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Does ultrasound equally improve the quality of beef? An insight into *longissimus lumborum*, *infraspinatus* and *cleidooccipitalis*

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ABSTRACT

Quality of bovine *longissimus lumborum, infraspinatus* and *cleidooccipitalis* muscles after high-intensity ultrasound (HIU; 40 kHz and a power of 11 W/cm^2 for 0, 40, 60, and 80 min) and aging (0, 7 and 14 d) was evaluated. The effects of HIU on pH and color of meat were not considered negative. HIU improved water holding capacity (WHC) of *l. lumborum* and *infraspinatus* only after aging. Whereas, the WHC of *cleidooccipitalis* increased immediately after sonication. The total collagen of HIU treated samples was significantly lower compared to the untreated samples. Ultrasonication for 80 min was the most effective for *infraspinatus* and *cleidooccipitalis*. Toughness decreased with HIU, *Infraspinatus* and *l. lumborum* tenderized more than *cleidooccipitalis*. HIU application and 7 d aging is an excellent combined treatment to improve tenderness of the three muscles. *Infraspinatus* was the most tender meat. HIU could help industry to improve the quality of beef as it helps in tenderization and accelerates maduration particularly of *l. lumborum*.

High-intensity ultrasound; Beef quality; Muscles; Ultrastructure

1. Introduction

Consumer perception relates to access and availability to high quality, healthy, nutritious and environmentally friendly foods. Awareness of health and nutrition has led to the search for alternative processes that maintain and improve the characteristics of food. Ultrasonic treatment is an innovative technology that has been increasingly used in food preservation and analysis. Ultrasound is an acoustic energy, and is considered mechanical, nonionizing, and nonpolluting (Ünver, 2016) with great potential for use in high-quality food production processes. Ultrasound changes the physical, chemical, and functional properties of food products (Terefe, Sikes, & Juliano, 2016) and it has potential uses of high-intensity ultrasound on fresh meat (Alarcón-Rojo, Janacua, Rodríguez, Paniwnyk, & Mason 2015). Applications have been published with interesting advantages in freezing (Zheng & Sun, 2006), thawing (Miles, Morley, & Rendell, 1999), meat brining (Kang et al., 2016), bacterial inhibition (Piñon, Alarcon-Rojo, Renteria, & Carrillo-Lopez, 2018) and tenderizing (Peña-Gonzalez et al., 2017). The basis of the applications of ultrasound at a frequency range of 20 kHz to 1 MHz is acoustic cavitation, which occurs in regions under rapidly alternating high-amplitude pressure waves and consists of the growth and collapse of gas bubbles within a liquid medium (Leong, Ashokkumar, & Kentish, 2011) resulting in physical modifications of muscular tissues. The controversy regarding the benefits of high-intensity ultrasound (HIU) is associated with multiple factors influencing its applications. The discrepancies in the results are due to intrinsic (species, age, aging, type of muscle) and extrinsic factors (ultrasonic systems, time, intensity and frequency) (Carrillo, Alarcon-Rojo, Luna-Rodriguez, & Reyes-Villagrana, 2017). Some studies have highlighted the positive effects of ultrasound on the conservation of nutritional and organoleptic properties of meat products (Ünver, 2016) and microstructural changes to the myofibrils in beef (Stadnik, Dolatowski, & Baranowska, 2008) that may have beneficial tenderizing actions and increasing the perception of tenderness of meat while significantly reducing aging time (Peña-Gonzalez et al., 2017). Tenderness of skeletal muscle is determined mainly by myofibrillar proteins and connective tissues (Purslow, 2005). Hence, tenderness differs between muscles (Hildrum et al., 2009), not only due to collagen but also due to differences in the *post-mortem* proteolytic activity. When meat is exposed to HIU proteolysis increases during the postmortem storage as reflected by an increased degradation of desmin and troponin-T (Wang et al. 2018).

However very little information is available on the effect of HIU on the different muscles themselves. Therefore, the objective of this study was to determine the effect of HIU and

aging on quality parameters and microstructure of beef *longisimus lumborum, infraspinatus* and *cleidooccipitalis*.

2. Material and methods

2.1. Animals and meat samples

Three bovine muscles (*longissimus lumborum, infraspinatus* and *cleidooccipitalis*) were obtained from Hereford carcasses of 450 ±15 kg live weight slaughtered at a commercial meat processing company under standard commercial conditions. These muscles were chosen because they are from very different anatomical regions within the carcass and have different quality. *L. lumborum* (from the loin) is a tender muscle, *infraspinatus* (from the chuck) is a very tender muscle, and *cleidooccipitalis* (from the neck) is considered a tough muscle.

The *L. lumborum* and *infraspinatus* muscles were excised from the left and right sides of 3 carcasses and the *cleidooccipitalis* muscle from the left and right sides of 6 carcasses at 24 h *post-mortem* in a 4 °C chiller and then trimmed of subcutaneous fat and connective tissue. Since the *cleidooccipitalis* is a smaller muscle, it was necessary to use 3 more muscles (from 3 additional carcasses) to have enough meat for the study. Each muscle was cut into 12 experimental pieces of 13.0 cm x 9.0 cm x 2.5 cm for *l. lumborum* and *infraspinatus*, and 4 pieces of 6.0 cm x 7.0 cm x 2.5 cm for *cleidooccipitalis*. Samples were randomly assigned to one of 12 treatments (four ultrasonication times x three aging time periods). In total, 108 muscle portions were used (three repetitions/treatment). After treatment samples were vacuum packed, ultrasonicated and stored at 4 °C according to each treatment group.

2.2. Application of HIU and treatments

The effective power introduced to the system by the HIU was measured using the calorimetric technique described by Margulis & Margulis (2003). Sample sonication was performed in a US bath (Branson 1510R-MTH, USA) with a total capacity of 2.25 L using distilled water (400 mL) as a diffusion media. HIU treatment was performed at a frequency of 40 kHz and a power of 11 W/cm^2 which is in the category of high-intensity (> 5 W/cm2 or 10–1000 W/cm2) and low-frequency (20–100 kHz) ultrasound (Ashokkumar & Mason, 2007). Once the vacuum packed samples were placed in the HIU bath, they were sonicated by applying half of the assigned time to each side, that was, 0, 20, 30, or 40 min on each

side. The total time sets for the sonication were thus 0, 40, 60, and 80 min. Treatment 0 was the control treatment or non-ultrasonicated muscles. The bath temperature was maintained constant at 4 ℃ during the HIU application (González-González et al., 2017). After ultrasonication the samples were stored at 4 ℃ for 0, 7 and 14 d periods. Shear force of 0 d HIU treated samples was determined immediately after sonication.

2.3. pH measurement

The pH was recorded randomly at three locations along the muscle length using a pH-meter at 24 h *post-mortem* (Hanna Instruments 99163, Rumania). The measurement depth was 5 cm. The probe was previously calibrated at 4 ºC with calibration solutions with a pH of 4.0 and 7.0. Three random readings were performed (in three different parts of the sample) and the averages were recorded.

2.4. Color measurement

Color was measured using a Minolta colorimeter (Model CR-400, Konica Minolta Sensing, Inc., Osaka, Japan. Illuminant C. 2° observer angle of measurement) based on the color co-ordinates, namely L*, a*, b*, C* and Hº. Before the measurement, the instrument was calibrated using a white calibration tile with specifications C: Y=94.2, x=.3130 and y=.3190 and an aperture of 8 mm. Color values were expressed as L* (brightness/darkness), a* (redness/greenness), b* (yellowness/ blueness), C* (chroma) $(a^{*2} + b^{*2})^{1/2}$ and Hº (hue angle) = arctan (b*/a*). The measurements were performed on parts of the sample that were free of visible connective and adipose tissue, allowing the oxygenation of the myoglobin for 30 min at 4 ºC. Three measurements *per* sample were performed and the averages were recorded.

2.5. Water holding capacity determination (WHC)

WHC was determined by the press method (Grau & Hamm 1953), as modified by Tsai & Ockerman (1981). A sample of approximately 0.3 g was placed between two filter papers (Number 1, Whatman®), which were in turn placed between two plexiglass plates, upon which a constant weight pressure of 10 kg was exerted for 15 min. WHC was later calculated by measuring the weight difference and was expressed as a percentage.

2.6. Shear force measurement

To determine shear force (SF) in the samples the methodology of the American Meat Science Association Guidelines (AMSA, 1995) was followed. Samples were placed in sealed plastic bags and cooked on a water bath (Fisher Scientific® mod. Isotemp 215) until an internal temperature of 72± 1 ºC was reached at the geometric center. Subsequently, samples were stored at 4 ± 1ºC for 24 h after which 8 cylinders with a diameter of 12.7 mm *per* sample were obtained using a manual corkscrew, taking care that the blocks were collected parallel to the longitudinal orientation of the muscle fibres. The cylinders were cut using a "V" form Warner Bratzler knife (triangular aperture of 60º) at a speed of 2.0 mm/sec. The peak force (in N) to cut each cylinder transversally was recorded with a TA-TX-plus texture analyser (Stable Micro Systems Ltd., Surrey, UK).

2.7. Collagen determination

The quantification of collagen was performed by the methodology of the International Organization for Standardization (ISO 3496: 1994) which is based on the hydrolysis of 4 g of sample in 30 mL of sulfuric acid solution (3 M) at 105 °C for 16 h, filtration and dilution of the hydrolyzate. The oxidation reaction was carried out with 4 mL of the dilution and 2 mL of chloramine-T, mixed and left to rest for 20 min. Then, 2 mL of the color reagent (p-dimethylamino-benzaldehyde solution) was added, mixed and placed in a water bath at 60 °C for 20 min. Samples were then placed on ice for 3 min, followed by 30 min at 25 °C and finally, the absorbance was measured in a spectrophotometer (Thermo Spectronic, 4001/4, USA) at 558 nm. A standard curve of hydroxyproline was prepared with concentrations of 0.5 µg / mL, 1 µg / mL, 1.5 µg / mL and 2 µg / mL. The content of hydroxyproline was calculated using the equation:

$$W_n = \frac{6.25\ C}{m \times V}$$

Where *Wn* is the content of hydroxyproline expressed as a percentage by mass; *C* is the concentration of hydroxyproline in µg/mL; *m* is the mass in grams of sample and *V* is the volume in mL of the aliquot of the hydrolyzate taken to complete to 250 mL. Subsequently, a conversion factor of 7.25 was used to calculate the collagen content (Palka, 1999).

2.8. Scanning electron microscopy

Four representative treatments were selected for SEM analysis (control and 80 min HIU treatment at 0 and 14 d of storage). Cubes (0.5 cm^3) samples were cut under a stereoscope (Carl Zeiss®) from the surface of the sonicated and non-sonicated samples and fixed in a 2.5% glutaraldehyde solution diluted in Sorensen phosphate buffer (pH 7.2). Subsequently, the cubes were dehydrated in an ethanol series (30, 40, 50, 60, 70, 80, 90, 2x100 %) for 40 min. The samples were dried at critical point using CO_2 as transitional fluid (Samdri-780A Tousimis dryer) after which they were covered with gold-palladium (JEOL, Fine CoatSputter JFC-1100, Japan) to favor the conduction in the scanning electron microscope (JEOL JSM 6390 SEM), using an accelerating voltage of 5 kV and obtaining micrographs with magnifications of 200X to characterize fibres and spaces between muscle fibres.

2.9. Area between the muscle fibres

Micrographs were analyzed with the *ImageJ* software (Rasband, 2018). A 100 x 100 µm grid was drawn over the axial images and three grids of each image were selected randomly and placed over a binary image in each 100 x 100 µm grid. This was used in line with the scaled axial images to measure the interfibrilar areas. Three frames of each image were selected randomly and passed to a binary image in each area, the region of interest (ROI) was then calculated. The difference between the ROI and the total area was reported as the fibre area of the cooked meat. The images were taken with a magnification of 300x with a scale 50µm bar.

3. Statistical analysis

A completely randomized factorial design was used with three replications in which three muscles (*l. lumborum, infraspinatus* and *cleidooccipitalis*) were evaluated for four sonication times each (0, 40, 60 and 80 min) at three storage times (0, 7 and 14 ds). The treatments and their interaction effects were estimated on pH, color characteristics (CIE L*, a*, b*, hue, chroma), WHC, SF, collagen content and interfibrillar area. All the tests used three samples for each treatment except SF measurement (8–9 samples). Analysis of variance (ANOVA) was performed and the difference between samples was determined using Tukey tests (significance level $P < 0.05$) (SAS, 2006). The micrograph images were segmented manually using *ImageJ* software. The segmentation and area measurements have been speedup by the application of *ImageJ* macros which were defined for this purpose. The reported values are means ± SE.

4. Results and discussion

The aim of this study was to evaluate the changes in physical parameters and microstructure of three beef muscles (*longisimus lumborum*, *infraspinatus* and *cleidooccipitalis*) after the application of ultrasound and aging. The main effects and interaction of treatment factors of measured variables on beef muscles are presented in Table 1.

4.1. pH

Fig. 1 shows the pH of beef samples. Although pH values were numerically very close to each other, there were statistical differences ($P < 0.05$) among treatments. A significant interaction ($P < 0.05$) between ultrasonication and aging times (0, 7 and 14 d) was observed for pH of *longissimus lumborum* and *cleidooccipitalis*, while *infraspinatus* only showed effect of ultrasound ($P < 0.05$) but not of storage time ($P > 0.05$). The pH of *longissimus lumborum* increased ($P < 0.05$) with the application of 40 and 80 min of ultrasound at 7 and 14 d. In general, the pH (5.41 to 5.56) was within the normal values for fresh beef. The pH of *infraspinatus* increased with sonication time. Samples treated with 60 and 80 min of ultrasound presented higher pH than when sonicated for 0 and 40 min ($P < 0.05$). However, no significant changes in pH ($P < 0.05$) can be noticed during aging time. The highest pH was observed in the sample sonicated for 80 min (5.73). The pH of m. *Cleidooccipitalis* increased ($P < 0.05$) as the time of ultrasonication increased from 0 to 80 min (Fig. 1) and with the time period ($P < 0.05$) or aging. These changes could be due to a release of ions from the cell structure to the cytosol, and changes in the structure of the proteins causing a change in the position of some ionic groups (Got et al., 1999). Immediately after slaughter, the pH of all muscles decreases due to accumulation of lactic acid produced during anaerobic glycolysis, that allows subsequent maturation processes. Muscles such as *Longissimus* with a high percentage of type II white fibers, tend to have a faster decrease of pH during early *postmortem* times, in comparison to muscles with lower type II fibers (Choi, Hwang, & Lee, 2016). The activity of endogenous enzymes, mainly calpains, continues at *post-mortem* pH and participate in maturation of meat (Lawrie & Ledward, 2006). Increases in the pH of the meat with ultrasound treatment and aging have previously been observed by Peña-Gonzalez et al. (2019). A normal pH range for high-quality beef is 5.4–5.8 and these values are indicative of high quality of meat, a normal *post-mortem* aging process and adequate cold storage conditions.

4.2. Water holding capacity (WHC)

The WHC of the three muscles showed a significant interaction ($P < 0.05$) between ultrasonication time and aging time (Table 1, Fig. 2). For *l. lumborum* and *infraspinatus* there was no effect on WHC immediately after the application of ultrasound ($P > 0.05$), but after 7 and 14 d storage the WHC of both sonicated muscles significantly increased ($P < 0.05$) with 40 and 80 min HIU, respectively. An immediate effect of HIU was observed in the WHC of *cleidooccipitalis* where treated muscle showed higher WHC than the control and it increased steadily up to 14 d storage except the 80 min ultrasound treatment which did not show any further change after 7 d storage (see Fig. 2). In general, the m. *infraspinatus* and *cleidooccipitalis* showed higher WHC than *l. lumborum*. It is evident that ultrasound has a clear benefit on WHC of *cleidooccipitalis* since similar WHC value (56%) was observed in sonicated meat without aging than that of the no sonicated meat after 14 d of storage (Fig. 2).

The WHC expresses the ability of the material to retain water on its spatial structure with myofibrillar/cytoskeletal proteins as the essential constituents. The lower the pH of the muscle, the lower their ability to bind water. In the present study, *l. lumborum* has the lowest pH and it also showed the lowest WHC, compared to *infraspinatus* and *cleidooccipitalis* with higher WHC. Since most of the muscle water is present within the myofibrils, a general hypothesis for explaining drip loss is that it originates from shrinkage of myofibrils after death (with lower pH), causing the water to be expelled into the extracellular space (Offer & Trinick, 1983). In cold-stored meat, the spatial arrangement and the electrostatic charge of myofibrils change its WHC (Offer & Knight, 1988). WHC in sonicated *l. lumborum* was lower than in *infraspinatus* and *cleidooccipitalis,* probably because ultrasound effects in the chemical structure of the myofibrillar proteins of *l. lumborum* are lower than in the other two muscles and causes the reduction in the spaces among the sarcomere filaments. In the present study, the application of ultrasound for 40 min in any of the three muscles increased their WHC. Similarly, it was observed that the ultrasonic treatment could significantly reduce the pressure loss and free water content, while improving the immobilised water content during cooking of beef (Zou, Zhang, Kang, & Zhou, 2018). They also reported that the myofibrils of beef were ruptured by ultrasonic treatment along with the Z-lines, leading to the muscle swelling.

4.3. Color (L, a*, b*, C*, y h*)*

Color is a key factor in meat quality since consumers greatly rely on fresh meat color as an indicator of wholesomeness. The color characteristics of meat are presented in Tables 1, 2

and 3. The ultrasonicated *l. lumborum* showed similar L* values ($P > 0.05$) compared to untreated control samples (Table 1). Meat was slightly darker ($P < 0.05$) after storage for 7 d, but lightness increased by d 14 to similar values than unaged samples. Redness (a*) of that muscle decreased ($P < 0.05$) with increased aging and ultrasonication times with no effect on yellowness (b*) of meat and chroma (C*) decreased ($P < 0.05$) with both, ultrasound and aging times of 7 and 14 d ($P < 0.05$). The hue angle (h*) showed a tendency to shift the meat color from red to yellow as time of sonication and aging increased.

Infraspinatus aged for 7 d showed higher ($P < 0.05$) a* and C*, but similar L*, b* and h* ($P > 0.05$) than control. However, when meat was aged for 14 d there was no effect ($P > 0.05$) on color (L*, a*, b*, C* and Hue), showing similar values than the control meat. The application of 40 min of ultrasound had a tendency to reduce the color of *infraspinatus* (lower C* and higher h*), whereas, the ultrasonication for 60 or 80 min did not have effect ($P > 0.05$) on the color of this muscle (Table 2).

Lightness, redness and yellowness of *cleidooccipitalis* decreased with aging ($P < 0.05$). Ultrasound treated meat showed higher ($P < 0.05$) L*, b*, total color (C*) and h*, but a* was lower than ultrasonicated samples. However, meat sonicated for 40 min had lower L*, a*, b*, C* and h*. As a relevant result, the application of ultrasound for 80 min did not drastically affect the color of *cleidoccipitalis* (Table 3).

The increases observed in the L* of aged meat are associated with the reduction of the respiratory activity of the mitochondria that provides a greater oxygenation of the myoglobin molecule and results in a higher formation of oxymyoglobin. This was observed particularly in *l. lumborum* (Lee, Apple, Yancey, Sawyer, & Johnson, 2008) resulting in higher L* values. Some authors have also observed increases in L* of meat during aging (Vitale, Pérez-Juan, Lloret, Arnau, & Realini, 2014) but not changes in L* were observed after ultrasound application (Caraveo, Alarcón-Rojo, Rentería, Santellano, & Paniwnyk, 2015). The decrease in the red color in *l. lumborum* and *cleidooccipitalis* can be associated with the increase in water loss, consequently increasing the loss of myoglobin during the storage time, which favors the reduction of redness.

Chroma represents the intensity of the color, that is, it describes how clear or dull the color is (AMSA, 2012) and is a good indicator of the oxygenation of the meat recently exposed to the air. In the presented study *l. lumborum* showed a decrease in C* with ultrasound and aging time, whereas *cleidooccipitalis* saturation decreased only with longer aging time.

Nevertheless, none of the values were under the threshold value of 18 for visual acceptability in beef (MacDougall, 1982). Hence, HIU treatment may not represent a disadvantage for beef color saturation on consumer discrimination.

4.4. Collagen

The total collagen content of samples of the three muscles of beef is presented in Fig. 3. A significant reduction was observed with aging time ($P < 0.05$) and with ultrasound application ($P < 0.05$). The total collagen of sonicated beef samples is significantly lower compared to the unsonicated beef at all treatment times. From the data, it is apparent that US treatment for 80 min is slightly more effective in reducing the total collagen. The micrograph shown in Fig. 4 provides further support to this observation. It was found that the change in microstructure of the 80 min-sonicated samples followed a similar pattern in all the treatment times. A higher content of total collagen was observed in *cleidooccipitalis* and *infraspinatus* muscles, but it showed a decline during storage. In the case of *l. lumborum*, the decrease in total collagen was minimum during storage. Similar results have been reported by Colle et al. (2015) who did not find differences in soluble or insoluble collagen between aging periods for beef *l. lumborum*. Therefore, the improvement in *l. lumborum* mechanical tenderness and consumer tenderness is likely due to post-mortem proteolysis. The decrease observed in total collagen of *infraspinatus* and *cleidooccipitalis* might be caused by the type of muscle and their individual properties. M. *infraspinatus* is characterized by high content of total and soluble collagen (Hildrum et al., 2009; Modzelewska-Kapituła & Nogalski, 2014). Although total collagen of meat seems to change relatively little during aging (Starkey, Geert, Geesink, Oddy, & Hopkins, 2015) the solubility of collagen increases in infraspinatus between the 5th and 10th d of aging when meat tenderness increases (Modzelewska et al., 2015). The synergic effects of the myofibril and the intramuscular connective tissue determine the tenderness of aged beef (Li, Zhou, & Xu, 2018). Since *infraspinatus* and *cleidooccipitalis* have large amounts of total and soluble collagen, these components might play a more important role in postmortem aging than that of *l. lumborum* muscle.

Chang et al. (2015) reported that low frequency and high intensity ultrasound (40 kHz, 1500 W) had no effect on the content of insoluble collagen in bovine *semitendinosus*, however, they found minor effects on total collagen and on solubility when using 50 min of sonication time but observed a decrease in the mechanical resistance of the connective tissue due to

ultrasound. Got et al. (1999) reported results similar to those of Chang et al. (2015) in *semimembranosus*. Contrarily Lyng, Allen, & Mckenna (1997) did not observe significant changes in soluble collagen due to the effect of ultrasound on *l. thoracis et lumborum*, *semitendinosus* and *biceps femoris* 2.5 cm thick and using three ultrasonic baths of different intensities. Important report is that of Purslow (2018) who pointed out that although the percentage of soluble collagen is often taken as a measure when assessing contributions of collagen to cooked meat texture, in reality it is the resistive proportion of the intramuscular collagen that resists both aging and cooking that should be our focus.

4.5. Shear force

Shear force (SF) decreased ($P < 0.05$) with storage time and with sonication time ($P < 0.05$) in all muscles studied. Immediately after application of ultrasound (40, 60 and 80 min) SF was reduced. This effect is clearly marked in the three evaluated muscles; *l. lumborum, infraspinatus and cleidooccipitalis* at time 0 of aging (Fig. 4). At 0 d the highest reduction in SF (6.3 N) was observed in *l. lumborum* reaching a SF value (33.1 N) similar to that of 7 d aged meat without ultrasound (34.8 N). SF declined further as sonication time increased until 7 d aging when tenderization seemed to stop with the exception of *cleidooccipitalis* treated with 40 min HIU which tenderized slightly until 14 d aging period. In general, the lowest SF value (18.9 N) was observed in *infraspinatus* after 80 min HIU with lower value than the control (26.0 N) at the same aging time.

During aging proteolysis of specific structural muscle proteins occurs as a result of the action of endogenous proteinases (Hopkins & Thompson, 2002). The calcium activated proteases (calpains), in particular μ-calpain, are largely responsable for the degradation of myofibrillar proteins during aging (Geesink, Kuchay, Chishti, & Koohmaraie, 2006). Degradation of the myofibrillar proteins leads to a weakening of the myofibrils and therefore tenderization. The endogenous proteases activity of the calpain system seems to differ among muscles due to differences in fiber type (Anderson et al., 2012; Laville et al., 2009; Stolowski et al., 2006). During aging, both sarcoplasmic and myofibrillar proteins from slow-twitch oxidative muscle or fiber (type I, red) seemed to be much less susceptible to proteolysis than proteins from fast-twitch glycolytic muscles (type IIb, white) (Ouali, 1991). This means that aging rate would be higher in white muscles than in red muscles (Muroya, Ertbjerg, Pomponio, & Christensen, 2010; Muroya et al., 2012).

The differences between fibres might help to explain the variations observed in the SF in the muscles of the present study after aging and/or ultrasound treatment (Fig. 4). The no sonicated *l. lumborum* tenderizes first with 43% type IIB fibre (white), then *infraspinatus* with 47% type I fibre (red) and finaly the *cleidooccipitalis* muscle with 58% type I fibre (red) (Honikel, 1986; Kirchofer, Calkins, & Gwartney. 2002; Totland & Kryvi, 1991). Totland & Kryvi, 1991). *Cleidooccipitalis* is a red muscle, it contains similar high myoglobin concentrations than sternomandibularis (Honikel, 1986) which has 58% type I fibres (Totland & Kryvi, 1991). However, when HIU is applied the tenderization rate seems to be increased for *cleidooccipitalis* when meat tenderized faster (Fig. 4).

In the present study M. *infraspinatus* presented the lowest values of SF. *Infraspinatus* is known as the second most tender (WBS < 28 N) beef muscle in the carcass next to M. psoas major (Rhee et al., 2004) with the highest sensory quality (Hildrum et al., 2009; Modzelewska-Kapituła & Nogalski, 2014; Von Seggern et al., 2005). In fact, chuck muscles, such as *infraspinatus*, tenderizes better than round muscles after aging and other enhancement treatments (Stetzer, Tucker, McKeith & Brewer, 2007). An interesting observation is that SF of the three muscles does not largely decrease between 7 and 14 d aging (Fig. 4), which is in accordance with the findings of Cassens et al. (2018) who did not find differences in SF between 7 and 14 d of aging treatments in beef and the observations of Nishimura et al. (1998) who emphasizes that increases in meat tenderness are generally highest during the first 10 days of postmortem aging.

The tenderization of meat by HIU has mainly been attributed to the rupture of protein structures by proteolysis (Stadnik et al., 2008) caused by cathepsins and/or calpains (Chang et al., 2015) and shortens aging periods (Chandrapala, 2015). Recently, Wang et al. (2018) observed a fast rate of desmin and troponin-T degradation in ultrasound-treated samples and attributed these changes to the activation of μ-calpain. Ultrasound is known to cause cell rupture and water hydrolysis affecting the structural stability and catalytic functions of proteins (Awad et al., 2012). We previously reported that HIU tenderizes beef *l. dorsi* and accelerates aging, effects which have been evidenced here but with different muscles (Peña-Gonzalez et al., 2019).

4.6. Microstructure

Fig. 5 shows the microstructural changes in the three beef muscles having been subjected to

80 min HIU and/or aged for 14 d. Some structural alterations can be seen in the three muscles during aging and with the application of 80 min HIU. On the overall integrity of muscle cells seem to be disrupted where muscle fibres tend to separate from each other in the three muscles when treated with 80 min ultrasound, but differences between muscles appear not to be large. It is well known that aging of meat is characterized by various ultrastructural changes produced by the action of different proteolytic systems showing myofibrillar ruptures along the Z lines (Listrat et al., 2016).

Fig. 6 shows the interfibrillar area of the muscles after the application of 80 min ultrasound and 14 d aging. The changes observed by SEM in the meat structure could not be reflected in the interfibrillar area, since it decreased with ultrasound application in all muscles probably because the technique used to calculate the area between muscle fibres was not sensitive enough to detect changes as previously observed (Carrillo et al., 2019) in SEM images of beef samples treated with 16, 28 and 90 Wcm^{-2}. The only increase in area was observed in *l. lumbroum* and *infraspinatus* after 14 d aging (Fig. 6).

Several authors have studied the effect of ultrasound on the meat structure. Siro et al. (2009) reported an increase in the distance between fibers when applying ultrasound for 90 and 180 min. Stadnik, Dolatowski, & Baranowska (2008) also reported that in samples sonicated at 45 kHz for 2 min and after a period of maturation of 72 h, the samples presented alterations in the sarcomere, with fragmentation of the Z line. Kang et al. (2015) observed that low-frequency, high-power ultrasound (40 kHz, 1,500 W) applied to beef for 30 min had significant effects on meat texture and connective tissue properties resulting in tender meat. In another study, Kang et al. (2016) reported that beef muscle, when treated with ultrasound (2.39, 6.23, 11.32 and 20.96Wcm^{-2}) during the brining process, resulted in protein oxidation and changes in the secondary structures of the beef proteins. More recently Kang et al. (2017) demonstrated that the improved tenderness of sonicated (150 and 300 W) beef was attributed to the increased myofibrillar index and the proteolysis of desmin and troponin-T which were verified by TEM images. Finally, published work of dela Malva et al. (2019) has proven that differences in tenderness could be related to the integrity of muscle tissue myofibers during proteolytic degradation (della Malva et al., 2019).

The findings of the present study give an insight of the influence of ultrasound and aging in three bovine muscles. Differences in SF, total collagen and structure of the three studied muscles might respond to the individual properties of these muscles. Collagen might play a

different role in the quality of each muscle as these responded differently to aging and ultrasound.

5. Conclusion

Although there have been some studies about the effect of ultrasound on meat, there are few reports exploring the use of ultrasound on different muscles of the same species. Ultrasound and aging did not cause negative effect on pH and color but it improved WHC of *l. lumborum*, *infraspinatus* and *cleidooccipitalis*. Collagen decreases with aging and ultrasound except in *l. lumborum*. Ultrasound application and 7 d aging is an excellent combined treatment to improve tenderness of the three muscles. The most tender meat was that of *infraspinatus* at any time of sonication but *l. lumborum* appear to benefit the most from ultrasound application. These findings could bring beneficial impact on the meat industry since ultrasound could help industry to establish effective and efficient process conditions for improving the quality of meat to address consumer demands.

Founding sources

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Conflict of interest

The authors declare no conflict of interest.

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Table 1

Significance probability associated with the *F* statistic (*P*-values) by ANOVA analysis. Values equal or lower than 0.05 dennotes significance of main effects and interactions of treatment factors of measured variables for three aging times (0, 7 and 14 days) with four ultrasonication times (0, 40, 60 and 80 min) on three bovine muscles (*Longissimus lumborum*, *Infraspinatus* and *Cleidooccipitalis*).

Variable	Factor / Interaction		
	Aging	US time	Aging*US
	Longissimus lumborum		
pH	0.2197	0.0331	0.0301
WHC	0.0009	0.2651	0.0297
L*	0.0297	0.8736	0.4748
a*	<0.0001	<0.0001	<0.0001
b*	0.3324	0.5139	0.8542
Chroma	<0.0001	<0.0001	<0.0001
Hue	<0.0001	0.0003	<0.0001
Collagen	0.0004	0.0014	0.0013
Shear force	<0.0001	<0.0001	<0.0001
Interfibrillar area	0.2991	0.0008	0.0012
	Infraspinatus		
pH	0.8619	0.0051	0.1372
WHC	<0.0001	0.5757	0.0027
L*	0.5737	0.0985	0.4244
a*	0.0067	0.0349	0.0564
b*	0.0921	0.0027	0.0042
Chroma	0.0033	0.0328	0.0065
Hue	0.1833	0.0076	0.0204
Collagen	<0.0001	<0.0001	<0.0001
Shear force	<0.0001	<0.0001	<0.0001
Interfibrillar area	0.0736	0.2419	0.0158
	Cleidoccipitalis		
pH	<0.0001	0.0004	0.0001
WHC	0.0025	0.0013	0.0039
L*	0.0027	0.0003	0.002
a*	0.0035	0.0147	0.02
b*	0.0003	<0.0001	<0.0001
Chroma	0.0013	0.0037	0.0057
Hue	0.5904	0.0002	0.0085
Collagen	<0.0001	<0.0001	<0.0001
Shear force	0.0003	0.0007	0.0005
Interfibrillar area	0.2259	0.4165	0.5139

Table 2

Color parameters (CIE L*a*b*, chroma and Hue)[1] of beef *m. Longissimus lumborum* treated with high-intensity ultrasound (0, 40, 60 and 80 min) after aging for 0, 7 and 14 d at 4 ºC (mean values ± standard error).

Factor	CIE L*a*b*				
Aging (d)	L*	a*	b*	C*	Hue
0	35.24±0.9[b]	21.15±1.52[a]	11.41±0.73[a]	24.06±1.51[a]	28.48±0.52[b]
7	36.81±0.61[a]	19.23±0.98[b]	11.26±0.54[a]	22.31±1.0[b]	30.43±0.66[b]
14	36.62±0.73[b]	13.92±1.55[c]	11.98±0.64[a]	18.45±1.33[c]	41.16±2.74[a]
Ultrasound (min)	L*	a*	b*	C*	Hue
0	36.43±0.52[a]	21.18±2.03[a]	12.06±0.23[a]	24.44±1.71[a]	30.12±2.45[c]
40	35.98±0.84[a]	18.11±1.31[b]	11.25±0.64[a]	21.37±1.16[b]	32.09±2.79[b]
60	36.42±0.92[a]	16.84±2.38[c]	11.55±0.74[a]	20.62±1.81[b]	35.47±3.47[b]
80	36.06±1.10[a]	16.27±2.18[c]	11.35±0.82[a]	19.99±1.77[b]	35.73±3.73[a]

[1] L* is a measure of darkness to lightness (greater L* values indicate a lighter color); a* is a measure of redness (greater a* values indicate a redder color); and b*is a measure of yellowness (greater b* values indicate a more yellow color). Chroma (C*) is a measure of the total, or vividness of color (greater C* values indicate greater total color and/or a more vivid color). Hue angle represents the change from the true red axis (larger angle indicates a greater shift from red to yellow).

[a, b, c] Column means with different superscripts differ significantly ($P < .05$).

Table 3

Color parameters (CIE L*a*b*, chroma and Hue)[1] of beef *m. Infraspinatus* treated with high-intensity ultrasound (0, 40, 60 and 80 min) after aging for 0, 7 and 14 d at 4 ºC (mean values ± standard error).

Factor	CIE L*a*b*				
Aging (d)	L*	a*	b*	C*	Hue
0	28.53±0.81[a]	19.43±1.17[b]	10.1±0.70[a]	21.94±1.29[b]	27.38±1.85[a]
7	29.69±1.27[a]	21.85±1.16[a]	11.29±0.76[a]	25.30±1.33[a]	27.14±1.34[a]
14	29.18±1.83[a]	20.67±1.03[b]	10.08±0.66[a]	23.01±1.16[b]	25.83±1.38[a]
Ultrasound (min)	L*	a*	b*	C*	Hue
0	27.56±1.33[a]	19.96±1.01[a]	9.61±0.50[b]	23.07±1.11[b]	25.60±1.37[b]
40	30.70±1.41[a]	21.65±1.22[a]	12.03±0.40[a]	24.80±1.24[a]	28.99±1.74[a]
60	29.68±1.38[a]	21.48±1.45[a]	10.94±0.93[b]	24.12±1.67[b]	26.94±1.09[b]
80	28.59±1.8[a]	19.52±0.95[a]	9.39±0.90[b]	21.68±1.16[b]	25.59±1.22[b]

[1] L* is a measure of darkness to lightness (greater L* values indicate a lighter color); a* is a measure of redness (greater a* values indicate a redder color); and b*is a measure of yellowness (greater b* values indicate a more yellow color). Chroma (C*) is a measure of the total, or vividness of, color (greater C* values indicate greater total color and/or a more vivid color). Hue angle represents the change from the true red axis (larger angle indicates a greater shift from red to yellow).

[a, b, c] Column means with different superscripts differ significantly ($P < .05$).

Table 4

Color parameters (CIE L*a*b*, chroma and Hue)[1] of beef *m. Cleidoccipital* treated with high-intensity ultrasound (0, 40, 60 and 80 min) after aging for 0, 7 and 14 d at 4 ºC (mean values ± standard error).

Factor	CIE L*a*b*				
Aging (d)	L*	a*	b*	C*	Hue
0	33.50±1.28[a]	27.97±1.66[a]	13.99±1.57[a]	31.31±2.06[a]	26.39±1.83[a]
7	31.11±1.01[b]	23.63±2.63[b]	11.84±1.28[b]	26.50±2.67[b]	26.83±2.16[a]
14	31.61±1.19[b]	22.68±2.46[b]	10.92±1.21[b]	25.20±2.68[b]	25.73±1.29[a]
US (min)	L*	a*	b*	C*	Hue
0	33.00±1.32[a]	26.94±2.55[a]	12.10±1.26[b]	29.57±2.69[a]	24.32±1.71[b]
40	29.76±0.96[b]	21.17±2.18[b]	9.35±1.05[c]	23.15±2.39[b]	23.84±0.97[c]
60	32.29±0.92[a]	25.83±2.22[b]	13.34±1.10[b]	29.09±2.41[a]	27.39±0.70[b]
80	32.25±0.93[a]	25.11±2.50[b]	14.21±1.11[a]	28.88±2.62[a]	29.72±1.70[a]

[1] L* is a measure of darkness to lightness (greater L* values indicate a lighter color); a* is a measure of redness (greater a* values indicate a redder color); and b*is a measure of yellowness (greater b* values indicate a more yellow color). Chroma (C*) is a measure of the total, or vividness of, color (greater C* values indicate greater total color and/or a more vivid color). Hue angle represents the change from the true red axis (larger angle indicates a greater shift from red to yellow).

[a, b, c] Column means with different superscripts differ significantly ($P < .05$).

Fig. 1. pH values of beef *m. Longissimus lumborum, Infraspinatus* y *Cleidoccipital* treated with high-intensity ultrasound (0, 40, 60 and 80 min) after aging for 0, 7 and 14 d at 4 ºC. Asterisks (*) indicate significant differences ($P < .05$). Bars refer to standard error of mean.

Fig. 2. Water holding capacity (WHC, %) of beef *m. Longissimus lumborum, Infraspinatus* y *Cleidoccipital* treated with high-intensity ultrasound (0, 40, 60 and 80 min) after aging for 0, 7 and 14 d at 4 ºC. Asterisks (*) indicate significant differences ($P < .05$). Bars refer to standard error of mean.

Fig. 3. Total collagen (µg /mL de hydroxyproline) of beef *m. Longissimus lumborum, Infraspinatus* y *Cleidoccipital* treated with high-intensity ultrasound (0, 40, 60 and 80 min) after aging for 0, 7 and 14 d at 4 ºC. Asterisks (*) indicate significant differences ($P < .05$). Bars refer to standard error of mean.

Fig. 4. Shear force of beef *m. Longissimus lumborum, Infraspinatus* y *Cleidoccipital* treated with high-intensity ultrasound (0, 40, 60 and 80 min) after aging for 0, 7 and 14 d at 4 ºC. Asterisks (*) indicate significant differences ($P < .05$). Bars refer to standard error of mean.

Fig. 5. Images of bovine meat *Longissimus lumborum*, *Infraspinatus* and *Cleidooccipitalis* without ultrasound and aging (0/0), without ultrasound and 14 d aging (0/14), with 80 min ultrasound without aging (80/0) and with 80 min ultrasound and 14 d of aging (80/14). Scale bar = 50µm; 300 x magnification.

Fig. 6. Interfibrillar spaces (µm2) in an area of 10,000 (µm2) for m. *Longissimus lumborum*, *Infraspinatus* and *Cleidoccipital* from beef treated with high-intensity ultrasound (0 and 80 min) after aging for 0 and 14 d at 4 ºC. Asterisks (*) indicate significant differences ($P < .05$). Bars refer to standard error of mean.

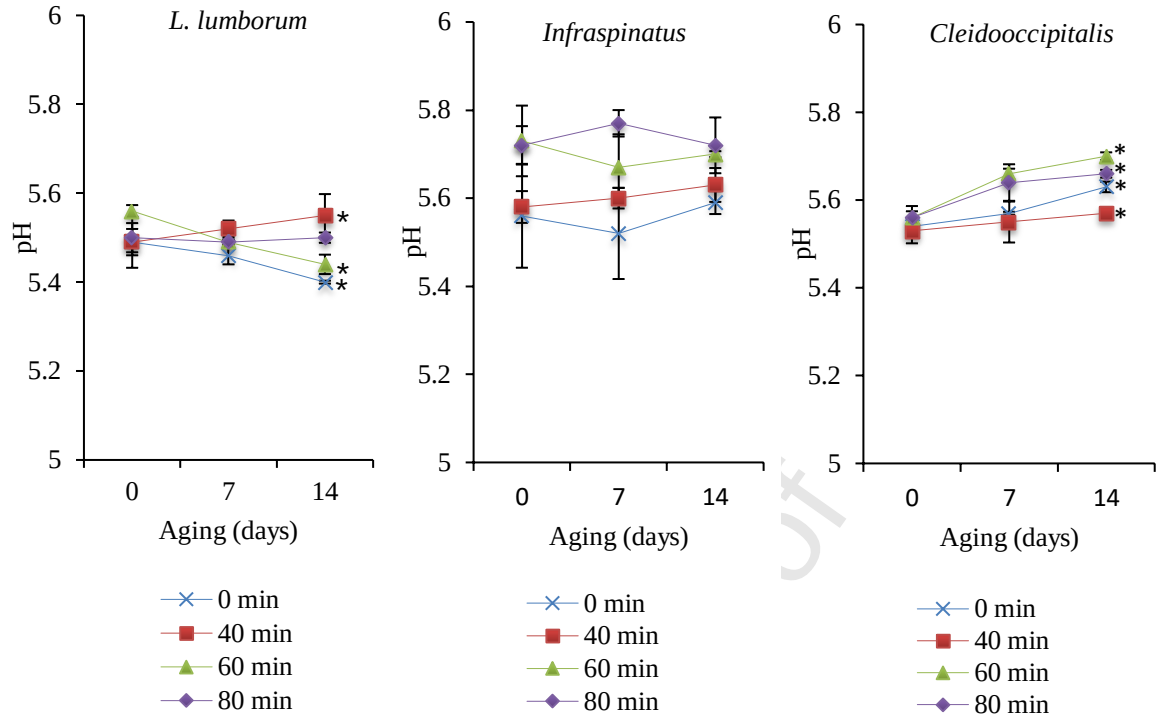


Fig. 1

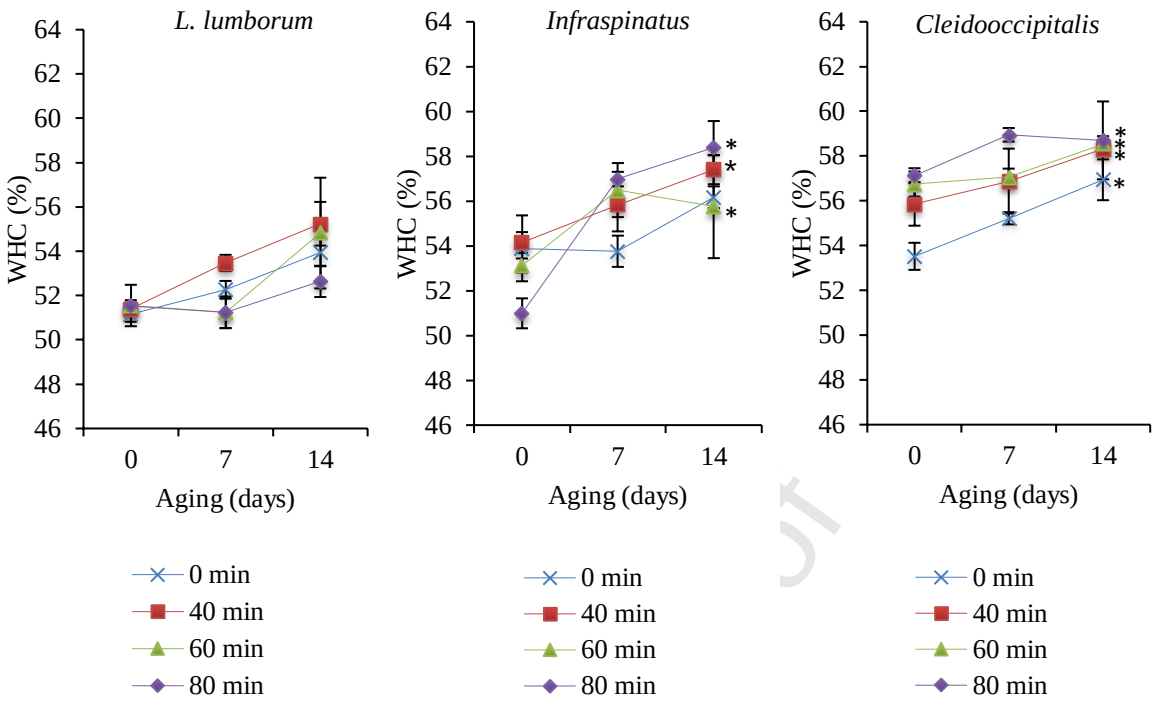


Fig. 2

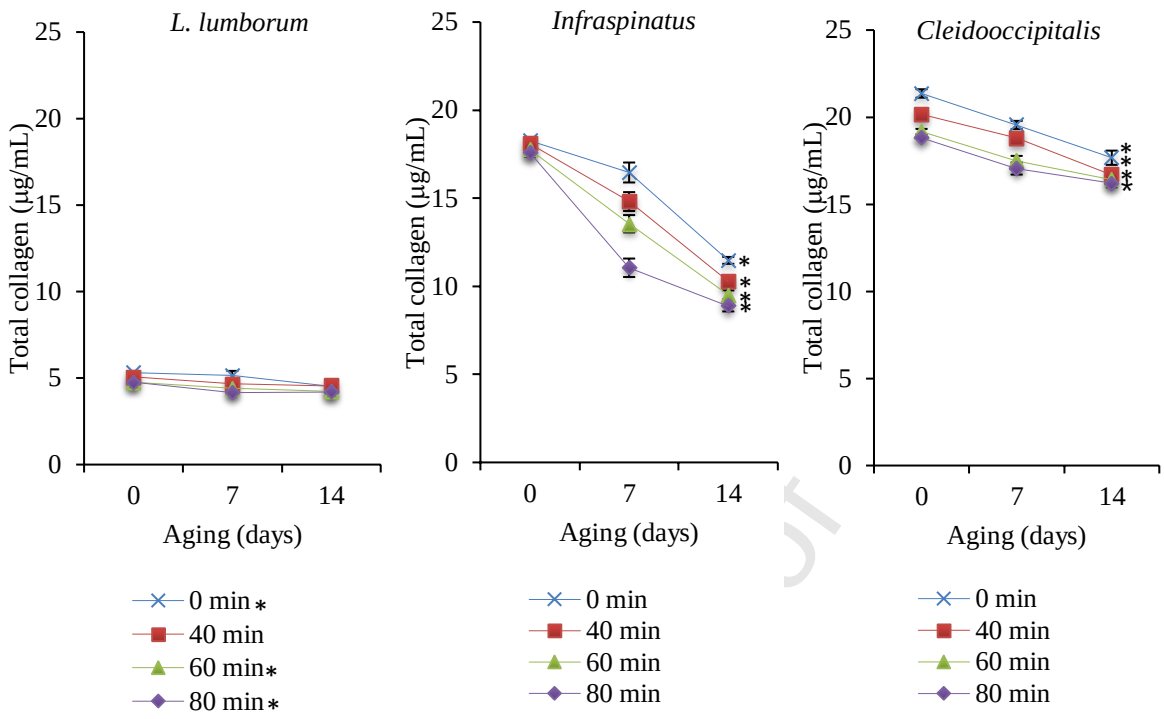

L. lumborum
Infraspinatus
Cleidooccipitalis
Total collagen (μg/mL)
Aging (days)
0
5
10
15
20
25
0
7
14
0 min *
40 min
60 min*
80 min *
0 min
40 min
60 min
80 min
0 min
40 min
60 min
80 min

Fig. 3

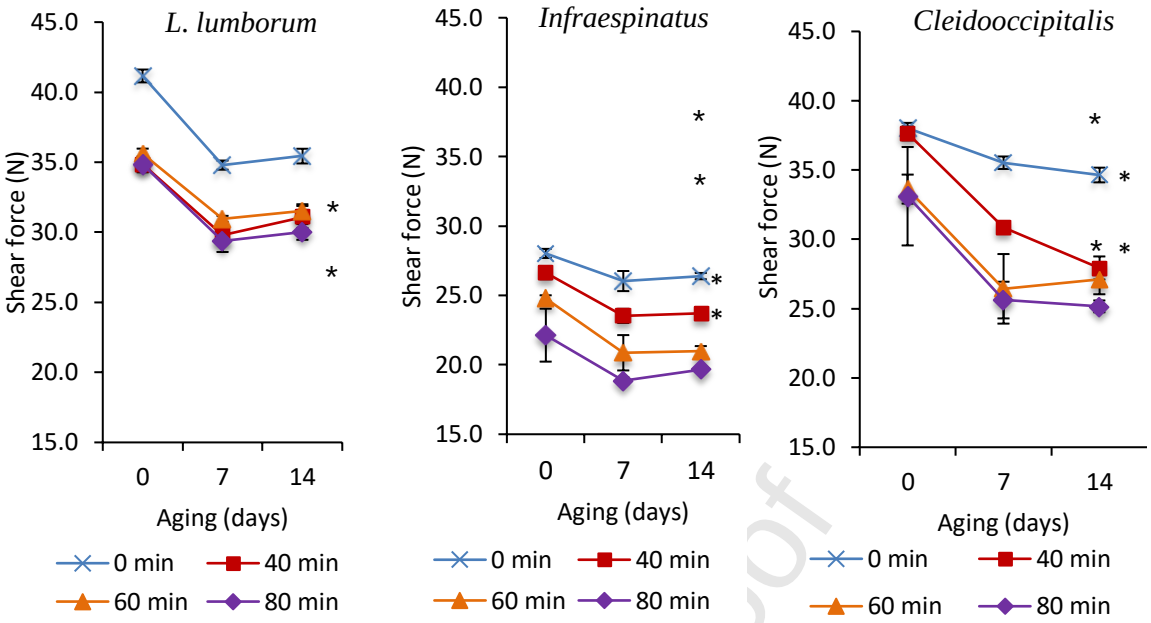
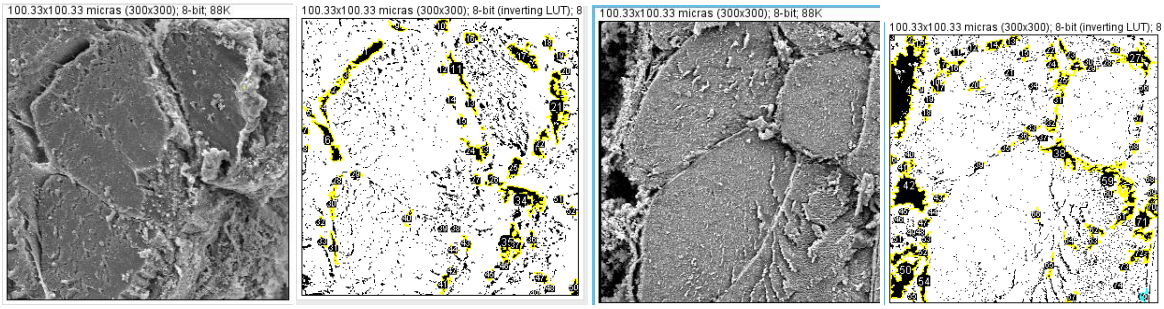
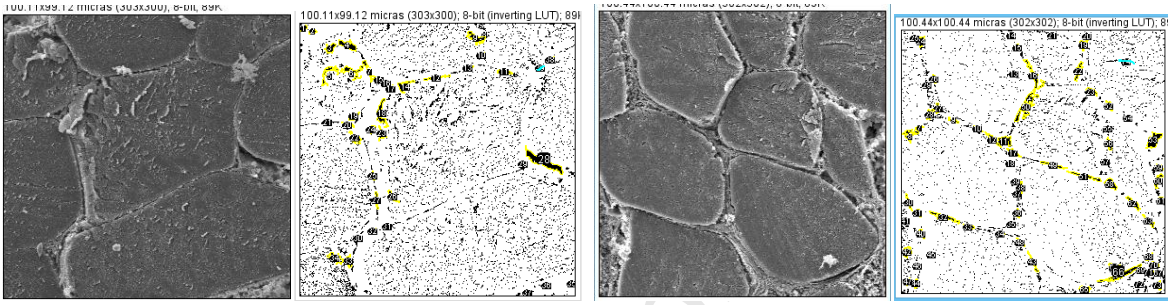


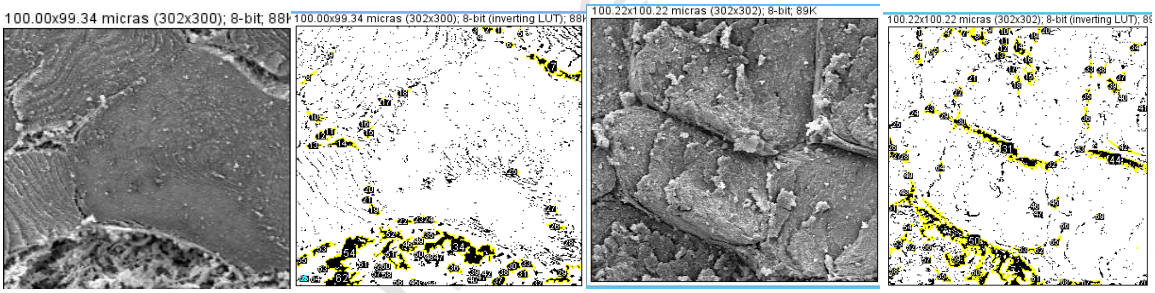
Fig. 4



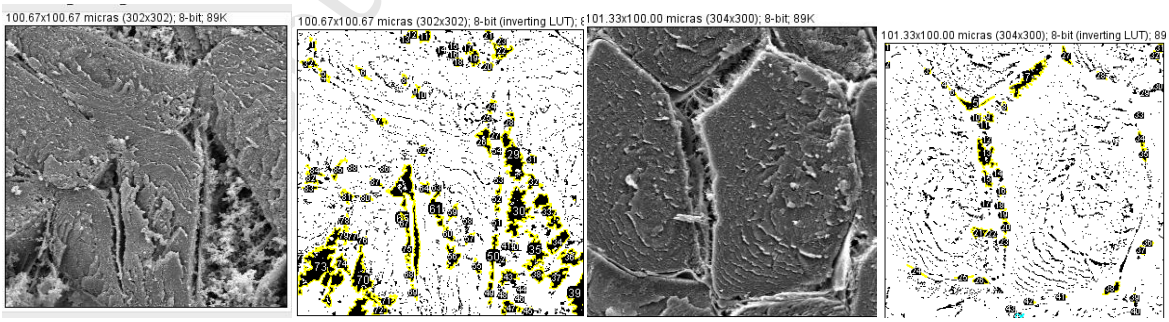
Longissimus lumborum 0/0 *Longissimus lumborum* 0/14



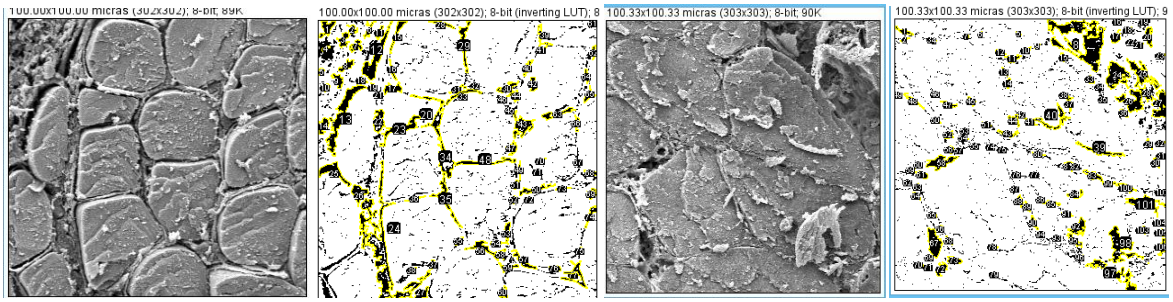
Longissimus lumborum 80/0 *Longissimus lumborum* 80/14



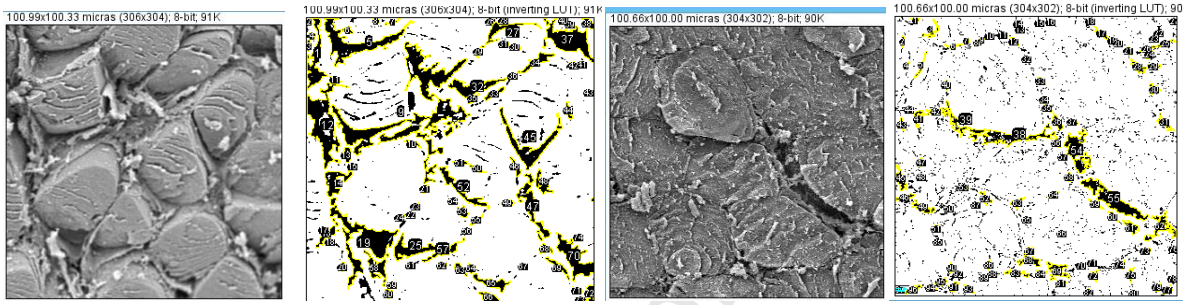
Infraspinatus 0/0 *Infraspinatus* 0/14



Infraspinatus 80/0 *Infraspinatus* 80/14



Cleidooccipitalis 0/0 *Cleidooccipitalis* 0/14



Cleidooccipitalis 80/0 *Cleidooccipitalis* 80/14

Fig. 5

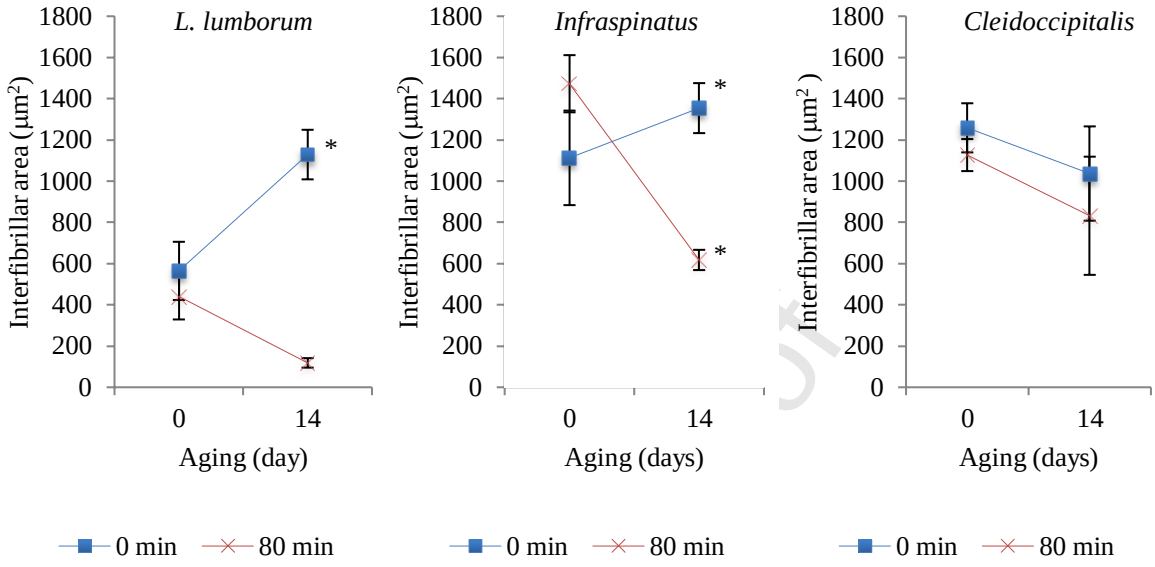


Fig. 6

HIGHLIGHTS

- Ultrasound accelerates the ageing process of bovine muscles
- Water holding capacity of *Cleidooccipitalis* increases immediately after sonication
- Sonication for 80 min is the most effective treatment for reducing the total collagen of meat
- *Infraspinatus* is the most tender muscle however *l. lumborum* appear to benefit the most from ultrasound application.

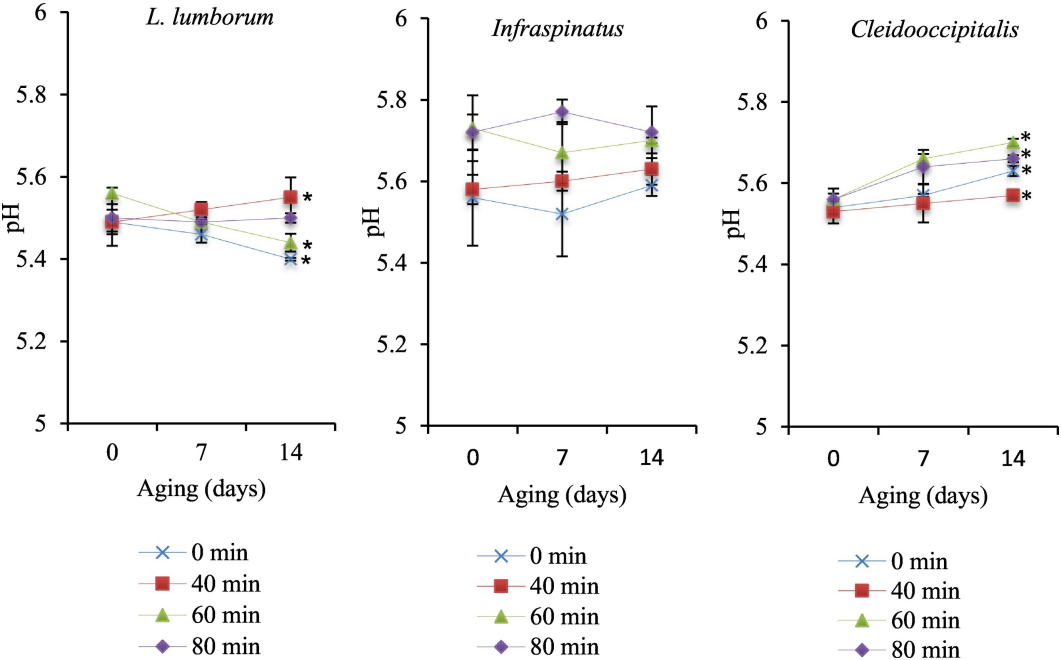

L. lumborum
Infraspinatus
Cleidooccipitalis
pH
6
5.8
5.6
5.4
5.2
5
0
7
14
Aging (days)
0 min
40 min
60 min
80 min
*

Figure 1

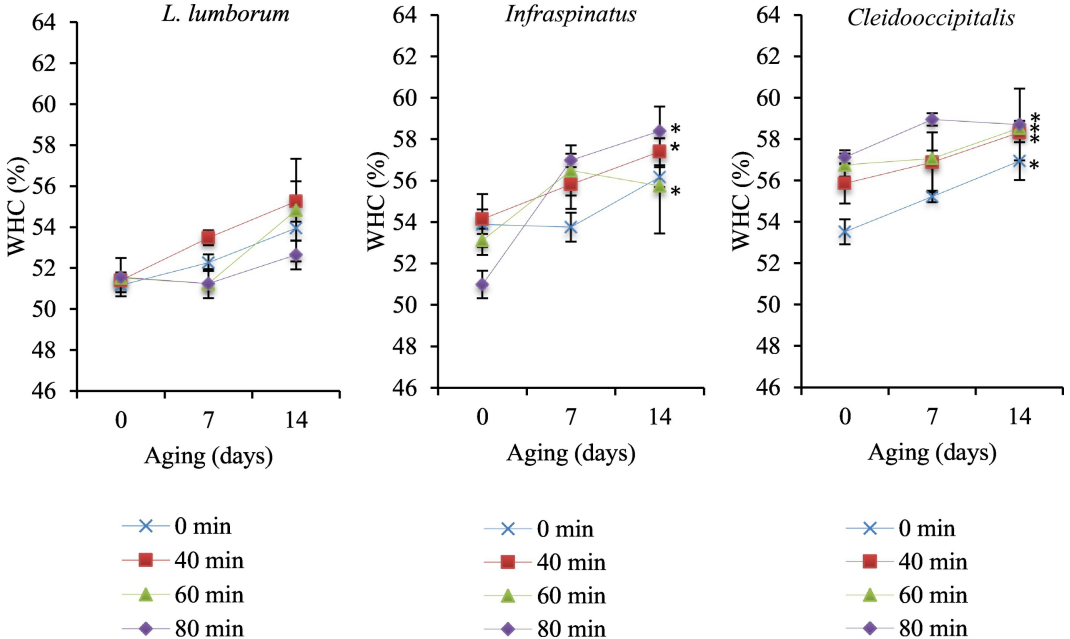

L. lumborum
Infraspinatus
Cleidooccipitalis
WHC (%)
64
62
60
58
56
54
52
50
48
46
0
7
14
Aging (days)
*
*
*
*
**
***
0 min
40 min
60 min
80 min

Figure 2

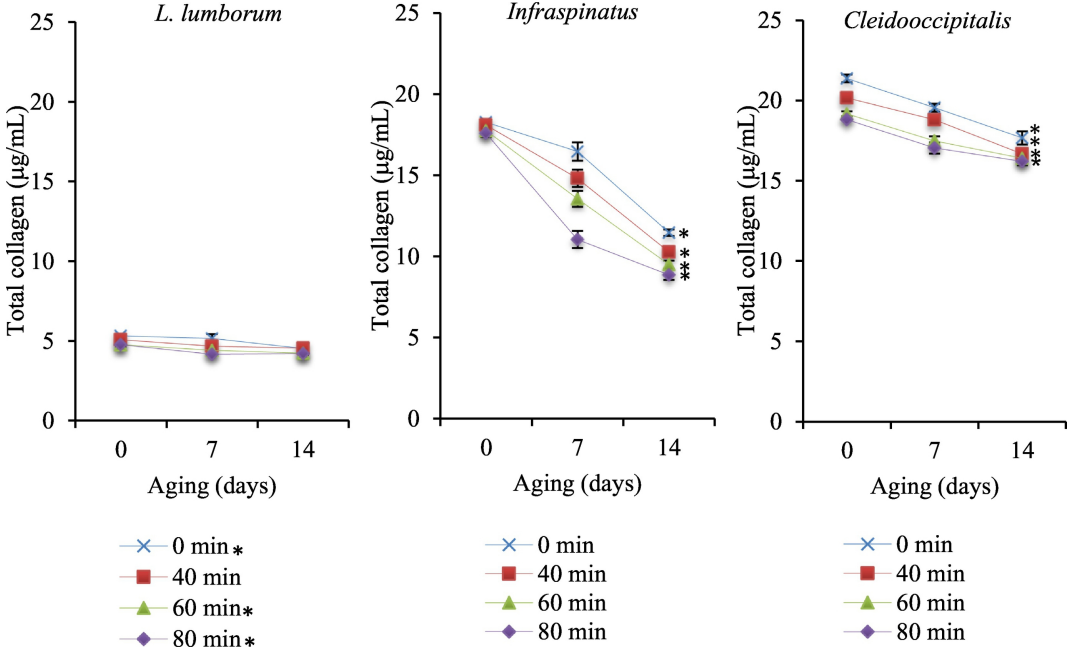

L. lumborum
Infraspinatus
Cleidooccipitalis
Total collagen (µg/mL)
Aging (days)
0
7
14
5
10
15
20
25
0 min *
40 min
60 min*
80 min *
0 min
40 min
60 min
80 min
0 min
40 min
60 min
80 min
*
***
***

Figure 3

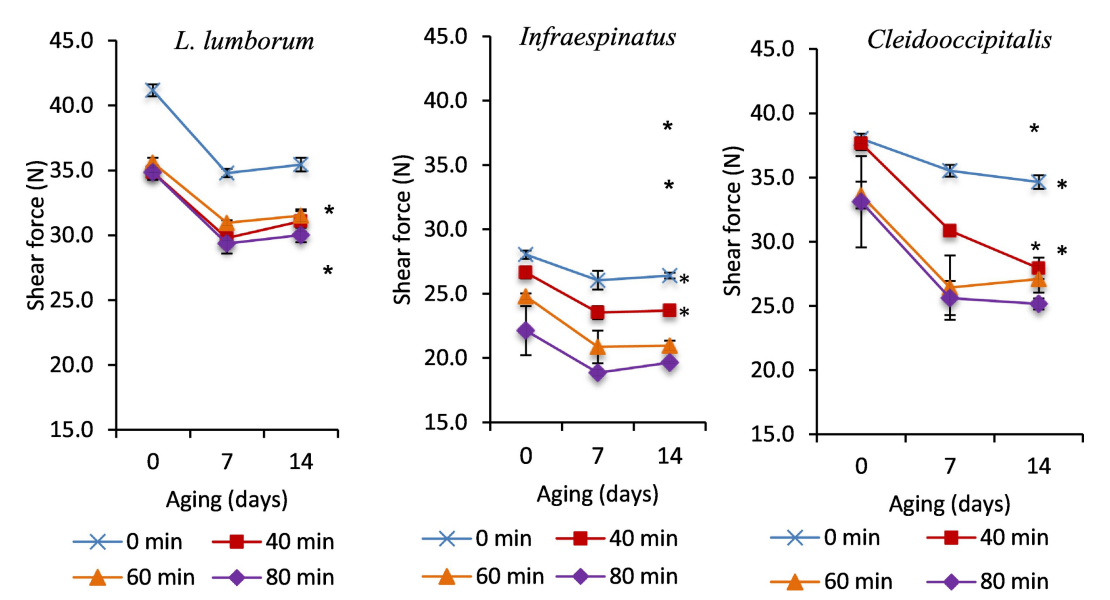
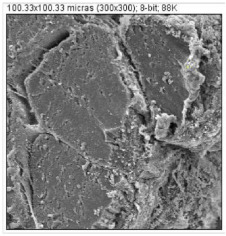
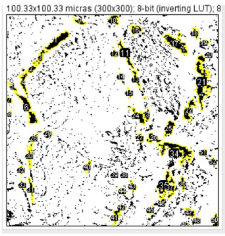
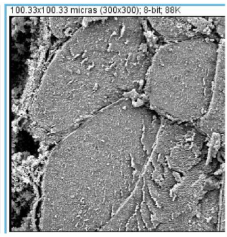

L. lumborum
Infraespinatus
Cleidooccipitalis
Shear force (N)
45.0
40.0
35.0
30.0
25.0
20.0
15.0
0
7
14
Aging (days)
0 min
40 min
60 min
80 min
*

Figure 4

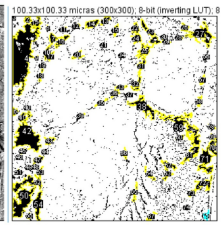


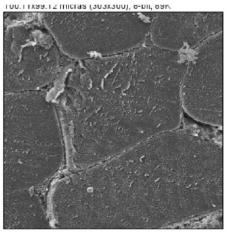
Longissimus lumborum 0/0



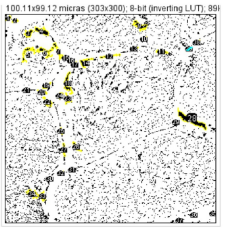


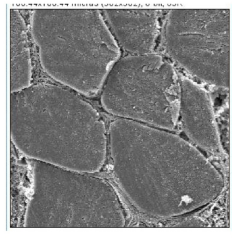
Longissimus lumborum 0/14



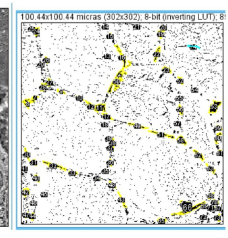


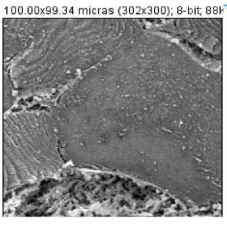
Longissimus lumborum 80/0



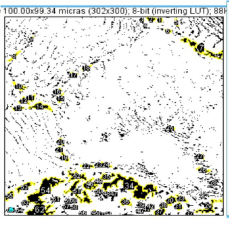


Longissimus lumborum 80/14

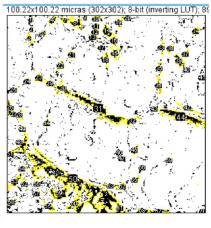


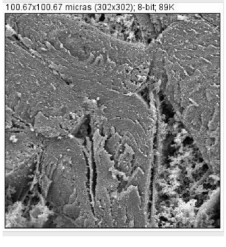


Infraspinatus 0/0

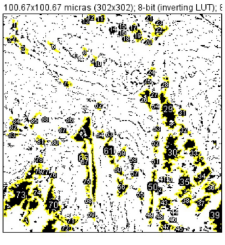


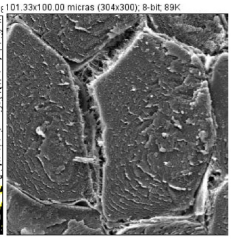
Infraspinatus 0/14



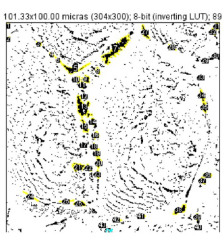


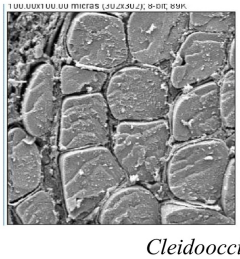
Infraspinatus 80/0



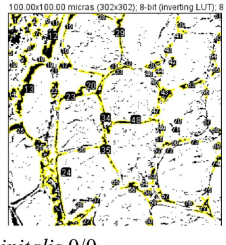


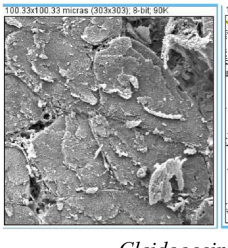
Infraspinatus 80/14



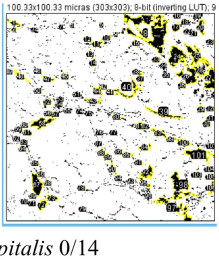


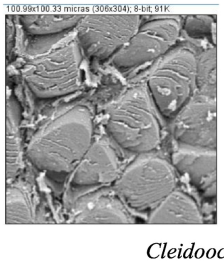
Cleidooccipitalis 0/0



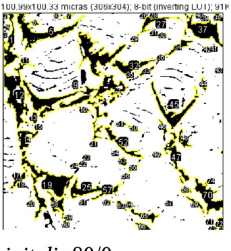


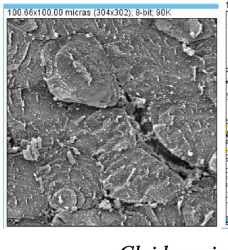
Cleidooccipitalis 0/14





Cleidooccipitalis 80/0





Cleidooccipitalis 80/14

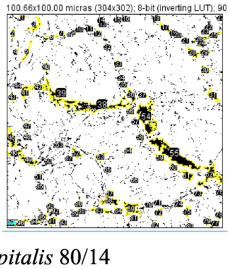


Figure 5

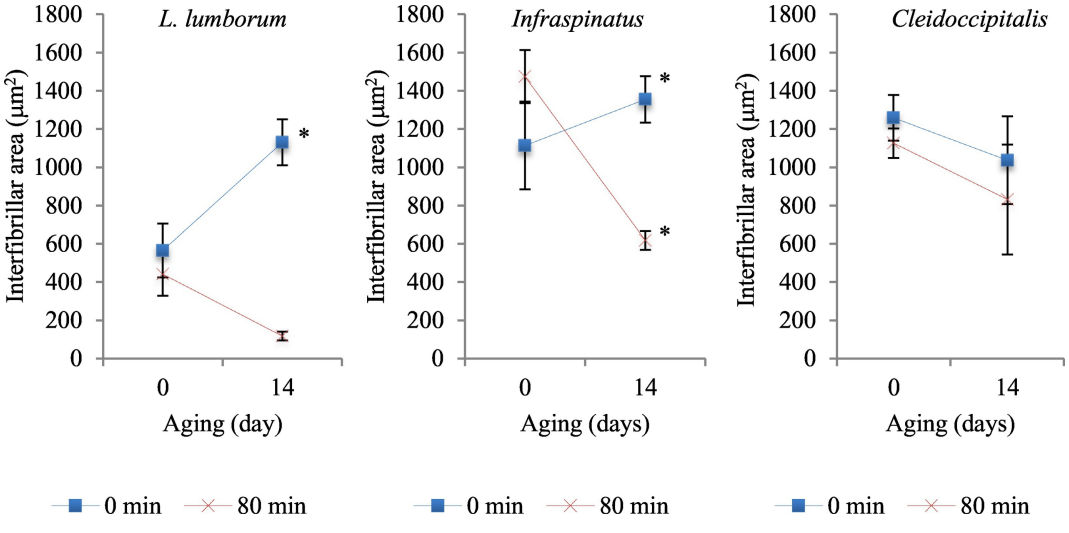

L. lumborum
Infraspinatus
Cleidoccipitalis
Interfibrillar area (μm²)
1800
1600
1400
1200
1000
800
600
400
200
0
*
0
14
Aging (day)
Aging (days)
0 min
80 min

Figure 6