE	0.0139	<0.001	<0.001	0.1620	0.0073	<0.001	0.0021
ΔE	0.0801	<0.001	0.5612	0.3063	<0.0001	0.9844	0.7458
DL	0.996	0.214	0.6719	0.2178	0.1039	0.0155	0.4338
WHC	0.0539	0.0580	0.0030	0.2117	0.0056	0.0247	0.1970
SF	0.0096	<0.001	<0.001	0.8602	0.0005	0.0023	0.0011
Fsoluble	0.1675	<0.001	<0.001	0.2206	0.2666	<0.001	0.4050
Finsoluble	0.1690	<0.001	<0.001	0.2191	0.2653	<0.001	0.4073
Total collagen	0.1680	<0.001	<0.001	0.2182	0.2643	<0.001	0.4080

[1] L* = lightness; a* = redness; b* = yellowness; chroma (C*) = total colour; hue angle = the angle with the a* axis. Larger values of the hue angle indicate less red colour; ΔE = colour/energy difference; Fsoluble = soluble fraction of collagen; FIinsoluble = insoluble fraction of collagen. HIU= high-intensity ultrasound; DL = drip loss; WHC = water-holding capacity of meat; SF = shear force.

3.1. Colour

An effect of ultrasound treatment and muscle type ($p < 0.05$) on some colour parameters was found (Table 2). Sonicated meat had higher yellowness (b* value) than non-sonicated meat. Furthermore, b* was higher in *L. dorsi* than *T. brachii*. Changes in beef colour could be attributed to factors inherent to the breed such as the concentration and chemical state of pigments, structural attributes within the muscle cell, and the amount of carotenoids deposited in intramuscular fat due to grazing [27]. This could indicate that ultrasound intensity affected the colour due to chemical changes in tissue pigments [28]. Reports of the effect of HIU on beef colour are highly variable.

Table 2. Colour parameters (CIE L*a*b*, Chroma, HUE and ΔE) of muscles treated with high-intensity ultrasound (HIU) and stored at different times (least square means ± standard error).

	CIE L*a*b* [1]					
Factor	**L***	**a***	**b***	**C***	**HUE**	**ΔE**
HIU (min)						
0	31.67 ± 0.26 ^{b}	13.90 ± 0.29 ^{a}	9.12 ± 0.14 ^{b}	16.66 ± 0.30 ^{a}	33.80 ± 5.30 ^{b}	0.93 ± 0.024 ^{a}
20	32.81 ± 0.26 ^{a}	13.72 ± 0.29 ^{a}	9.60 ± 0.14 ^{a}	16.79 ± 0.30 ^{a}	35.30 ± 4.67 ^{a}	0.87 ± 0.024 ^{a}
Muscle (M)						
T. brachii	32.62 ± 0.26 ^{a}	13.93 ± 0.29 ^{a}	8.82 ± 0.14 ^{b}	16.52 ± 0.30 ^{a}	32.73 ± 3.62 ^{b}	0.89 ± 0.024 ^{a}
L. dorsi	31.93 ± 0.26 ^{a}	13.69 ± 0.29 ^{a}	9.90 ± 0.14 ^{a}	16.93 ± 0.30 ^{a}	36.37 ± 5.58 ^{a}	0.91 ± 0.024 ^{a}
Storage (d)						
0	32.62 ± 0.45 ^{a}	14.71 ± 0.49 ^{ab}	9.20 ± 0.25 ^{ab}	17.38 ± 0.52 ^{a}	34.47 ± 3.64 ^{c}	0.00 ± 0.04 ^{f}
3	32.58 ± 0.45 ^{a}	12.66 ± 0.49 ^{bc}	9.54 ± 0.25 ^{ab}	15.98 ± 0.52 ^{ab}	37.83 ± 7.42 ^{a}	0.52 ± 0.04 ^{e}
6	31.74 ± 0.45 ^{a}	13.89 ± 0.49 ^{abc}	9.40 ± 0.25 ^{ab}	16.81 ± 0.52 ^{ab}	34.22 ± 4.00 ^{bc}	1.10 ± 0.04 ^{c}
9	32.29 ± 0.45 ^{a}	12.44 ± 0.49 ^{c}	8.77 ± 0.25 ^{b}	15.13 ± 0.52 ^{b}	35.55 ± 3.48 ^{ab}	0.76 ± 0.04 ^{d}
12	31.93 ± 0.45 ^{a}	14.06 ± 0.49 ^{abc}	9.20 ± 0.25 ^{ab}	16.90 ± 0.52 ^{ab}	33.46 ± 3.95 ^{bc}	1.36 ± 0.04 ^{b}
15	32.11 ± 0.45 ^{a}	15.10 ± 0.49 ^{a}	9.20 ± 0.25 ^{a}	18.16 ± 0.52 ^{a}	33.78 ± 5.54 ^{bc}	1.68 ± 0.04 ^{a}
HIU * storage						
0 min						
0 d	32.56 ± 0.63 ^{a}	15.19 ± 0.70 ^{a}	9.30 ± 0.35 ^{a}	17.87 ± 0.74 ^{a}	32.23 ± 0.14	0.00 ± 0.06 ^{g}
3 d	32.04 ± 0.63 ^{a}	12.38 ± 0.70 ^{a}	9.38 ± 0.35 ^{a}	15.65 ± 0.74 ^{a}	37.63 ± 0.07	0.61 ± 0.06 ^{e}
6 d	31.01 ± 0.63 ^{a}	14.43 ± 0.70 ^{a}	8.62 ± 0.35 ^{b}	16.84 ± 0.74 ^{a}	31.18 ± 0.14	0.86 ± 0.06 ^{d}
9 d	31.79 ± 0.63 ^{a}	12.92 ± 0.70 ^{a}	8.61 ± 0.35 ^{b}	15.31 ± 0.74 ^{a}	33.89 ± 0.11	0.77 ± 0.06 ^{de}
12 d	30.76 ± 0.63 ^{a}	12.84 ± 0.70 ^{a}	8.76 ± 0.35 ^{a}	15.58 ± 0.74 ^{a}	34.29 ± 0.05	1.39 ± 0.06 ^{c}
15 d	31.87 ± 0.63 ^{a}	15.63 ± 0.70 ^{a}	10.07 ± 0.35 ^{a}	18.73 ± 0.74 ^{a}	33.58 ± 0.04	1.59 ± 0.06 ^{b}
20 min						
0 d	32.67 ± 0.63 ^{a}	14.23 ± 0.70 ^{a}	9.11 ± 0.35 ^{a}	16.91 ± 0.74 ^{a}	32.71 ± 0.14	0.00 ± 0.06 ^{g}
3 d	33.12 ± 0.63 ^{a}	12.94 ± 0.70 ^{a}	9.69 ± 0.35 ^{a}	16.31 ± 0.74 ^{a}	38.04 ± 0.13	0.42 ± 0.06 ^{f}
6 d	32.47 ± 0.63 ^{a}	13.34 ± 0.70 ^{a}	10.17 ± 0.35 ^{a}	16.79 ± 0.74 ^{a}	37.27 ± 0.17	1.34 ± 0.06 ^{c}
9 d	32.80 ± 0.63 ^{a}	11.95 ± 0.70 ^{b}	8.93 ± 0.35 ^{a}	14.95 ± 0.74 ^{a}	37.21 ± 0.06	0.74 ± 0.06 ^{e}
12 d	33.46 ± 0.63 ^{a}	15.28 ± 0.70 ^{a}	9.88 ± 0.35 ^{a}	18.22 ± 0.74 ^{a}	32.64 ± 0.08	1.33 ± 0.06 ^{c}
15 d	32.34 ± 0.63 ^{a}	14.57 ± 0.70 ^{a}	9.81 ± 0.35 ^{a}	17.59 ± 0.74 ^{a}	33.87 ± 0.05	1.77 ± 0.06 ^{a}
M * storage						
L. dorsi						
0 d	31.21 ± 0.63 ^{b}	16.13 ± 0.70 ^{a}	9.58 ± 0.35 ^{a}	18.80 ± 0.74 ^{a}	31.16 ± 3.84	0.00 ± 0.06 ^{a}
3 d	32.72 ± 0.63 ^{ab}	10.2 ± 0.70 ^{d}	9.99 ± 0.35 ^{a}	14.30 ± 0.74 ^{c}	44.45 ± 3.50	0.51 ± 0.06 ^{a}
6 d	30.74 ± 0.63 ^{b}	14.4 ± 0.70 ^{b}	9.71 ± 0.35 ^{a}	17.50 ± 0.74 ^{ab}	34.07 ± 5.39	1.10 ± 0.06 ^{a}
9 d	30.7 ± 0.63 ^{b}	13.84 ± 0.70 ^{bc}	9.07 ± 0.35 ^{a}	16.33 ± 0.74 ^{ab}	33.59 ± 3.10	0.78 ± 0.06 ^{a}
12 d	33.34 ± 0.63 ^{a}	14.17 ± 0.70 ^{b}	10.49 ± 0.35 ^{a}	17.65 ± 0.74 ^{a}	36.60 ± 3.20	1.36 ± 0.06 ^{a}
15 d	32.84 ± 0.63 ^{a}	13.33 ± 0.70 ^{c}	10.54 ± 0.35 ^{a}	17.02 ± 0.74 ^{a}	38.38 ± 3.23	1.71 ± 0.06 ^{a}
T. brachii						
0 d	34.02 ± 0.63 ^{a}	13.2 ± 0.70 ^{c}	8.83 ± 0.35 ^{a}	15.97 ± 0.74 ^{b}	33.78 ± 3.20	0.00 ± 0.06 ^{a}
3 d	32.44 ± 0.63 ^{ab}	15.13 ± 0.70 ^{ab}	9.09 ± 0.35 ^{a}	17.65 ± 0.74 ^{a}	31.22 ± 1.97	0.51 ± 0.06 ^{a}
6 d	32.75 ± 0.63 ^{a}	13.31 ± 0.70 ^{c}	9.09 ± 0.35 ^{a}	16.13 ± 0.74 ^{b}	34.37 ± 2.48	1.10 ± 0.06 ^{a}
9 d	33.83 ± 0.63 ^{a}	11.04 ± 0.70 ^{d}	8.47 ± 0.35 ^{a}	13.92 ± 0.74 ^{c}	37.51 ± 2.78	0.73 ± 0.06 ^{a}
12 d	30.87 ± 0.63 ^{b}	13.95 ± 0.70 ^{b}	8.15 ± 0.35 ^{a}	16.15 ± 0.74 ^{b}	30.33 ± 0.77	1.36 ± 0.06 ^{a}
15 d	31.37 ± 0.63 ^{b}	16.87 ± 0.70 ^{a}	9.34 ± 0.35 ^{a}	19.30 ± 0.74 ^{a}	29.17 ± 2.50	1.65 ± 0.06 ^{a}

[1] L* = luminosity, a* = redness, b* = yellowness, C* = chroma, HUE= hue angle, ΔE = delta E, the colour difference. $^{a–g}$ Column means within each variable or interaction with different superscripts differ significantly ($p < 0.05$). HIU * storage = high intensity ultrasound × storage combination. M * storage = Muscle × storage combination.

The values found in this experiment were similar to those reported by Orellana et al. [29], who compared the meat colour parameters of Argentine Creole cattle with European cattle, determining that the meat of Creole cattle had a darker colour than meat

from European-breed cattle. Similarly, Sañudo et al. [30] reported that rustic breeds such as Creole have darker meat than genetically improved breeds. In this regard, meat from *Raramuri Criollo* steers is dark; nevertheless, this meat could have market opportunities to be sold in the local market. In the present study, significant differences ($p < 0.05$) in the total color change (delta E, ΔE) (Table 2) were observed due to ultrasound treatment, muscle type and days of storage. The ΔE of the sonicated meat was lower than the control treatment, and it was greater for *T. brachii* than *L. dorsi*.

According to the international regulation [31], the ΔE values were in the ranges from excellent to good, so it could be inferred that the difference observed in colour was due to ultrasound. Although effect was significant, it was mild and may not affect meat colour perception. The variability of the L*, a* and b* values is attributed to factors that influence the meat colour, such as muscle, packaging, and storage time [32]. Meat usually has average L* values of 35 and positive values of a* and b* [7]. Differences in meat colour could also be explained by structural attributes within the muscle cell [28].

3.2. pH

Table 3 shows the pH values, water-holding capacity, drip loss and shear force of the meat. No significant differences were detected in meat pH ($p > 0.05$), but there were significant effects ($p < 0.05$) on muscle type, storage time and the interactions of ultrasound × storage and muscle × storage. *T. brachii* had higher pH values than *L. dorsi*. The variations in pH can be attributed to the release of ionic groups from protein structures into the extracellular environment. The pH values from this study are within the normal range (5.00–5.50) for fresh beef [32]. Some authors reported values similar to the present study (5.42) in the *L. dorsi* muscle of Argentine Creole and Bradford cattle [29] as well as in Creole steers and Creole crosses with Angus cattle [33].

3.3. Drip Loss

No effects of ultrasound treatment, type of muscle, storage time, and their interactions on drip loss (DL) ($p > 0.05$) were observed (Table 3). The use of ultrasound did not show an effect ($p > 0.05$) on WHC, but effects of muscle type and storage and their interactions were found ($p < 0.05$). Drip loss is an important quality criterion for meat processing and for consumers and is defined as the red aqueous protein solution that emerges on the meat surface. Drip loss measures the amount of exudated water from the extracellular matrix of meat [32]. Although no effect of HIU was found in the present study, some authors such as Chang et al. [34] mentioned that ultrasound could lead to higher exudate in beef treated with 40 kHz and 1500 W from 10 to 60 min.

3.4. Water-Holding Capacity

The *L. dorsi* had higher WHC values than *T. brachii*, and the highest WHC value was observed on day 0 with a tendency to decrease with storage time (Table 3). These variations in WHC can be attributed to the denaturation of myofibrillar proteins caused by ultrasound. Through cavitation, HIU produces physical forces such as shock waves and microjets that cause a change in the functional properties of proteins [35]. Ultrasound can change the spatial conformation and solubility of the myofibrillar protein that bind and intercept water and thus affect WHC. This may explain the values at day 0 (immediately after sonication) when proteins were still intact, since that day, the highest WHC value was observed. The values of WHC (Table 3) are higher than those reported by Orellana et al. [29] for Argentine Creole breeds and crosses with zebu. It should be noted that in the study by Orellana et al. [29], beef was not sonicated.

3.5. Shear Force

For SF, the effect of ultrasound treatment, muscle type, storage time and their interaction was observed ($p < 0.05$). In general, the sonicated samples had lower SF than the control. Among the different muscles, *T. brachii* had higher SF values than *L. dorsi* during

storage, and the lowest value was observed on day 0, with a tendency to increase on day 3 and remain constant during the storage period (Table 3).

Table 3. Physicochemical variables of muscles treated with high-intensity ultrasound (HIU) and stored at different times (least square means ± standard error).

	Physicochemical Variables			
Variable [1]	**pH**	**DL**	**WHC**	**SF**
HIU (min)				
0	5.33 ± 0.004 ^{a}	1.79 ± 0.13 ^{a}	69.13 ± 0.57 ^{a}	3.75 ± 0.08 ^{a}
20	5.35 ± 0.004 ^{a}	1.78 ± 0.13 ^{a}	67.54 ± 0.57 ^{a}	3.46 ± 0.08 ^{b}
Muscle (M)				
Triceps brachii	5.41 ± 0.004 ^{a}	1.83 ± 0.13 ^{a}	67.08 ± 0.57 ^{b}	4.15 ± 0.08 ^{a}
L. dorsi	5.27 ± 0.004 ^{b}	1.75 ± 0.13 ^{a}	69.59 ± 0.57 ^{a}	3.07 ± 0.08 ^{b}
Storage (d)				
0	5.34 ± 0.004 ^{b}	1.71 ± 0.23 ^{a}	70.83 ± 0.98 ^{a}	2.49 ± 0.13 ^{b}
3	5.35 ± 0.004 ^{ab}	1.92 ± 0.23 ^{a}	67.33 ± 0.98 ^{ab}	3.66 ± 0.13 ^{a}
6	5.28 ± 0.004 ^{b}	1.68 ± 0.23 ^{a}	67.87 ± 0.98 ^{ab}	3.57 ± 0.13 ^{a}
9	5.32 ± 0.004 ^{b}	2.28 ± 0.23 ^{a}	66.64 ± 0.98 ^{b}	3.95 ± 0.13 ^{a}
12	5.33 ± 0.004 ^{b}	1.47 ± 0.23 ^{a}	69.22 ± 0.98 ^{ab}	4.06 ± 0.13 ^{a}
15	5.43 ± 0.004 ^{a}	1.68 ± 0.23 ^{a}	68.12 ± 0.98 ^{ab}	3.92 ± 0.13 ^{a}
HIU * storage				
0 min				
0 d	5.26 ± 0.03 ^{c}	1.68 ± 0.32 ^{a}	72.50 ± 1.39 ^{a}	2.31 ± 0.18 ^{c}
3 d	5.35 ± 0.03 ^{b}	1.50 ± 0.32 ^{a}	67.15 ± 1.39 ^{b}	4.11 ± 0.18 ^{a}
6 d	5.28 ± 0.03 ^{c}	1.61 ± 0.32 ^{a}	66.31 ± 1.39 ^{c}	3.49 ± 0.18 ^{b}
9 d	5.32 ± 0.03 ^{bc}	2.85 ± 0.32 ^{a}	69.43 ± 1.39 ^{ab}	4.38 ± 0.18 ^{a}
12 d	5.34 ± 0.03 ^{bc}	1.45 ± 0.32 ^{a}	68.38 ± 1.39 ^{b}	4.45 ± 0.18 ^{a}
15 d	5.43 ± 0.03 ^{a}	1.64 ± 0.32 ^{a}	71.00 ± 1.39 ^{a}	3.77 ± 0.18 ^{b}
20 min				
0 d	5.41 ± 0.03 ^{a}	1.74 ± 0.32 ^{a}	69.16 ± 1.39 ^{ab}	2.66 ± 0.18 ^{c}
3 d	5.35 ± 0.03 ^{b}	2.34 ± 0.32 ^{a}	67.51 ± 1.39 ^{b}	3.22 ± 0.18 ^{b}
6 d	5.28 ± 0.03 ^{c}	1.75 ± 0.32 ^{a}	69.44 ± 1.39 ^{ab}	3.64 ± 0.18 ^{b}
9 d	5.32 ± 0.03 ^{bc}	1.70 ± 0.32 ^{a}	63.84 ± 1.39 ^{d}	3.52 ± 0.18 ^{b}
12 d	5.32 ± 0.03 ^{bc}	1.48 ± 0.32 ^{a}	70.05 ± 1.39 ^{a}	3.67 ± 0.18 ^{b}
15 d	5.43 ± 0.03 ^{a}	1.72 ± 0.32 ^{a}	65.26 ± 1.39	4.07 ± 0.18 ^{a}
M * storage				
L. dorsi				
0 d	5.24 ± 0.03 ^{b}	1.52 ± 0.32 ^{b}	74.91 ± 1.39 ^{a}	2.31 ± 0.18 ^{d}
3 d	5.27 ± 0.03 ^{b}	2.47 ± 0.32 ^{a}	68.30 ± 1.39 ^{c}	2.92 ± 0.18 ^{cd}
6 d	5.14 ± 0.03 ^{c}	1.52 ± 0.32 ^{b}	67.48 ± 1.39 ^{c}	3.06 ± 0.18 ^{c}
9 d	5.27 ± 0.03 ^{b}	1.62 ± 0.32 ^{b}	68.70 ± 1.39 ^{c}	3.63 ± 0.18 ^{c}
12 d	5.30 ± 0.03 ^{b}	1.54 ± 0.32 ^{b}	70.29 ± 1.39 ^{b}	3.13 ± 0.18 ^{c}
15 d	5.41 ± 0.03 ^{a}	1.82 ± 0.32 ^{a}	67.86 ± 1.39 ^{c}	3.34 ± 0.18 ^{c}
T. brachii				
0 d	5.43 ± 0.03 ^{a}	1.90 ± 0.32 ^{a}	66.75 ± 1.39 ^{c}	2.66 ± 0.18 ^{d}
3 d	5.43 ± 0.03 ^{a}	1.37 ± 0.32 ^{b}	66.36 ± 1.39 ^{c}	4.40 ± 0.18 ^{b}
6 d	5.41 ± 0.03 ^{a}	1.84 ± 0.32 ^{a}	68.27 ± 1.39 ^{c}	4.07 ± 0.18 ^{b}
9 d	5.37 ± 0.03 ^{a}	2.94 ± 0.32 ^{a}	64.58 ± 1.39 ^{d}	4.28 ± 0.18 ^{b}
12 d	5.36 ± 0.03 ^{b}	1.40 ± 0.32 ^{b}	68.15 ± 1.39 ^{c}	4.99 ± 0.18 ^{a}
15 d	5.45 ± 0.03 ^{a}	1.53 ± 0.32 ^{b}	68.39 ± 1.39 ^{c}	4.50 ± 0.18 ^{b}

[1] DL = drip loss (%); WHC = water-holding capacity (%); SF = shear force (N). ^{a,b,c,d} Column means within each variable or interaction with different superscripts differ significantly ($p < 0.05$). HIU * storage = high intensity ultrasound × storage combination. M * storage = Muscle × storage combination.

The application of HIU has been studied by Peña-Gonzalez et al. [7] as a method to improve the tenderness of fresh meat by reducing meat SF. Those authors reported no detrimental effect of HIU on other quality parameters such as colour, WHC and DL.

This reduction in SF has been attributed to a number of physical and chemical factors triggered by ultrasound treatment. Such effects result in the alteration and breakdown of the structure of muscle fibers that promote proteolysis and denaturation of proteins due to the activation of endogenous enzymes such as cathepsins and/or calpains [34], which accelerate the ageing processes and the fragmentation of collagen macromolecules [36].

In the present study, SF decreased immediately after sonication, with lower values than the control treatment (Table 3). These results are consistent with those observed previously [7,37] when applying HIU to beef. The favorable effects reported in meat tenderness after a short time of HIU application were also demonstrated in the present study. This is a very promising result since the tenderness of *Raramuri Criollo* meat could be improved with the use of ultrasound. The tenderizing effect of HIU on meat could be related to the activation of calpains, the proteolytic activity involved in protein degradation that induces meat tenderization [38].

In this study, an important factor that influenced beef toughness was the type of muscle, leading to differences in SF between *L. dorsi* and *T. brachii* (Table 3). Different types of muscle fibers can help explain variations in SF. During storage, sarcoplasmic proteins and slow-twitch fibers (type I or red) are less susceptible to proteolysis than fast-twitch glycolytic muscle proteins (type IIb or white) [39]. This means that white fiber muscles are softer than red fiber muscles. The observations of the present study agree with this since *L. dorsi*, a predominant white muscle, was more tender than *T. brachii*, which is predominantly a red muscle and tougher than *L. dorsi*.

The SF values of this study are lower than those reported by Orellana et al. [29] for both the control and sonicated treatments. The tenderness values found in this study allowed the beef of both breeds to be classified as 'tender' [40]. The difference between the values can be attributed to the type/breed of cattle, feeding system, and ultrasound treatment.

3.6. Collagen

No effect of ultrasound treatment was observed on any type of collagen ($p > 0.05$). The *T. brachii* muscle had a higher amount of soluble collagen, insoluble collagen and total collagen than *L. dorsi*. During the storage period, the three types of collagen showed a marked tendency to be reduced ($p < 0.05$) during storage from 0 to 12 d (Table 4).

There are several studies reporting the effect of ultrasound on beef collagen. Gonzalez-Gonzalez et al. [6] applied HIU (40 kHz, 11 W/cm^2 and 80 min) and observed changes in collagen at 7 and 14 d of storage. They reported that sonicated samples had a decrease in the concentration of total collagen compared to control samples. Similar results were observed by other authors [41], who found effects at 28 kHz and 40 kHz at 20 min on the ultrastructure and infrastructure of collagen fibers in beef.

Chang et al. [34] found no effects of HIU (40 kHz, 1500 W) on the content of insoluble collagen in bovine *Semitendinosus* muscle, but they observed a small increase in the concentration of soluble collagen with 50 min of sonication. This effect was not observed in the present study, probably because the meat was sonicated only for 20 min. This time may have been too short to induce effects on the collagen molecule. Differences were observed in collagen concentrations between *T. brachii* and *L. dorsi.* The decrease in collagen solubility occurs simultaneously with the increase in shear force, which also increases with the animal's age.

During storage, muscles undergo a series of physical and biochemical changes related to the weakening of myofibrillar proteins. It has been reported that proteoglycans, which bind collagen fibrils, remain unchanged up to 10 d of storage, but a progression of structural alterations is clearly visible after 14 d [42]. This agrees with the results of the present study, where a decrease in collagen was observed during storage, and this decrease was more visible after 6 d of storage (Table 4).

Table 4. Collagen and total collagen fractions of muscles treated with high-intensity ultrasound and stored at different times (least square means ± standard error).

	Collagen µg /mL		
Variable	**Soluble Fraction**	**Insoluble Fraction**	**Total Collagen**
HIU (min)			
0	3.60 ± 0.016 [a]	3.47 ± 0.015 [a]	7.06 ± 0.031 [a]
20	3.63 ± 0.016 [a]	3.50 ± 0.015 [a]	7.13 ± 0.031 [a]
Muscle type			
Triceps brachii	4.23 ± 0.016 [a]	4.08 ± 0.015 [a]	8.31 ± 0.031 [a]
L. dorsi	2.99 ± 0.016 [b]	2.88 ± 0.015 [b]	5.88 ± 0.031 [b]
Storage (d)			
0	5.19± 0.028 [a]	5.00 ± 0.027 [a]	10.2 ± 0.054 [a]
3	3.05± 0.028 [e]	2.94± 0.027 [e]	6.00 ± 0.054 [e]
6	3.24± 0.028 [d]	3.13± 0.027 [d]	6.37 ± 0.054 [d]
9	3.49± 0.028 [c]	3.37± 0.027 [c]	6.86 ± 0.054 [c]
12	1.83 ± 0.028 [f]	1.77± 0.027 [f]	3.60 ± 0.054 [f]
15	4.86 ± 0.028 [b]	4.69± 0.027 [b]	9.55 ± 0.054 [b]
HIU * storage			
0 min			
0 d	5.14 ± 0.039 [a]	4.96 ± 0.038 [a]	10.1 ± 0.077 [a]
3 d	3.05 ± 0.039 [a]	2.94 ± 0.038 [a]	5.99 ± 0.077 [a]
6 d	3.26 ± 0.039 [a]	3.14 ± 0.038 [a]	6.41 ± 0.077 [a]
9 d	3.50 ± 0.039 [a]	3.37 ± 0.038 [a]	6.87 ± 0.077 [a]
12 d	1.77 ± 0.039 [a]	1.71 ± 0.038 [a]	3.49 ± 0.077 [a]
15 d	4.86 ± 0.039 [a]	4.68 ± 0.038 [a]	9.54 ± 0.077 [a]
20 min			
0 d	5.25 ± 0.039 [a]	5.06 ± 0.038 [a]	10.3 ± 0.077 [a]
3 d	3.06 ± 0.039 [a]	2.95 ± 0.038 [a]	6.00 ± 0.077 [a]
6 d	3.22 ± 0.039 [a]	3.11 ± 0.038 [a]	6.33 ± 0.077 [a]
9 d	3.48 ± 0.039 [a]	3.36 ± 0.038 [a]	6.84 ± 0.077 [a]
12 d	1.89 ± 0.039 [a]	1.82 ± 0.038 [a]	3.71 ± 0.077 [a]
15 d	4.87 ± 0.039 [a]	4.69 ± 0.038 [a]	9.56 ± 0.077 [a]
M * storage			
L. dorsi			
0 min			
0 d			
3 d	5.75 ± 0.039 [a]	5.55 ± 0.038 [a]	11.30 ± 0.077 [a]
6 d	2.54 ± 0.039 [d]	2.45 ± 0.038 [c]	4.99 ± 0.077 [d]
9 d	1.81 ± 0.039 [d]	1.74 ± 0.038 [d]	3.55 ± 0.077 [d]
12 d	2.69 ± 0.039 [d]	2.59 ± 0.038 [c]	5.28 ± 0.077 [d]
15 d	1.67 ± 0.039 [d]	1.61 ± 0.038 [d]	3.27 ± 0.077 [d]
T. brachii	3.50 ± 0.039 [c]	3.38 ± 0.038 [b]	6.88 ± 0.077 [c]
0 min			
0 d	4.63 ± 0.039 [b]	4.46 ± 0.038 [b]	9.09 ± 0.077 [b]
3 d	3.57 ± 0.039 [c]	3.44± 0.038 [b]	7.01 ± 0.077 [c]
6 d	4.68 ± 0.039 [b]	4.51 ± 0.038 [b]	9.19 ± 0.077 [b]
9 d	2.30 ± 0.039 [d]	4.14 ± 0.038 [b]	8.44 ± 0.077 [b]
12 d	2.00 ± 0.039 [d]	1.93 ± 0.038 [d]	3.93 ± 0.077 [d]
15 d	6.22 ± 0.039 [a]	6.00 ± 0.038 [a]	12.22 ± 0.077 [a]

[a–f] Column means within each variable or interaction with different superscripts differ significantly ($p < 0.05$). HIU * storage = high intensity ultrasound × storage combination. M * storage = Muscle × storage combination.

3.7. Microstructure

Figures 2 and 3 show the microstructural changes in *L. dorsi* and *T. brachii* muscles after ultrasound treatment and storage for 0, 6 and 15 d. In these figures, some structural alterations such as disorganization in the myofibrillar arrangement in both muscles can be observed immediately after ultrasound treatment and during storage. In general, muscle fibers tended to separate from each other in both muscles under ultrasound treatment for

20 min. Samples that were sonicated for 20 min showed an increase in the interfibrillar area of both muscles with the same storage time. This indicated an effect on the structure of the connective tissue, since in both cases, the interfibrillar space of the treated samples of both muscles had a tendency to increase with storage time. It was also found that the interfibrillar area decreased only on day 6 in *L. dorsi* muscles.

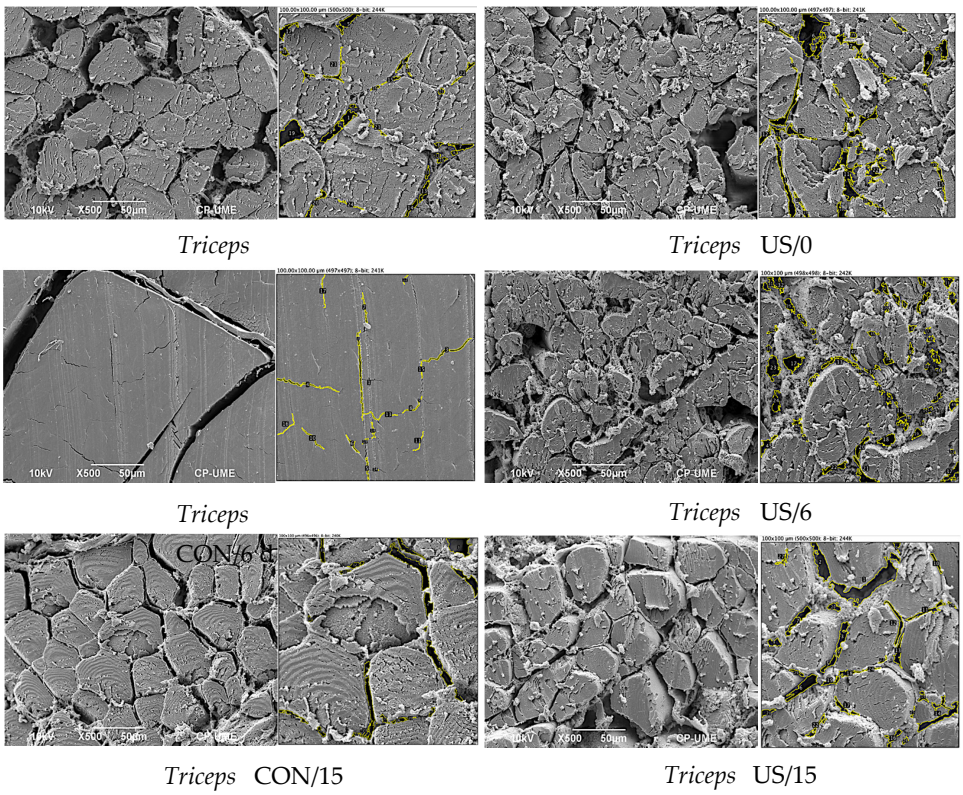

Figure 2. Microstructural changes in Longissimus dorsi from Creole Raramuri cattle without ultrasound (CON) and with ultrasound (US) during the storage period (0, 6 and 15 d). Scale bar = 50 μm, magnification 500×. The areas marked in yellow correspond to the areas of interfibrillar spaces in an area of 10,000 μm^2.

The decrease found in the interfibrillar area of both *L. dorsi* and *T. brachii* on day 6 of storage can be attributed to the low amount of connective tissue in this muscle. These results agree with those reported by Carrillo-Lopez et al. [43], who observed an increase in the interfibrillar space in *L. dorsi* immediately after the application of ultrasound at 37 kHz with intensities of 16 and 28 W/cm^2. On the contrary, González-González et al. [6] observed visible changes in the structure of the fibers of 3 muscles (*Longissimus lumborum*, *Infraspinatus* and *Cleidoccipital*) subjected to ultrasound at 40 kHz with an intensity of 11 W/cm^2 for 80 min. However, they did not report the treatment effect over the interfibrillar space.

The compact structure at 6 d of storage seems to be related to changes in the structure of myofibrillar proteins (myosin). According to Li et al. [44], ultrasound treatment of chicken paste suspensions increases gel strength, uniformity and compactness due to changes in the secondary structure of proteins (fewer alpha helices and more beta sheets). Ultrasound seems to induce protein oxidation due to cross-linking and protein aggregate formation, with negative effects on tenderness [45]. Table 3 confirms the increase in the *L. dorsi* shear force along with storage time. Although changes in interfibrillar space were observed at 15 d of storage, muscle hardness did not decrease at the end of the storage period. There is evidence that low-frequency and high-intensity ultrasound induces disruption of the muscle structure [7,34]. Peña-Gonzalez et al. [7] observed an increase in the distance between fibers and an increase in *L. dorsi* tenderness when applying ultrasound for 60 min after storage. However, those authors [7] applied ultrasound after the meat had been stored.

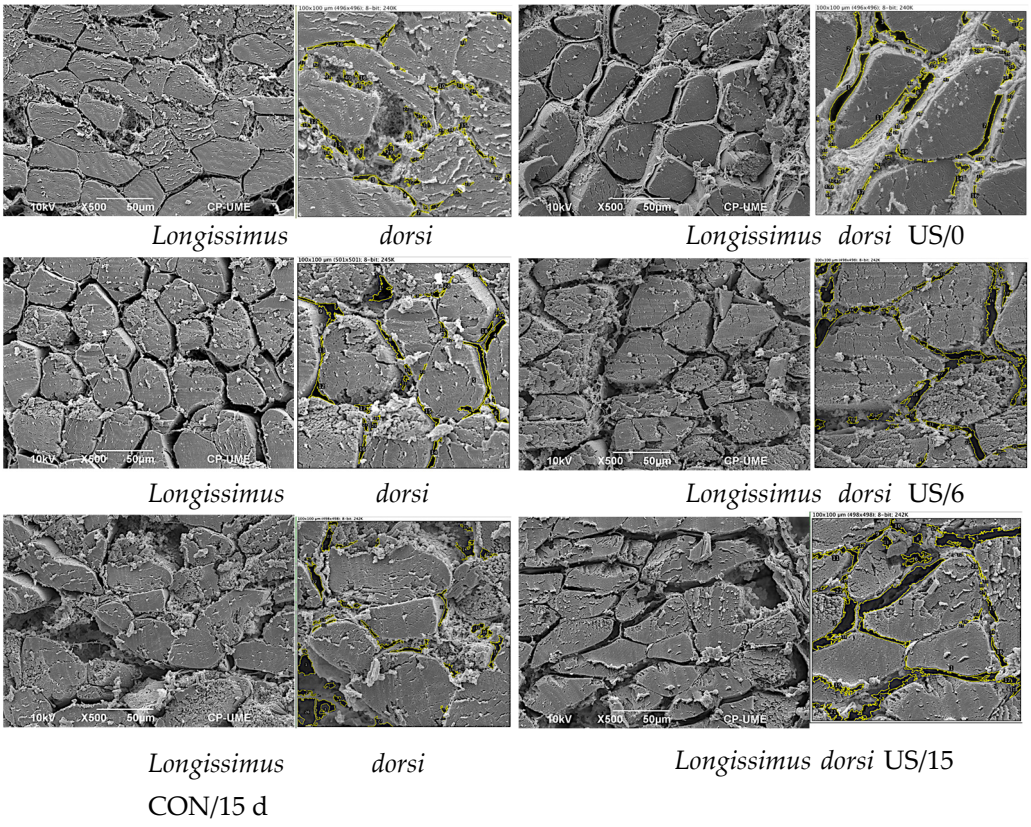

Figure 3. Microstructural changes in *Triceps brachii* from *Raramuri Criollo* cattle without ultrasound (CON) and with ultrasound (US) during the storage period (0, 6 and 15 d). Scale bar = 50 µm, magnification 500×. The areas marked in yellow correspond to the areas of interfibrillar spaces in an area of 10,000 μm^2.

4. Conclusions

The use of high-intensity ultrasound for 20 min on the *Triceps brachii* and *Longissimus dorsi* showed a decrease in shear force without negative effects on the colour, pH, water-holding capacity and drip loss of the meat. The use of ultrasound at a frequency of 45 kHz and intensity of 11 W/cm^2 for 20 min offers an alternative technique for tenderizing meat. More research is needed to characterize other meat quality characteristics from *Raramuri Criollo* cattle, as information is still scarce.

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