PAPER

QUALITY AND SENSORY PROFILE OF ULTRASOUND-TREATED BEEF

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ABSTRACT

The effects of high-intensity ultrasound treatment on beef (M. *longissimus dorsi*) quality and sensory attributes were evaluated. Ultrasound treatment (40 kHz, 11 Wcm²) was applied for 60 min. Control and ultrasound-treated samples were stored at 4°C and evaluated at 0, 7, and 14 days. After 14 days of storage, lipid oxidation of the ultrasound-treated samples increased (p < 0.0089), shear force decreased (p < 0.0001), and the treated meat was perceived as more tender and juicy. The application of ultrasound increased perception of tenderness without changing other sensory attributes.

Keywords: lipid oxidation, meat aging, meat tenderness, sensory attributes, ultrasonic treatment

1. INTRODUCTION

Various technological alternatives have been explored to enable minimally processed meat preservation, including novel thermal and nonthermal processing tools that have been successfully applied throughout the food supply chain (DEMIRDÖVEN and BAYSAL, 2009) without affecting the functional or sensory properties of fresh meat and meat products. Sensory attributes are important quality factors in the meat industry and are responsible for consumers' meat choices (MANDOUR et al., 2014). For this reason, methods are needed to ensure the safety, nutritional, and sensory qualities of meat. The use of ultrasound technology in meat processing is emerging (GALLEGO-JUÁREZ, 2010; CHEMAT et al., 2011). Ultrasound is an acoustic energy, and is considered mechanical, nonionizing, and nonpolluting (UNVER, 2016) with great potential for use in high-quality food production processes. Ultrasound changes the physical, chemical, and functional properties (TEREFE et al., 2016) of food products; can therefore, influence the quality of various food systems (KENTISH and FENG, 2014). Low intensity ultrasound has been used to evaluate the composition of meat, fish, and poultry products through food quality analysis (KNORR *et al.*, 2004) but also it has been reported as successful in the processes of mass transfer (CÁRCEL et al., 2007), marination, softening, and inactivation of microorganisms (ÜNVER, 2016). Ultrasound is an alternative to traditional meat aging methods for the tenderization and improvement of meat quality. Exposure to highintensity ultrasound can induce tenderness due to the cavitation effects that weaken the cell structure, release lysosomes and proteases, and cause protein denaturation (SIRÓ et al., 2009). The muscle tissue can be weakened to increase meat tenderness (STADNIK and DOLATOWSKI, 2011; HAI-JUN et al., 2012). Therefore, the aging period can also be reduced while preserving the quality parameters of meat (DOLATOWSKI et al., 2007) without compromising the oxidative stability of meat (STADNIK et al., 2008). However, this method must be developed further before it can be considered for industry-wide use. To date, no study has examined changes in the sensory properties of fresh or aged meat caused by high-intensity ultrasound. Thus, the aim of this study was to evaluate the effects of high-intensity ultrasound treatment on sensory quality, texture, and lipid oxidation (LO) of beef stored at 4°C.

2. MATERIALS AND METHODS

2.1. Meat and sample preparation

The samples for all experiments were beef from *M. Longissimus dorsi* (Hereford), obtained from a local supplier 2 days *post mortem* and then vacuum-packed. Muscles were stored at 4° C for 24 h prior to treatment. The pH of the meat was between 5.6-5.9. Visible fat was manually removed from each muscle prior to treatment. Samples were sliced similarly in terms of weight and size ($130 \times 90 \times 25$ mm, length × width × height). The location of the sample was randomly assigned to each treatment and a new muscle was used for each experimental replication. A total of 12 replicates were used.

2.2. Treatments

Samples were designated as; control (C) and ultrasound-treated (U). Based on the storage length (0, 7, or 14 days at 4°C), samples were further identified as; C_{v} , C_{τ} , and $C_{\iota_{4}}$ and U_{v} , $U_{\tau_{7}}$ and $U_{\iota_{4}}$, respectively. Ultrasound treatment (40 kHz, 11 Wcm²) was applied to the U samples at the end of each storage period. The samples were treated for 60 min (30

min/side) in a modified-intensity ultrasonic bath (Branson^{*} 1510 model 1510R-MTH; Branson Ultrasonics Corporation, Danbury, CT, USA) using distilled water as the diffusion medium. The effective power of the ultrasound system was determined using a calorimetric technique previously described (MARGULIS and MARGULIS, 2003). Temperature was kept constant at 4°C and the intensity was modified to obtain 11 Wcm². After sonication (or no treatment), meat was vacuum packed and prepared for analysis.

2.3. Shear force measurements

Shear force (SF) was measured using the method outlined by MAHER *et al.* (2004); the samples were placed in airtight plastic bags and cooked in a water bath (Isotemp 215: Fisher Scientific, Pittsburgh, PA, USA) until the temperature at the geometrical center of the sample reached 72°C. Cooked samples were tempered at room temperature and cooled at 4°C overnight, then drained and stored at 4°C for 24 h. After this period, 1 cm diameter cylinders were cut in the muscle parallel to the fibers using a punch. Cored samples were sheared using a TA-XT2i (Stable Micro Systems, Surrey, UK) with a V-shaped blade (Warner-Bratzler meat shear-compression) attached to a 100 N load cell and a crosshead speed of 200 mm min⁴. Average values of 8 replicates for each sample were performed and the SF values were reported as Newtons.

2.4. Lipid oxidation measurement

The degree of lipid oxidation (LO) was determined by measuring thiobarbituric acid (TBA)-reactive substances (TBARS) according to the technique described by PICCINI *et al.* (1986). Ten grams of muscle were homogenized (ESGE Bio Homogenizer model M133/1281-0; Bio Spec products Inc., Bartlesville, OK, USA) with a 10% solution of 6 N HCl for 40 s, the resulting suspension was subjected to distillation and 50 ml aliquots were collected. Afterwards a 2.5 ml of distillate was taken and mixed with 2.5 mL TBA at 0.02 M TBA. The mixture was incubated in a boiling water bath (100°C) for 40 min. A sample containing 2.5 mL of distilled water and 2.5 mL of TBA was used as a blank. Both were cooled for 10 min in running tap water and absorbance was measured at 535 nm on a spectrophotometer (Genesys 20, model 4001/4; Thermo Spectronic, Waltham, MA, USA). The results were plotted against a standard curve prepared with known concentrations of tetraethoxypropane. This determination was performed in triplicate and the results expressed as mg of malondialdehyde (MDA) per kg of meat (mg MDA/kg meat).

2.5. Sensory evaluation

2.5.1 Selection and training of panellists

Twelve panellists were recruited and trained using the quantitative descriptive analysis technique described by STONE *et al.* (2004). The panellists were selected by the basic taste test. In the second stage of selection, the Farnsworth-Munsell 100 Hue test for color sensitivity and test was used for taste, using the triangular test (International Organization for Standardization, ISO, 8586-1, 1993). Total training duration was 80 h, training included familiarization with relevant descriptive terms and ways of perceiving the selection and quantification of the sensory characteristics of cooked meat as well as the use of intensity scales ISO 4121 (2003). Representative samples were offered to the panel to determine relevant attributes. For the evaluation of appearance (i.e., color), a modified version of the AMSA (2012) protocol was used. Meat color was evaluated using reference scales. The panellists then generated individual lists of descriptors for each sensory characteristic (i.e.,

odor, appearance, flavor and texture) to characterize the meat samples (ISO 8586-2, 1994). Next, consensus lists of five descriptors per sensory modality were created and these terms were used for preparation of a lexicon that was used for further panellist training. The selected attributes were: whitish, pink, grayish, light-brown, and pale appearance; raw meat, grilled meat, fresh-cooked meat, boiled meat, and metallic odors; fresh bovine cooked meat, greasy, dry meat, bovine meat, and metallic flavors; soft, juicy, fibrous, tough, and elastic textures. The descriptor intensities per attribute were evaluated on a 10 cm linear scale with two anchor points. The final lexicon terms (descriptors) and definitions used to train the panellists are shown in Table 1.

Attribute	Descriptor	Definition
Appearance	Whitish	Perception of greater amount of white light on the surface of the meat.
	Pink	Pale shade of red.
	Grayish	Meat with less intense hue and brown tone.
	Light-brown	Brown hue reflecting more light.
	Pale	Meat color is observed to be less saturated.
Odor	Raw meat	Amount of beef odor in the sample; beef identity.
	Grilled meat	Full aromatic generally associated with beef suet that has been grilled.
	Fresh-cooked meat	Odor or note of aromatic fresh-cooked beef.
	Boiled meat	Aromatic notes associated with boiled meat or soup stock.
	Metallic	Aromatics associated with impression of slightly oxidized metal.
Flavor	Metallic	Taste associated with undercooked meat (bloody taste).
	Fresh bovine cooked meat	Taste characteristic of all meat, the aromatics associated commonly in partially cooked meat.
	Greasy	Flavor associated with fat heated to a high temperature.
	Dry meat	Flavor associated with meat that is overcooked and charred on the outside.
	Bovine meat	The aromatics commonly associated with matured cooked beef muscle products (boiled beef broth).
Texture	Soft	Describes beef meat that is easy to bite between the teeth (low hardness).
	Juicy	Perception of the amount of water released by the product during the first bites.
	Fibrous	Indicate that the orientation of particles in meat beef is similar to that perceived in celery.
	Tough	The number of chews required to masticate beef meat into a state ready for swallowing is similar to that necessary for old cow meat.
	Elastic	Describes the rapidity of recovery from a deforming force.

Table 1. Lexicon developed (descriptors that characterize a beef sample) by panellists and used in the evaluated quantitative descriptive analysis.

Sources: AMSA, 1995; BYRNE *et al.*, 2001; NOLLET and TOLDRÁ, 2011.

Panellists' performance was evaluated by applying a test of homogeneity of variances using the PROC GLM procedure in the SAS statistical package (SAS Institute, Cary, NC, USA). Consistency among the panel on sensory modalities was statistically significant for appearance (color) (p < 0.0001), odor (p < 0.05), flavor (p < 0.0001), and texture (p < 0.0001). The coefficient of the descriptors was estimated using XLSTAT-Sensory software (version 2015.6.01.25740; Addinsoft, Paris, France).

2.5.2 Sensory test

Samples were cooked in an oblong electric skillet (The West Bend Company, West Bend, WI, USA) to an internal temperature of 72 °C, following AMSA (1995)-established methods. Samples were cut into six equal pieces and maintained at 35°C until sensory analysis (\leq 30 min). The test was conducted under white light. Panelists were instructed to cleanse their palates with water between samples. The sensory tests were conducted for the sonicated (U) and untreated (C) samples in three sessions. In each session, panelists received randomly a (30 g) sample from each treatment, identified by a three-digit code. The panellists evaluated the samples using an unstructured 10-cm linear scale (0 = none, 10 = very). Data were recorded (values in cm) as intensity points for each descriptor.

2.6. Statistical analyses

The test variables (SF, LO, and sensory attribute intensity) were analyzed using the generalized linear model procedure (SAS software, SAS Institute) and the statistical model

$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + E_{ijk_i}$$

where Y_{ii} = response variables, μ = general average, A_i = effect of ultrasound treatment, B_i = effect of storage time, $(AB)_{ii}$ = effect of interaction between ultrasound treatment and storage time, and E_{iii} = random error. When the effect of a factor or interaction on one or more variables was significant (p ≤ 0.05), Tukey's statistical test was performed to compare the averages. Analysis of variance was also performed to determine the discriminant power of the descriptors and their estimated coefficients, using the XLSTAT-Sensory software package (version 2015.6.01.25740; Addinsoft).

3. RESULTS AND DISCUSSIONS

3.1. Shear force

SF differed significantly between treatments and storage periods (p < 0.0001; Fig. 1). SF values were higher on day 0 of storage and declined significantly by day 14 for both U and C samples. U samples had significantly reduced SF (p < 0.0001) compared to the C samples, which showed higher SF at all storage times. These results corroborate those reported previously (JAYASOORIYA et al., 2007; ZHOU et al., 2010). STADNIK and DOLATOWSKI (2011) highlighted the potential of using low-frequency and low-intensity *postmortem*. They found reduced meat toughness at 48 and 72 h postmortem. The effect of high-intensity ultrasound on SF reduction has also been reported for the following parameters: 24 kHz and 12 Wcm² for 4 min in bovine meat (JAYASOORIYA et al., 2007), 24 kHz and 12 Wcm² for 4 min in poultry after 7 days of storage (XIONG *et al.*, 2012), and 2.5-3 Wcm² for 180 min in pork (SIRÓ *et al.,* 2009). SIKES *et al.* (2014) also observed a reduction in SF with aging at 4° C for 7 days (p < 0.001), but no interaction between ultrasound treatment and storage. Other results have differed; as no effect was observed on SF with 62 Wcm-2 (LYNG et al., 1998), 22 Wcm-2 (POHLMAN et al., 1997a), or 4-19 Wcm² (MCDONNELL et al., 2014), although ultrasound treatment decreased gumminess and cohesiveness of salted pork in the latter study.

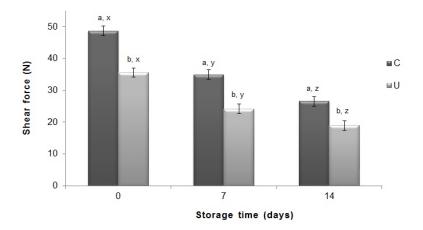


Figure 1. Effects of treatment and storage time on instrumental texture, measured as shear force (N) for bovine M. *Longissimus dorsi* treated and not treated with ultrasound (40 kHz, 11 Wcm³) and stored at 4°C for 0, 7, or 14 days (mean ± standard error bars). C= control (no ultrasound); U= ultrasound-treated.

^{a,b} Different letters indicate significant differences with ultrasound (p < 0.0001).

 $x_{y,z}$ Different letters indicate significant differences between storage time (p < 0.0001).

Ultrasound treatment affects meat tenderization via acoustic cavitation, as bubble formation, growth, and eventual collapse have thermal, chemical, and mechanical effects (YUSAF and AL-JUBOORI, 2014). Asymmetric collapse causes an eruption of fluid, producing a microburst affecting the integrity of muscle structure (BHASKARACHARYA *et al.*, 2009). This process is associated with *postmortem* hydrolysis of myofibrillar proteins in the aging stage, which leads to greater meat tenderization (GEESINK *et al.*, 2001) and explains the SF reduction observed in the present study. Likewise, depending on ultrasound frequency, alternating positive and negative pressures are produced, causing expansion or compression and resulting in cell rupture. This process also causes water hydrolysis (AWAD *et al.*, 2012), leading to the formation of chemically active free radicals (H and OH), which intervene in the structural stability and catalytic functions of proteins. Thus, ultrasound treatment may modify the availability of adenosine triphosphate in the *pre-rigor* muscle (SIKES *et al.*, 2014), which also accelerates the start of *rigor mortis* (DOLATOWSKI *et al.*, 2004; STADNIK and DOLATOWSKI, 2011) and therefore increases the aging rate of meat (CHANDRAPALA, 2015).

3.2. Lipid oxidation

The degree of LO in the samples differed significantly according to the interaction of treatment and storage factors (p < 0.01; Fig. 2). Both ultrasound and control meat presented lower lipid oxidation at day 0 of storage and these values increased significantly after 14 days of storage in treated samples (p < 0.01). The degree of LO in all samples fell below the rancidity threshold of 1-2 mg MDA/kg (VIEIRA *et al.*, 2009) and was also lower than the oxidation odor detection threshold (0.5-1 mg MDA/kg) (TARLADGIS *et al.*, 1960). These results agree with those reported by STADNIK (2009), who obtained TBARS values that indicated no compromise to the oxidative stability of ultrasound-treated (45 kHz, 2 Wcm² for 120 s) meat samples stored under refrigeration. Ultrasound breaks down cell membranes, fragments collagen, denatures proteins by bubble pulsation and cavitation, and promotes the formation of free radicals (KUIJPERS *et al.*, 2002). Consequently, it intensifies meat oxidation by increasing the speed of chemical reactions (AWAD *et al.*, 2003).

2012). Furthermore, aging represents a change in both structures and chemical composition of beef. For example, free radicals are produced during aging, mainly from metal release. Furthermore, fat and fat-like membrane molecules are degraded to fatty acids during aging (DASHDORJ *et al.*, 2016). These two factors together may explain why ultrasonicated meat is slightly more oxidized after 14 days of storage. Possibly, polyunsaturated fatty acids (PUFAs) released from phospholipids (membranes) during aging become more exposed to released free radicals during sonication (i.e., iron), interacting more rapidly during the same processes. Since lipid peroxidation is more strongly influenced by oxidation of membrane components such as PUFAs (FAUSTMAN *et al.*, 2010), the exposition of these fatty acids could be responsible for the slight increase in sonicated meat. However, the values obtained from treated samples in our study indicated minimal changes in LO during storage.

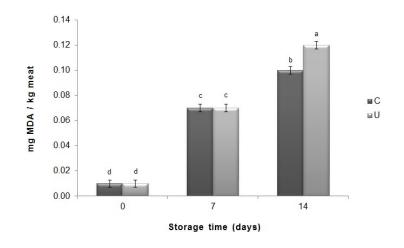


Figure 2. Effects of treatment and storage times on the lipid oxidation index (mg MDA/kg of meat) for bovine M. *Longissimus dorsi* treated and not treated with ultrasound (40 kHz, 11 Wcm³) and stored at 4°C for 0, 7 or 14 days (mean ± standard error bars). C= control (no ultrasound); U= ultrasound-treated. ^{a,b,c}Different letters indicate significant differences by interaction treatment of ultrasound and storage time (p < 0.0089).

3.3. Sensory properties

The effects of ultrasound treatment and storage on odor and flavor characteristics differed significantly with an interaction between these factors (p < 0.01 and p < 0.0001, respectively; Fig. 3A and 3C). After 7 and 14 days of storage, untreated samples had a more intense odor and flavor (raw meat odor, p < 0.0001; fresh-cooked meat odor p < 0.0006; and fresh bovine cooked meat flavors, p < 0.0001) than meat without storage, but also a more intense pleasant boiled meat odor (p < 0.0001) compared to samples treated with ultrasound. Ultrasound treatment also increased the perception of unpleasant greasy flavor (p < 0.0034), which was more noticeable after storage for 14 days (p < 0.0001).

The untreated samples were perceived as less greasy on day 0 (p < 0.0001) maybe because of the structural damage or the liberation of cooked-meat flavor precursor lipids. Metallic flavor, dry meat flavor, and metallic odor showed no significant difference according to storage period and treatment. The intensity of fresh-cooked meat odor was lower after storage for 7 days in ultrasound-treated samples (p < 0.0001), which may be due to the concentration of volatile compounds (aromatic molecules) that may be lower during this period. STETZER *et al.* (2007, 2008) reported that positive flavor compounds decrease with aging (between 7 and 14 days of storage) and negative compounds increase. Pentanal and 3-hydroxy-2-butanone decrease with aging while nonanal, butanoic acid and 1-octene-3-ol increase. Both sonicated and untreated meat showed more whitish and pink colors at 14 days of storage (p < 0.0001; Fig. 3B) compared to treated and untreated samples in other periods of storage.

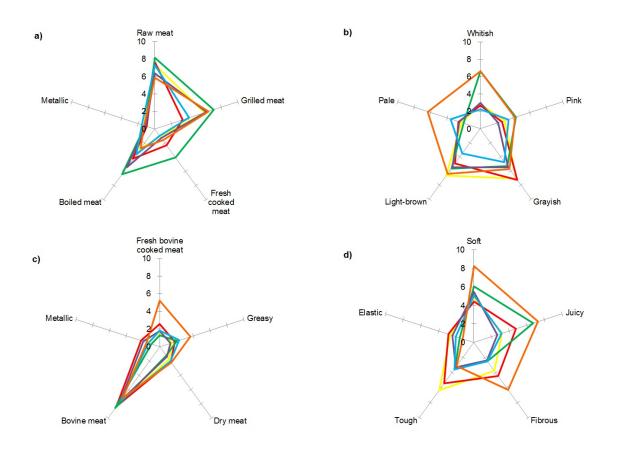


Figure 3. Quality descriptors for bovine M. *Longissimus dorsi* with and without ultrasound treatment (40 kHz, 11 Wcm²) after storage at 4 °C for 0, 7 and 14 days. a) Odor descriptors, b) Color descriptors, c) Flavor descriptors, d) Texture descriptors. C_7 = control (not ultrasound, yellow); 0 days of storage; C_7 = control (not ultrasound, red); 7 days of storage; C_{14} = control (not ultrasound, green); 14 days of storage; U_9 = ultrasound, 0 day of storage, (purple); U_7 = ultrasound, 7 days of storage, (blue); U_{12} = ultrasound, 14 days of storage, (orange).

Untreated meat tended to have a grayish color, with significant interaction observed for 0 and 7 days of storage (p < 0.0001). On day 0, untreated meat had a more intense lightbrown color (p < 0.0002) than sonicated meat; contrarily, the lowest intensity of this attribute was observed in ultrasound-treated samples after 7 days of storage. The palest color was registered for ultrasound-treated meat at 14 days of storage (p < 0.0001). This may be related to the results obtained by JAYASOORIYA *et al.* (2007) and HAI-HUN *et al.* (2012), who indicated that ultrasound application generates an increase in muscle temperature. Therefore, the thermal denaturation and oxidation of the meat pigments could affect the color of the meat, making it paler and less red. The ultrasound-treated meat stored for 14 days was the softest and juiciest of all samples (p < 0.0111 and p < 0.004, respectively; Fig. 3C), but it had a more fibrous texture, associated with lower SF values. Meat not treated with ultrasound and stored for 7 days had the most elasticity (p < 0.0058). Untreated meat on day 0 of storage had the toughest perceived texture, in agreement with the instrumental texture results (greater SF value). These results coincide with LYNG et al. (1998) who indicated that lamb treated with ultrasound and storage for 7 days was perceived as softer probably associated with the process of proteolysis during storage and the cavitation effect of ultrasound. In contrast, POHLMAN *et al.* (1997b) reported no effect of treatment with ultrasound and storage time on bovine M. *pectoralis* because of the greater presence of connective tissue. It has been asserted (DOLATOWSKI et al., 2007; STADNIK and DOLATOWSKI, 2011) that a softer meat texture after ultrasound can be explained by the physical weakening of the muscular structure, affecting the cellular membranes by accelerating proteolysis and releasing cathepsins from the lysosomes and/or calcium ions of the intracellular storage. The descriptors with the strongest discriminating factors for sample characterization were texture attributes, with the exception of untreated meat after 14 days of storage and ultrasound-treated meat after 7 days of storage (Fig 4).

Human perception is conditioned by the sensory interaction of physical processes, such as chewing; thus, sensory properties are linked to physical characteristics (CAINE *et al.*, 2003) and ultrasound wave propagation in meat depends on meat properties (DAMEZ and CLERJON, 2008). The results of the present study show that exposure to high-intensity ultrasound increases meat tenderness, as perceived by trained panellists who characterized the ultrasound-treated sample that had been stored for 14 days as the most tender. Sensory attributes resulting from proteolysis, such as odor, flavor, tenderness, and juiciness became evident due to the aging process, as the storage period increased. Ultrasound treatment resulted in additional softness and juiciness effects over the storage period. These panel results and SF values are similar to those obtained in other studies. POHLMAN *et al.* (1997a) conducted a sensory analysis of beef samples (M. *pectoralis* and M. Longissimus thoracis) subjected to ultrasound aging (20 kHz, 1000 Wcm²) or cooked by convection, and found increased myofibrillar tenderness (p < 0.05) and reduced flavor intensity in ultrasound-treated samples. Muscle treated with ultrasound had greater postcooking moisture, but no difference in juiciness was observed; the quantity of connective tissue and tenderness in general were unaffected by the aging method. LYNG et al. (1998) reported that sensory evaluation of bovine M. Longissimus thoracis, M. *lumborum*, and M. *semimembranosus* treated with ultrasound (20 kHz and 62 Wcm² for 15 s) showed no difference in tenderness, general texture, or global acceptance after 0, 3 or 14 days of storage. However, they found that storage time significantly improved chewability. In spite of the difficulties with comparing different experiments due to differences in frequency/intensity/time combinations of the ultrasound applied to meat, the majority of studies describe the favorable effects of ultrasound on meat texture (ALARCON-ROJO et al., 2015) and that effect has been corroborated herein.

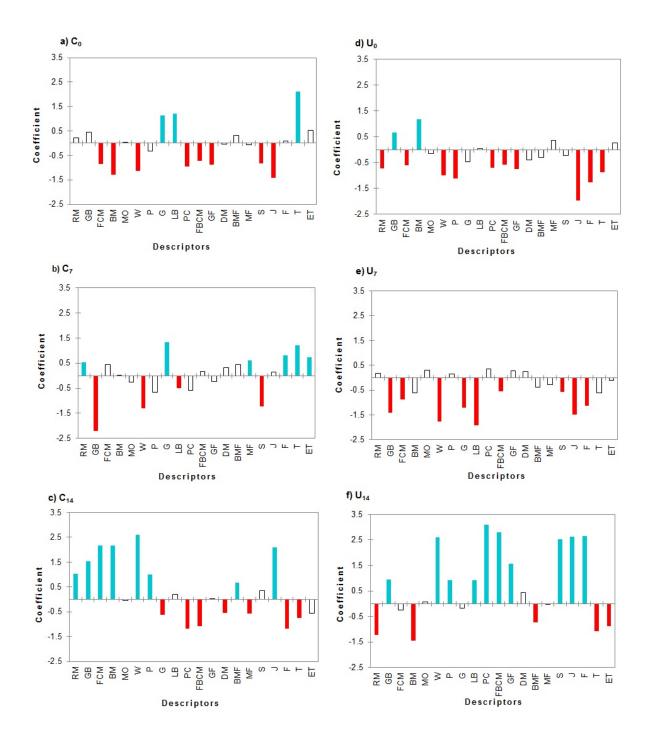


Figure 4. Estimated coefficient of descriptors for bovine M. *longissimus dorsi* with ultrasound treatment after storage at 4 °C for 0, 7, or14 days. (confidence interval 95 % model Y= P+J). C₀= control, 0 days of storage; C₇= control, 7 days of storage; C₁₄ = control, 14 days of storage; U₀ = ultrasound, 0 days of storage; U₇ = ultrasound, 7 days of storage; U₁₄ = ultrasound, 14 days of storage. RM= raw meat; GB= grilled beef; FCM= fresh-cooked meat; BM= boiled meat; MO= metallic odors; W= whitish; P= pink; G= grayish; LB= lightbrown; PC= pale color; FBCM= fresh bovine cooked meat; GF= greasy flavor; DM= dry meat; BMF= bovine meat flavor; MF= metallic flavor; S= soft; J= juicy; F= fibrous; T= tough; ET= elastic texture.

4. CONCLUSIONS

High-intensity ultrasound reduces the Warner-Bratzler SF of beef, and although meat LO increases, it does not negatively affect the quality. Thus, ultrasound application may be a feasible way to preserve the sensory properties of meat while significantly reducing aging time. Ultrasound technology can be applied to improve meat texture, as confirmed by our finding that high-intensity ultrasound increased meat tenderness. In this context, factors related to muscle (species, gender, age, diet or muscle type) and those related to ultrasound (frequency, intensity, time or ultrasound system) should be considered. Results of sensory analyses indicate that ultrasound does not change panellists' perception of beef quality. These findings should be complemented by consumer evaluation to rule out any detriment to meat quality.

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