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Physicochemical Properties and Young Adult Consumer Preference of Dry-Aged Beef after High-Intensity Ultrasonication

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Abstract: This study aimed to determine the effect of high-intensity ultrasound (HIU. F = 37 kHz, I = 28W/cm^2, bath for 30 min, 5 °C) on physicochemical characteristics and sensorial preference of seven aged (23 d ageing) bovine muscles (*L. dorsi lumborum, L. dorsi thoracis, Psoas major, Semimembranosus, Biceps femoris, Rectus femoris,* and *Gluteus medius*). Muscles were randomly distributed in two treatments: with and without ultrasonication. Colour (L*, a*, b* and C*), water-holding capacity (WHC), and shear force (N) were determined before and after simulated retail display (SRD) in modified atmosphere packing (MAP; 75% O$_2$: 25% CO$_2$, 3 °C, 13 h led light exposition) for 5 d. Sensorial toughness was also evaluated at the end of the SRD. Ultrasonication slightly reduces 6–9% WHC of beef. HIU did not affect ($p > 0.05$) water loss, meat colour, shear force and sensorial toughness of the meat. The *Semimembranosus* was the toughest muscle. Ultrasonication of 23-day-aged beef did not show improvements on quality characteristics, and despite minor changes in water loss and slight increase in shear force, consumers did not detect differences.

Keywords: high-ultrasonication; dry-aged beef; beef tenderness; beef colour; consumer preference

1. Introduction

Meat ageing is a worldwide process carried out to allow a natural enzymatic proteolysis that improves meat tenderness, flavour, and juiciness [1,2]. Two general methodologies for beef ageing are widely known in the industry. The wet ageing of meat consists of placing the tissue under vacuum conditions, usually placed on sealed bags under low temperatures (4–1 °C) for specific amounts of time. Meanwhile, dry ageing consists of placing unpacked whole carcasses, primal cuts, or pieces of meat under low temperatures within a controlled environment (relative humidity and airflow) for a defined time. Under these conditions, the liquid is absorbed by the meat and its flavour is concentrated [3]. Historically, dry-aged beef has a particular niche market in the USA and Europe, and demand for it is rapidly growing in other countries, particularly in high-end restaurants, traditional, and gourmet retailers [4,5]. This ageing process is uncommon in many countries due to its high cost derived from shrinkage, trim loss, risk of contamination, technology for ageing, and space [6]. Tenderness and flavour increase in aged beef, which results from calpain activity from myofibrillar and cytoskeletal proteolysis in sarcomeres, more specifically, rupture of z lines during the first 14 to 21 d post-mortem [7]. Positive effects of ageing in beef tenderness also depend on the biochemistry (calpain–calpastatin activity) of the muscle, which can be linked to the breed of the animal. Hence, breeds from *Bos indicus* cattle such as Brahman

have been identified to display high calpastatin activity, and, despite the ageing process, it reduces the calpain functionality on post-mortem tenderization (ageing) resulting in tougher meat [8,9].

High-intensity ultrasound (HIU, >5 W/cm^2 or 10–1000 W/cm^2) has been successfully tested in fresh meat and meat products, improving tenderness, water-holding capacity (WHC), and mass transfer [10–18]. In most of these studies the HIU application to the meat was performed between 24 h to five days post-mortem, or during the process of transformation (i.e., curing, smoking, ageing, and freezing). Additionally, the application of HIU on fresh beef has been investigated at different ultrasound intensities, exposition times, and sample arrangements (i.e., cubes, muscle steaks, and cuts) [19–24]. Research has been focused on the effect of HIU on *Longissimus dorsi, Semimembranosus* and *Semitendinosus* textural properties [10,11]. Positive effects on tenderness have been found in bovine *Semimembranosus* when applying ultrasound (US) at 45 kHz, reducing shear force from 48 to 72 h post-mortem [25]. Further results have found that HIU (40 kHz, 1500 W, 10–60 min) applied to bovine *Semitendinosus* affects meat texture, particularly toughness. Toughness was reduced from 5500 g in control samples to 1000 g in sonicated beef [26]. Recently, HIU used on bovine *L. dorsi* at 11 W/cm^2/60 min reduced shear force (more than 20 N) compared with control after 7–14 d of retail display [19].

The effects on meat tenderness by HIU can be summarized by: (a) structure disruption, including myofibrillar proteins and collagen molecules, and/or (b) chemical changes promoting proteolysis or protein denaturation [26,27]. Additional benefits of applying HIU to meat have been described in colour improvement [23,24] and microbial reduction [12,18]. There are also reports of negative effects on oxidative stability and water loss. Comparisons of the effects of HIU on various muscles (*L. dorsi* vs. *Semitendinosus*) have reported different effects; for example, *Semitendinosus* showed no effects from ultrasonication, while *L. dorsi* was reported to be negatively affected when ultrasonicated inside vacuum bags [24]. Since HIU promotes a higher proteolysis [28] by enzymatic release leading to break of desmin and troponin-T structures [29], HIU has been considered as a technology for application in beef to accelerate [30] or alternate to the ageing process [23]. Furthermore, there are no reports on the effects of HIU on aged meat.

There is a lack of research on the effects of HIU among different muscles in the same animal. This study aimed to determine if there were additional effects of HIU application on physicochemical properties and young adult consumer preference of seven different muscles of bovine after dry-ageing for 23 d.

2. Materials and Methods

2.1. General Sampling and Sample Management

Two male, grass-fed bovines (Hereford × Longhorn) raised at Sul Ross State University (SRSU) were used to perform this study. Animals were slaughtered under SRSU regulations (IACUC AUP# 2018-ALP-AS-101) at 20 m age. After 21 d post-mortem (dry ageing at 1 °C, 80% relative humidity and air flow range of 1.0 m/s), which is the most common ageing period in the United States, seven muscles (*L. dorsi lumborum, L. dorsi thoracis, Psoas major, Semimembranosus, Biceps femoris, Rectus femoris* and *Gluteus medius*) from the right half of the carcasses were dissected. Approximately 3 kg of each muscle/animal were separated and vacuum-packed. After one day of storage, all muscle sections were placed in insulated boxes with ice, sealed, and transported (3 °C for 4 h) to the meat laboratory in Universidad Autonoma de Chihuahua (UACH). Upon arrival, muscle sections were refrigerated at 3 °C and 55% relative humidity for one additional day until the laboratory analysis. The day of analysis, the muscles were unpacked, and the excess of fat and connective tissue was trimmed. Later, the pH was monitored with a digital pH meter (Hanna Instruments, HI99163, Nusfalau, Romania), to guarantee that the meat was adequate for the study. The pH average was 5.58 ± 0.03. Later, the muscles were sliced (perpendicular to fibre direction) into 2 cm thick, 10 × 12 cm area steaks. Eight steaks per muscle were sampled from each

animal (56 steaks/animal). All analyses were developed at environmental temperature in the laboratory (22 °C).

2.2. Ultrasonication

Steaks from all muscles were randomly assigned into two treatments: the control group steaks (NS, no sonication) were immersed in 4 L of distilled water (6 °C) for 30 min, while the sonicated (US, F = 37 kHz, I = 28 Wcm^{-2}) treatment group steaks were immersed in an ultrasonication bath (Elmasonic$^®$ S 40 H, Elma Schmidbauer GmbH, Singen, Germany) with 4 L of distilled water for 30 min (15 min/side). Ultrasonication parameters were selected based on previous studies on meat where the intensity had positive effects on tenderness [13,24,31,32]. Ultrasonication bath temperature was kept constant at 5 °C with a refrigeration probe Julabo $^®$ (FT200). After, steaks were placed on a tray with absorbent paper for 5 min to remove excess of water. All steaks were exposed to simulated retail display (SRD) for 5 d in modified atmosphere packaging (MAP, 75% O_2: 25% CO_2, 3 °C, 13 h led light exposition/d). The steaks were individually displayed in PVC trays (PVC cryovac$^®$ trays, 18 × 10 × 5 cm, with PET-PVDC-PE top lidding, Charlotte, NC, USA) during the SRD.

2.3. Physicochemical Analyses

Drip loss was determined in triplicate following the methodology of Honikel and Hamm [33]. Briefly, 3 g of muscle were suspended inside a plastic bag for 48 h at 4 °C. Loss was calculated by difference of weight of the steak before and after treatment and the SRD. Differences in weight were calculated to obtain percentage of loss. WHC was calculated by duplicate for all steaks, except those used for sensorial analysis according to the methodology by Tsai and Ockerman [34].

Colour measurements were repeated in triplicate at three instances for each sample, 20 min after dissection (to allow initial blooming), and during SRD (every 24 h). Colour was measured with a Minolta chromameter (Konica Minolta Camera, UK. Aperture, 8 mm. Illuminant C. Standard observer, C: Y = 94.2, x = 0.3130 and y = 0.3190) following CIE Lab methodology (Roufs, 1978), considering L*, a*, b* and C* coordinates. The total colour difference (ΔE) between the original colour and the colour at the end of the SRD was calculated with the equation ΔE* = [ΔL*^{2} + Δa*^{2} + Δb*^{2}]$^{1/2}$.

Meat shear force analysis was performed using the Warner–Bratzler method, in accordance with recommendations by AMSA (AMSA, 2016). A TA.XT2i texture-meter (Stable Micro Systems, Surrey, UK) containing a V-shaped blade attached to a 50 N load cell with a cross head speed of 200 mm min-1 was used to perform the shear force test. Six cores were evaluated and averaged from every sample. Results of the shear force tests were reported as Newtons (N).

2.4. Consumer Preference

Consumer preference test was carried out in the sensory analysis laboratory (designed according to Standard ISO 8589:2007) from the Animal Science and Ecology Department, UACH. The sensory laboratory is equipped with individual booths illuminated by white lighting and controlled temperature (22 °C). The preference was determined using a paired test with randomly selected consumers (N = 60, 35 males and 25 females, from 22 to 38 years old) who reported to consume beef regularly. The age range was chosen to represent a young adult cohort. Every cooked sample was placed in plastic containers, coded with three-digit numbers, and presented in a randomized order of two samples per muscle (sonicated and non-sonicated) to the consumers. Consumers reported their preference (which of both samples was preferred) based on their personal taste. A total of seven pairs of samples were offered to the consumers in two different sessions. In both sessions, the consumers were provided with water for oral cleaning before the evaluation of each pair of muscle samples [35].

2.5. Statistical Analyses

Initially, variables were analysed using a general linear model procedure with a factorial arrangement (muscle × treatment) to detect main effect differences and interactions ($p < 0.05$). Animals were considered in the initial statistical model as blocks to dismiss heterogeneity among them. Since they were found to not be different, block effect was eliminated from the model. Tukey's post hoc tests were performed to compare means when a main effect ($p < 0.05$) of muscle was detected. T-paired tests were performed for each variable to identify differences ($p < 0.05$) between before and after treatment. Colour analysis considered day as a fixed effect and sample as a random effect in a fixed model. Consumer preference data was analysed using a Chi-square test to detect differences between males and females; later, when sex of the consumer resulted as not significant ($p > 0.05$), the same test was used to detect differences between treatments. All statistical analyses were performed using the software SPSS®, version 25 [36].

3. Results and Discussion

3.1. Physicochemical Traits

No differences ($p > 0.05$) were found in the water dynamic among muscles after the SRD. However, SRD alone reduced the WHC in all sonicated and control (non-sonicated) samples (Table 1). Nevertheless, the WHC reduction by sonication (6–9%) seemed slightly more marked in the control group; therefore, water losses appeared slightly increased when sonication was performed (Table 1).

Table 1. Water dynamic of 23-day-aged beef before and after being immersed in deionized water or ultrasonicated (US, 28 W/cm^2) in deionized water and placed under simulated retail display (75% O_2: 25% CO_2, 3 °C) for 5 d. WHC = Water-holding capacity.

	Before	**After**	***p***
	Control		
Water loss	41.4 ± 0.98	45.1 ± 0.81	0.02
WHC	58.6 ± 0.98	54.9 ± 0.81	0.02
	Sonicated		
Water loss	41.0 ± 0.65	47.3 ± 0.65	0.000
WHC	59.0 ± 0.65	52.7 ± 0.65	0.000

Previous studies have reported meat decolouration by HIU application at the same (22 W/cm^2) [25,37] or lower intensities (11 W/cm^2) in fresh meat from three different muscles [15]. Beef colour depends greatly on the chemical state of myoglobin, a sarcoplasm protein that may be easily oxide-reduced by the oxygen exposition resulting from quotidian physical or chemical processes in meat [38,39] (i.e., ageing [40], ultrasonication [10] or packaging [1,41]). HIU may detrimentally reduce meat colour when ultrasonication time is longer than 20 min, reducing redness and chroma and increasing the lightness [14,18].

In the present study, there was no effect of HIU on individual colour parameters of the aged beef. After meat ageing, colour among muscles was homogeneous even after treatment and SRD in all samples. Dry ageing itself has the capacity to modify beef colour. Dry ageing reduces L*, a*, and b* values independently of percentage of relative humidity (50–85%) when compared with wet ageing [40]. Moreover, adverse effects on colour can be worse when considering that beef wet ageing for 14–21 d also reduces the stability of colour during retail display [42]. The change of colour in meat by ageing is the result of protein oxidation and reduction of water activity in the tissue [30]. Due to these changes in the myoglobin and the beef colour by ageing, some effects of HIU on colour may be inapparent in this evaluation.

WHC varied among muscles in the same carcass. Varying metabolism among different muscle fibres in each muscle contributes to its capacity for retain liquids. Hence,

predominant oxidative fibres within a muscle produced a higher post-mortem WHC. Dry ageing of beef carcasses typically produces dehydration of the tissue, moisture loss, weight loss, and increase in WHC of the tissue [7,43], and up to 10% of the original weight of the meat may be lost to shrinkable moisture losses through evaporation during all periods of dry ageing [7]. Additionally, the rate of intramuscular fat may play a role in increasing the WHC of a muscle [44]. In our study, it is plausible the 21 + 2 days of meat dry ageing could have homogenized the original WHC of the seven evaluated muscles to produce a similar WHC at the end of the trial.

Results of HIU effect on water retention in meat are very disparate. However, HIU has been reported to increase water losses in meat, particularly when ultrasonication time exceed 30 min. Ultrasonication of 40 kHz and 1500 W for periods longer than 20 min increases the exudate in beef [45] which can lead to higher water losses in the tissue. In the present study, the ageing of the carcass (23 d) could promote myofibrillar protein denaturation, changes in electrical charges, and disruption of cell structure, the same reported effects of HIU in meat [46]. However, HIU has been reported to improve the WHC of meat under conditions such as shorter times or during the use of salts in brining. HIU application for longer time periods may cause myosin oxidation which indirectly leads to increased WHC in meat [27,46]. In addition, long exposition to high O_2 concentrations is also commonly related to general protein oxidation in meat; in this case, myosin is frequently affected by that oxidation [47], leading to increase in liquid release by the tissue. In this study, water loss in the ultrasonicated meat was slight compared with the control group and was not perceived by consumers in the preference tests.

No differences in meat colour were found ($p > 0.05$) among muscles during the SRD. Sonication did not have an effect ($p > 0.05$) on any colour coordinates (L*, a*, b*, or C*) of aged beef during SRD. As expected, colour markedly deteriorated during SRD due to the presence of oxygen, reducing ($p < 0.05$) values of yellowness, redness, and chroma (Figure 1). The colour change was most evident in chroma and redness with reduction from 8 to 10 units after 24 h in both control and ultrasonicated meat. Chroma and redness were slightly reduced from 1 to 2 units over the next three days of SRD. In addition, the total colour change (ΔE) of meat after SRD was not affected by the muscle; however, the ultrasonication showed a significant increase in the ΔE (see Figure 1B) with a consequent decolouration of the product after SRD. Together, the ageing [1], the high exposition to O_2 [48] and the ultrasonication might contribute to total decolouration of beef.

Significant differences ($p < 0.05$) were found among muscles (Figure 2A). *Semitendinosus* was tougher than the rest of the analysed muscles, while *Psoas major* tended to be the tender ($p = 0.11$) muscle, which agrees with previous comparisons done for these muscles [49]. Positive effects of HIU on fresh beef tenderness have been documented [26,27]. Nevertheless, shear force characteristics of aged beef were not affected ($p > 0.05$) by HIU application in the present study. No added effect of HIU was observed on tenderness in addition to the tenderness provided by the ageing process (Figure 2).

Beef ageing has been widely recognized as a process to preserve and improve the tenderness of meat. The increase in meat tenderization by dry ageing results from a natural proteolytic enzyme post-mortem process by calpains and other proteases. These enzymes carry on an extensive proteolysis degrading myofibrillar proteins and cytoskeletal anchorage complexes in muscle fibres mainly during the first 15–21 d post-mortem [7,50]. Longer ageing periods usually result in higher tenderization of meat; for instance, from 14 to 35 days of ageing, a reduction of 17% in shear force may be observed [5,51].

The shear force values observed in the meat of this study because of the ageing process were extremely low (26.7 to 28.7 N). These Warner–Bratzler shear forces correspond to very tender beef according to pre-established values (under 58.83 or 45.11 N) differentiating tender and tough meat [52]. Consequently, potential effects on tenderness by HIU did not occur or were not evident after 23-day ageing.
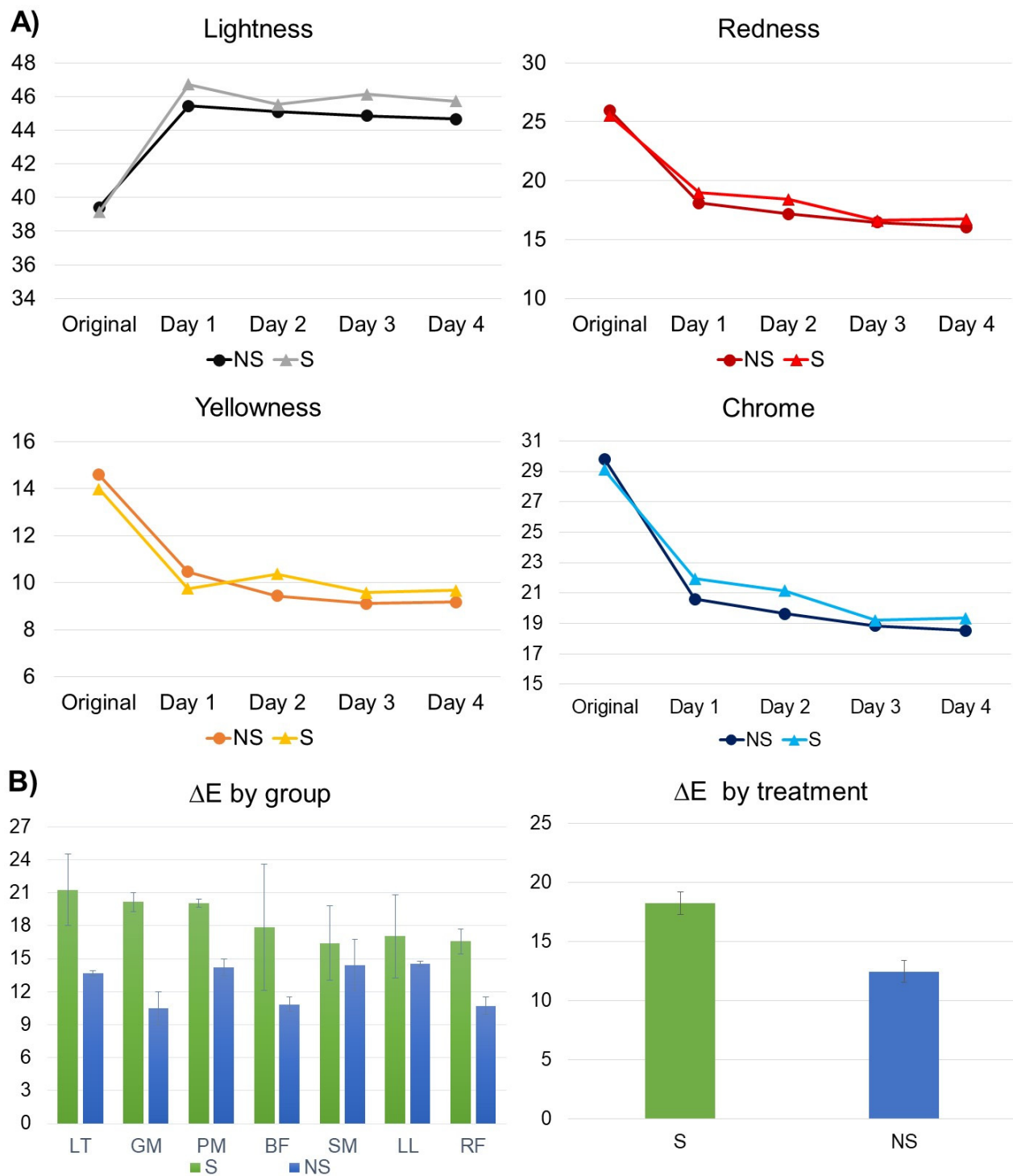
Figure 1. Colour change of 23-day-aged beef; (**A**) During simulated retail display (75% O_2: 25% CO_2, 3 °C) for 5 d; and (**B**) Total colour differences (ΔE) at the end of the retail display. NS = Not ultrasonicated. S = Ultrasonicated (US, 28 W/cm^2). BF = *Biceps femoris*. GM = *Gluteus medius*. LL = *Longissimus lumborum*. LT = *Longissimus thoracis*. PM = *Psoas major*. RF = *Rectus femoris*. SM = *Semitendinosus*.
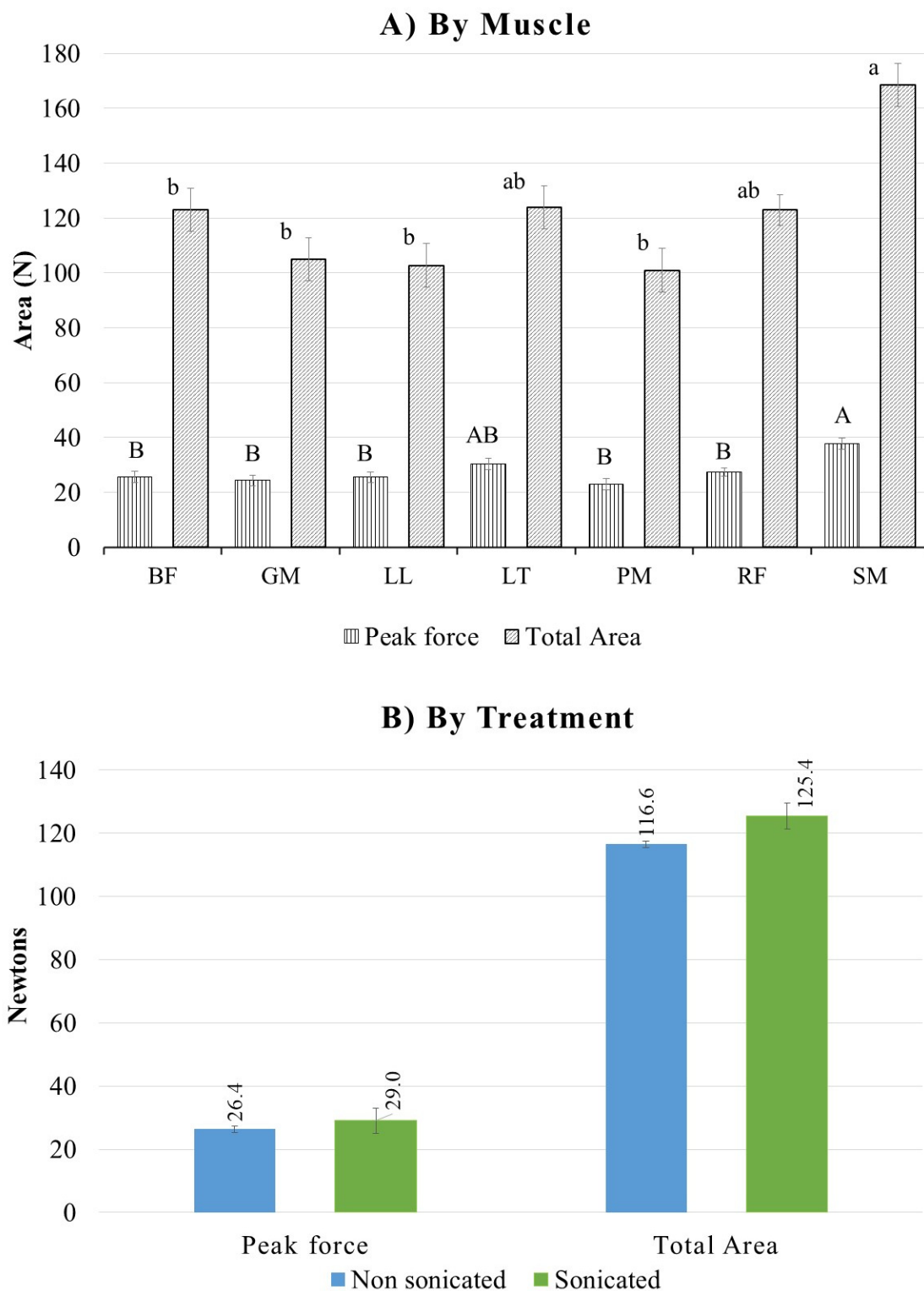
Figure 2. Shear force properties (total area and peak shear force) of 23-day-aged beef after simulated retail display (75% O_2: 25% CO_2, 3 °C) for 5 d. (**A**) corresponds to values by muscle: BF = *Biceps femoris*. GM = *Gluteus medius*. LL = *Longissimus lumborum*. LT = *Longissimus thoracis*. PM = *Psoas major*. RF = *Rectus femoris*. Different letters represent significant differences ($p < 0.05$) among muscles into the same parameter (Lowercase letters for area and capital letters for peak force). SM = *Semitendinosus*. (**B**) corresponds to values by treatment: Sonicated meat at 28 W/cm^2 after ageing.

3.2. Consumer Preference

Figure 3 shows the percentage of consumer preference of the seven evaluated muscles. There was not a significant effect ($p > 0.05$) by ultrasonication in any muscle. Furthermore, in the summary of preferences (Total), no significant differences were found between either control or ultrasonicated meat.
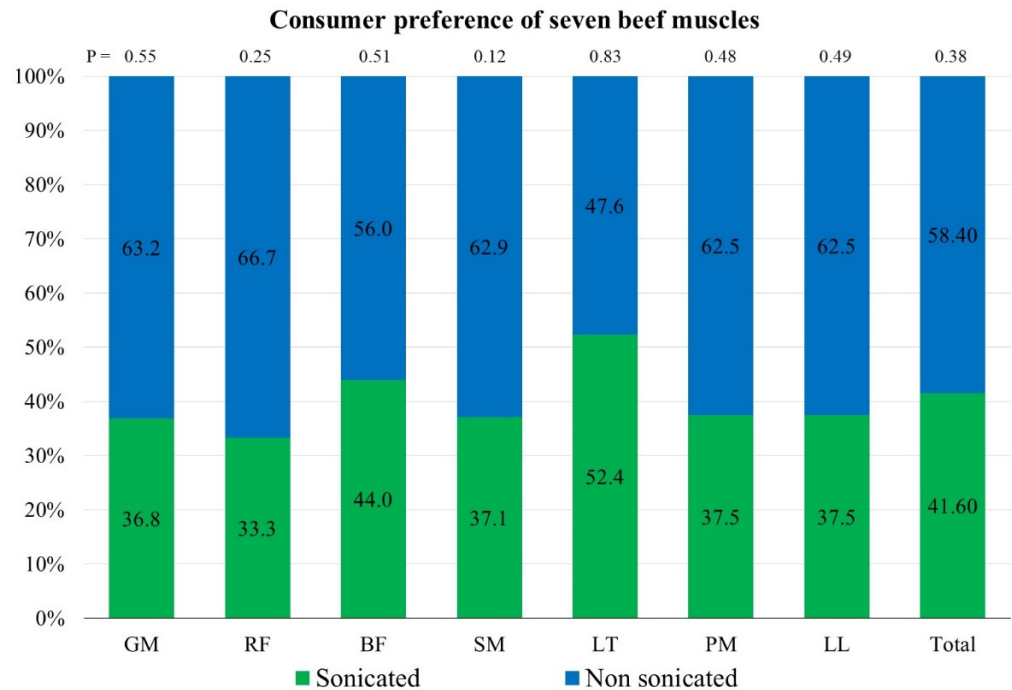

Figure 3. Percentage of consumer preference by tenderness of seven 23-day-aged beef muscles in simulated retail display (75% O_2: 25% CO_2, 3 °C) for 5 d. BF = *Biceps femoris*. GM = *Gluteus medius*. LL = *Longissimus lumborum*. LT = *Longissimus thoracis*. PM = *Psoas major*. RF = *Rectus femoris*. SM = *Semitendinosus*.

The results of tenderness preference by consumers were in accordance with instrumental shear force analysis. Additional comments by consumers pointed to the difficulty of stating their preference, indicating that both samples of the muscle were very tender. As described in the section of shear force, the values from 26.7 to 28.7 N (2.7 to 2.9 kgf, respectively) correspond to remarkably tender meat [53] granted by 23 d in dry ageing conditions. It is worth mentioning that this study is the first reporting consumer preference from dry-aged beef after high-intensity ultrasonication, and data from consumer preference must be interpreted carefully as a relatively small group of young adult consumers performed the evaluations. In Mexico, young adults represent the highest group of age [54] and have purchase power [55] that provides opportunities to reach niche products such as aged meat. Further research efforts should consider increasing the number of consumers or panellists to perform a full sensory analysis and/or consider having different age cohorts.

4. Conclusions

HIU (HIU, 28 W/cm^2) applied to beef aged for 23 d did not have additional effects on water-holding capacity, colour, shear force or young adult consumer preference of seven different muscles, yet the samples in this study exhibited colour stability and very low shear force values. Dry ageing of beef for 23 d provided benefits to colour and shear force that may be difficult to modify by ultrasonic cavitation. However, differences in shear force among various muscles may still be detected after 23 d of ageing. It is still necessary to delve into the implications of ultrasonicating aged meat and its effect on the microstructure and the potential modification of muscle proteins.

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