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High-Intensity Ultrasonication as an Innovative Approach for the Softening of Wooden Breast Meat in Broilers

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Abstract: Considering the remarkable implications exerted by the occurrence of wooden breast (WB) abnormality on meat tenderness and marketability, the poultry processing industry demands the implementation of postmortem procedures that can improve the textural traits of chicken breasts affected by this defect. Within this scenario, this study aimed at exploring the effectiveness of high-intensity ultrasonication (HIU) in attenuating the toughness of WB fillets and evaluating its effects on the main technological properties and quality characteristics of chicken breast meat. Overall outcomes showed that HIU significantly reduced ($P < 0.01$) the compression forces of both unaffected and WB raw meat without negatively affecting the main meat quality traits and technological properties, such as color and water holding capacity. The significant ($P < 0.05$) increase in myofibrillar fragmentation index (MFI) observed in unaffected fillets might hint at an alteration of myofibril integrity following the mechanical action of ultrasonic waves. However, despite the effectiveness of HIU in improving the textural traits of raw WB meat, overall outcomes obtained through western blot and MFI analyses suggested that HIU did not remarkably alter the microstructure of myopathic muscles. The myodegenerative lesions typically occurring in WB muscles may have partially disguised the mechanical effects of ultrasonic waves on muscle cells' structures, making the elucidation of the mechanisms that lead to the softening of WB meat particularly complex.

Key words: broiler breast meat, wooden breast, tenderness, high-intensity ultrasonication, water holding capacity
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Introduction

The available OECD-FAO forecasts disclose that poultry is the most consumed meat in both developing and developed countries, and its consumption is predicted to reach an average of 33 kg/capita by 2030 (OECD, 2023). To meet this growing demand for poultry meat, selection criteria have been addressed over the years to improve both the size and yield of *pectoralis major* muscles, which account for up to 25% of the animal's weight (Aviagen, 2022) and are considered the most valuable cut for both processed and fresh meat products (Baldi et al., 2020). The aforementioned selection procedures have undoubtedly brought great advancements in broiler production traits and efficiency, but on the other hand, they have also been accompanied by the appearance of abnormalities affecting the breast muscles of fast-growing broilers (Velleman and Clark, 2015; Petracci et al., 2019). It is widely believed that the occurrence of these defects severely affects meat quality, so abnormal meat is usually discarded or downgraded and used in the formulation of processed products, resulting in massive economic losses for the poultry industry (Huang and Ahn, 2018; Zanetti et al., 2018). With an average incidence of up to 58%, wooden breast (WB) is the muscular defect that most jeopardizes not only the technological characteristics and nutritional value of the meat (Kuttappan et al., 2017; Xing et al., 2020) but also its sensory properties

(Aguirre et al., 2018; Sun et al., 2018). Fillets affected by this condition usually present focally or diffusely hardened consistency, paleness, small hemorrhages, and a clear viscous exudate, features that all together make WB meat unattractive to consumers (Soglia et al., 2016). From a microscopic viewpoint, muscles affected by WB exhibit a profoundly altered muscular structure (e.g., giant and necrotic fibers, fiber lysis, infiltration of inflammatory cells, fat deposition) and a massive proliferation of connective tissue (Sihvo et al., 2017). The latter phenomenon, which often leads to fibrosis, is manifested by extreme and diffuse hardness of the pectoral muscle, which usually exhibits remarkably increased average compression and shear force values compared with unaffected meat (Soglia et al., 2016; Baldi et al., 2019). The severe hardness of WB meat is a relevant issue not only when meat is sold for fresh consumption but also when it is downgraded for further processing because the utilization of affected fillets might compromise the sensory properties and functionality of the final product. Because it is difficult to mitigate the occurrence of WB *in vivo*, the identification of postmortem strategies that can improve the textural properties of WB meat is urgently needed for the poultry processing industry. Within this context, high-intensity ultrasonication (HIU) is an emerging technology that is gaining interest in the meat industry because of its ability to improve the functional properties of meat through cavitation-induced mechanisms that can affect biological tissues at both macroscopic and microscopic levels (Al-Hilphy et al., 2020). In addition to the industrial applications of HIU (e.g., accelerating mass transfer, improving curing and marinating processes, etc.), this technology has been investigated for its ability to increase meat tenderness, either directly by physically weakening muscle structure or indirectly by increasing proteolysis through the release of cathepsins and/or calcium ions (Ca^{2+}) that activate calpains (Alarcon-Rojo et al., 2015; Chang et al., 2015). Although the efficacy of HIU on the tenderization of beef meat has been widely tested and reported (Jayasooriya et al., 2007; Stadnik et al., 2008; Barekat and Soltanizadeh, 2017; Wang et al., 2022), the effects on chicken meat, in general, and on the breast muscle affected by WB, in particular, are still partially unexplored and require further investigation. Within this context, this study aimed to explore whether HIU improves the texture of meat affected by WB defect and to investigate its effects on the main technological properties of chicken meat.

Materials and Methods

The study was divided into 2 experiments: the first was conducted to explore the efficacy of HIU in attenuating the toughness of WB fillets, whereas the second aimed to evaluate the effects of ultrasonication on the main technological and quality characteristics of chicken breast meat as well as to investigate the mechanisms that could be responsible for the tenderization of WB meat.

Experimental design and samples collection

For the first experiment, a total of 50 chicken butterfly fillets (*pectoralis major* muscle) were obtained from the same flock of broiler chickens (Ross 308 strain, males, 48 d of age, 2.8 kg body weight) that were farmed and harvested under commercial standard conditions. Butterfly fillets were collected at 24 h postmortem from a commercial processing plant, and categorized by 2 experienced people as unaffected (NORM) and WB ($n = 25$/group) following the standards suggested by Sihvo et al. (2014). More specifically, chicken breasts affected by WB were selected based on their palpable hardness and muscle rigidity, choosing those cases in which the condition was uniformly present at the same severity level throughout the whole butterfly fillet. After collection, the samples were transported under refrigerated conditions to the laboratory, where breast muscles were cut to obtain both the left and right fillets. One fillet was designated to HIU, whereas the other one served as control. The side receiving the treatment (either right or left) was randomly selected. Thus, fillets were divided into 4 experimental groups ($n = 25$ fillets/group), according to the presence of WB abnormality and HIU: NORM, WB, NORM+HIU (unaffected fillets subjected to HIU), and WB+HIU (WB fillets subjected to HIU). HIU was performed using an ultrasonic bath model Elmasonic xtra ST 600H (Elma Schmidbauer GmbH, Singen, Germany) filled with water (45 L). Samples were individually packed under vacuum and treated for 40 min at an operating frequency of 25 kHz and a power density of 17.8 W/cm^2. Treatment parameters were selected based on previous experiments aimed at evaluating the effects of treatment duration and frequency on chicken meat tenderness (data not shown). During the whole treatment, the temperature of the water was monitored with a temperature probe (model TESTO 445, Testo SE & Co., Titisee-Neustadt, Germany) and kept constant at 5 ± 2°C using a coil exchanger cooling chiller inserted into

the treatment tank. To avoid variation in terms of power and efficacy of the treatment, samples addressed to HIU were randomly divided into 2 batches (25 samples each) to maintain the same water:sample ratio in the treatment tank. Immediately after treatment, a meat sample was obtained from the cranial section of each fillet and used for texture analysis.

For the second experiment, a total of 30 chicken breasts (*pectoralis major* muscle) were obtained from a flock of broiler chickens that had the same characteristics as those used for Experiment 1. As described for the first trial, NORM and WB breasts ($n = 15$/group) were collected, handled, and randomly divided into 4 experimental groups ($n = 15$ fillets/group), according to the presence of WB abnormality and HIU. Meat samples, previously packed under vacuum, were treated with HIU under the same conditions as in Experiment 1 (40 min, 25 kHz, and 17.8 W/cm^2) and used immediately after treatment to assess meat texture, pH, color, drip and cooking losses, water mobility and distribution by time domain nuclear magnetic resonance (TD-NMR), myofibrillar fragmentation index (MFI), and expression level of desmin by western blot analysis.

Analytical Determinations

Texture analysis. The compression force test was performed using a TA-XT2i Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK) equipped with an aluminum flat square probe (4 × 4 cm). In detail, a 2 × 2 × 1 cm raw meat sample was obtained from the cranial section of each muscle, and the instrument was set to compress the sample to 40% of its initial height at a speed of 3 mm/s with the force perpendicular to the muscle fibers in order to assess the contribution of myofibrils to meat texture (Soglia et al., 2017).

pH and color measurements. The pH of meat samples was determined after the homogenization of 2.5 g of meat in a sodium iodoacetate solution as proposed by Jeacocke (1977). The color of breast muscles (Commission Internationale de l´Eclairage L^* = lightness, a^* = redness, b^* = yellowness) was measured in triplicate on the bone-side surface of each fillet using a CR-400 Chroma Meter (Konica Minolta, Tokyo, Japan) with an illuminant source C following the calibration with a reference color standard ceramic tile.

Drip and cooking losses. Parallelepiped meat cut (8 × 4 × 3 cm, weighing about 60 g) was excised from the cranial part of each fillet, placed over sieved racks inside plastic boxes, and stored for 48 h at 4 ± 1°C (Soglia et al., 2017). After storage, the samples were reweighed, and the weight difference was used to calculate drip loss. Subsequently, the same samples were individually placed in plastic bags, packed under vacuum, and cooked in a water bath at 80°C for 20 min. The difference in samples' weight before and after the cooking process was used to determine cooking loss.

Time domain nuclear magnetic resonance. Water mobility and distribution were assessed by TD-NMR. Proton transverse relaxation (T_2) decay curves in meat samples were recorded at an operating frequency of 20 MHz with a Bruker (Milan, Italy) Minispec PC/20 spectrometer using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence as previously described by Baldi et al. (2019). Briefly, measurements were carried out in duplicate and accomplished at a constant temperature (24°C) on meat samples weighing about 500 mg with a height that did not go above the active region of the radio frequency coil. Samples were taken from the cranial section of each fillet with the aid of a specific tool and inserted into a glass tube for TD-NMR assay. In order to have a total intensity of 100, the CPMG decays were normalized and then transformed into relaxograms, which were subsequently interpreted in terms of bound, intra-myofibrillar, and extra-myofibrillar water proton populations.

Myofibrillar fragmentation index. MFI was assessed following the procedure reported by Hopkins et al. (2000) with slight modifications. Specifically, 1.5 g of meat excised from the cranial section of each fillet was homogenized in 15 mL of cold buffer (0.1 M potassium chloride, 1 mM ethylenediaminetetraacetic acid, 1 mM sodium azide, and 25 mM potassium phosphate, pH 7.0) at 13,000 rpm using Ultra-Turrax T25 (IKA, Wilmington, NC). The homogenate was then centrifuged at 1,000 × g for 15 min at 4°C and, after the removal of the supernatant, the myofibrils were resuspended in 10 mL of cold buffer and centrifuged again at the same conditions. The aforementioned step was repeated twice, and the obtained myofibril suspension was filtered with a 1-mm pore-size sieve to remove connective tissue and debris. Finally, the protein concentration of myofibrillar suspension was determined by Bradford assay (Bradford, 1976), adjusted to 0.5 mg/mL with buffer, and then measured spectrophotometrically at 540 nm. MFI was calculated by multiplying the absorbance values by 200.

SDS-PAGE and western blot. Myofibrillar proteins were extracted as reported by Liu et al. (2014), and western blot analyses were carried out according to Soglia et al. (2022). Specifically, 15 µg of myofibrillar proteins were loaded into a gradient gel (4-20% Mini-PROTEAN TGX Stain-Free Gels, Bio-Rad Laboratories, Hercules, CA), and electrophoretic

separation was carried out at constant voltage (200 V) for about 30 min. After transferring the proteins, nitrocellulose membranes were incubated (45 min, at room temperature while shaking) with 15 mL tris-buffered saline with Tween 20 (20 mM Tris, 150 mM sodium chloride, 0.1% Tween 20; pH 7.4–7.6) with 5% skimmed milk powder. Membranes were then probed (60 min, room temperature while shaking) with a polyclonal rabbit anti-desmin (10570, PROGEN Biotechnik GmbH, Heidelberg, Germany) antibody (diluted 1:6,000) and a secondary anti-rabbit antibody for 60 min (1:15,000) (Merck Millipore, Burlington, MA) and treated with horseradish peroxidase–conjugated streptavidin (Merck Millipore) for 20 min. Final detection was performed with enhanced chemiluminescence (Clarity Western ECL Substrate) western blotting detection kit (Bio-Rad Laboratories). Images were obtained using the ChemiDoc MP Imaging System (Bio-Rad Laboratories). Densitometric differences were assessed using Image Lab software and normalized to total fluorescent protein signal intensity (Valli et al., 2018). Results were then expressed as percent, considering as 100% the intensity of the bands assigned to NORM and WB samples that were not exposed to HIU.

Statistical analysis

Overall data were tested for normality according to Shapiro–Wilk test (SAS Institute, Cary, NC), and variables showing a non-normal distribution were properly transformed. Then, in order to establish the effect of HIU on both unaffected (NORM) and affected (WB) muscles, data obtained from Experiments 1 and 2 were analyzed separately using the one-way analysis of variance option of the general linear model procedure of SAS software (SAS Institute), considering HIU as the main effect.

Results

Experiment 1

Figure 1 shows the results concerning the effect of HIU on the compression force (kilograms) measured on both NORM and WB raw meat. The application of HIU did not remarkably affect the compression force of NORM muscles ($P > 0.05$), whereas the hardness of WB fillets was significantly mitigated by the treatment. Indeed, WB+HIU samples showed significantly ($P < 0.01$) reduced compression forces if compared with their untreated counterparts (7.8 vs. 9.7 kg, respectively).

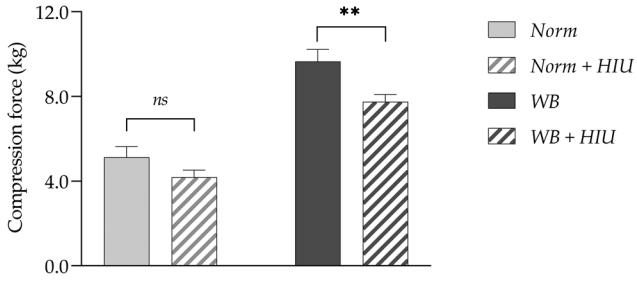

Figure 1. Average compression forces (kilograms) of unaffected (NORM) and wooden breast (WB) broiler *pectoralis major* muscles subjected to high-intensity ultrasonication (HIU) (NORM+HIU and WB+HIU) ($n = 25$/group) in Experiment 1. Data represent means ± SEM. Asterisks indicate a significant difference between the experimental groups (**$P < 0.01$). ns = not significant.

Experiment 2

As shown in Table 1, the ultrasonication process remarkably affected the texture of *pectoralis major* muscles, regardless of the presence of WB myopathy. Indeed, the compression forces of meat samples exposed to HIU were found to be significantly reduced by about 25% in both NORM+HIU and WB+HIU groups compared with their untreated counterparts ($P < 0.05$ and <0.01, respectively).

Results concerning the effect of HIU on pH, color, and water holding capacity (WHC; assessed by determining drip and cooking losses) of both NORM and WB fillets are shown in Table 1. The pH of *pectoralis major* muscles, whether unaffected or affected by WB, was not modified by the ultrasonication process. On the contrary, the application of HIU significantly

Table 1. Average compression force (kilograms), ultimate pH, color, drip, and cooking loss (%) values of unaffected (NORM) and wooden breast (WB) broiler *pectoralis major* muscles subjected to high-intensity ultrasonication (HIU) (NORM+HIU and WB+HIU) ($n = 15$/group) in Experiment 2.

	Compression force (kg)	pH	*L**	*a**	*b**	Drip loss (%)	Cooking loss (%)
NORM	6.9	5.88	53.0	1.70	3.85	3.33	22.6
NORM+HIU	5.3	5.80	56.0	1.90	5.44	2.66	21.5
SEM	0.50	0.04	0.52	0.21	0.32	0.24	0.80
P value	*	*ns*	***	*ns*	***	*ns*	*ns*
WB	10.2	6.10	52.7	1.95	5.56	3.40	27.7
WB+HIU	7.5	6.08	55.3	2.41	6.10	3.16	26.7
SEM	0.62	0.05	0.93	0.20	0.34	0.20	1.11
P value	**	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

***$P \leq 0.001$; **$P < 0.01$; *$P < 0.05$.

ns = not significant ($P > 0.05$).

changed the color of unaffected muscles, especially the lightness (L^*) and yellowness (b^*) values, which were significantly ($P \leq 0.001$) higher in the NORM+HIU samples compared with the untreated counterparts. As concerns WHC, no statistical differences have been detected among the experimental groups, suggesting that the ultrasonication process did not affect the ability of meat to retain water both during the refrigerated storage and the cooking process (i.e., drip and cooking loss, respectively), regardless of the occurrence of WB abnormality.

To better explore the effect of HIU on chicken breast WHC, TD-NMR technique was applied to measure water distribution and mobility in muscle tissue. The results obtained with TD-NMR confirmed that HIU had no effect on meat WHC under the conditions tested in this study, regardless of the presence of WB defect. Indeed, as shown in Table 2, the distribution and mobility (expressed as relative intensity and transverse relaxation time, respectively) of bound, intramyofibrillar, and extra-myofibrillar water fractions were not significantly modified by HIU.

Results concerning MFI assay are reported in Figure 2. NORM+HIU group showed significantly higher MFI values ($P < 0.05$) compared with their untreated counterpart (67.4 and 61.5, respectively), whereas the WB fillets showed no change in MFI values after ultrasonication treatment.

In western blot analysis, 2 electrophoretic fragments were detected (see supplementary material). No significant differences in the relative intensity of the desmin bands were detected when comparing treated and untreated samples, regardless of the occurrence of the WB defect (data not shown).

Table 2. Average relative intensities (*R.I.*, %) and relaxation times (T_2, ms) of the protons populations identified through TD-NMR, assessed on unaffected (NORM) and wooden breast (WB) broiler *pectoralis major* muscles subjected to high-intensity ultrasonication (HIU) (NORM+HIU and WB+HIU) ($n = 15$/group) in Experiment 2.

	Bound water		Intra-myofibrillar water		Extra-myofibrillar water	
	R.I. (%)	T_2 (ms)	*R.I.* (%)	T_2 (ms)	*R.I.* (%)	T_2 (ms)
NORM	2.36	3.19	76.4	42.9	18.7	112.6
NORM+HIU	2.30	2.93	75.9	42.8	22.8	114.7
SEM	0.10	0.23	2.53	0.91	1.54	3.97
P value	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
WB	2.62	3.19	63.7	46.1	33.7	135.9
WB+HIU	2.35	2.93	65.1	45.0	35.9	137.3
SEM	0.16	0.23	2.58	0.87	2.49	5.60
P value	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

ns = not significant ($P > 0.05$).

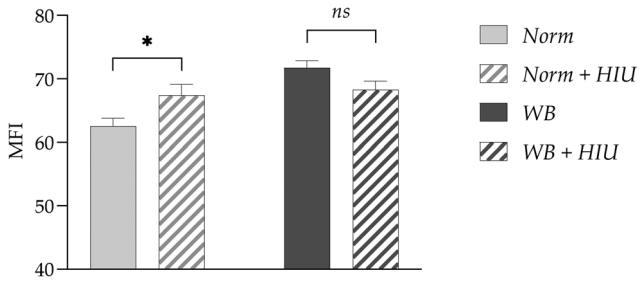

Figure 2. Average myofibrillar fragmentation index (MFI) of unaffected (NORM) and wooden breast (WB) broiler *pectoralis major* muscles subjected high-intensity ultrasonication (HIU) (NORM+HIU and WB+HIU) ($n = 15$/group) in Experiment 2. Data represent means ± SEM. Asterisks indicate a significant difference between experimental groups ($^*P < 0.05$). ns = not significant.

Discussion

The overall results regarding meat texture obtained in this study suggest that HIU is an effective strategy to attenuate the hardness associated with the occurrence of WB defect. Indeed, the application of HIU significantly reduced the compression forces of the WB fillets, so much so that the average compression force of the WB+HIU samples was, in absolute terms, quite similar to that observed in NORM meat (7.5 and 6.9 kg, respectively; Table 1). The potential of high-intensity ultrasound technology for meat tenderization has been previously demonstrated for pork (Siró et al., 2009), beef (Stadnik et al., 2008; Chang et al., 2015; Wang et al., 2022), rabbit (Gómez-Salazar et al., 2018), and chicken (Dickens et al., 1991; Meek et al., 2000; Chen et al., 2021) meat cuts and is already being used to improve the textural traits of "tough meats," such as those of spent hens and geese (Xiong et al., 2012; Li et al., 2018). Most authors agree that ultrasound treatment can tenderize meat by several mechanisms, including physical disruption of myofibrillar protein structures (Stadnik et al., 2008) and fragmentation of collagen macromolecules (Chang et al., 2012) as well as the acceleration of proteolysis through the release of lysosomal cathepsins and/or Ca^{2+} ions that activate calpains. These events are caused by HIU-induced acoustic cavitation, i.e., a phenomenon in which bubbles form, grow, and eventually collapse in the treatment medium (i.e., water), causing either mechanical and/or thermal

effects (Alarcon-Rojo et al., 2019). Although the former involves the implosion of bubbles, which generates shock waves that can affect biological tissues at a micro- and macro-scale, the latter is related to the critical temperatures reached during the process that can cause the denaturation of proteins and nucleic acids of muscular cells (Alarcon-Rojo et al., 2015; Jayasooriya et al., 2004). However, because the temperature of the treatment medium (i.e., water) was kept constant at 5 ± 2°C during the whole process, the modification in meat hardness observed within this study could not be attributed to the thermal effect induced by HIU. Therefore, we hypothesized that the lower compression forces observed in NORM+HIU and WB+HIU groups might be due to the mechanical effect of cavitation on muscle cell structure, which may have triggered myofibrils fragmentation.

To test our hypothesis, MFI assessment was performed to determine whether HIU may have altered myofibril integrity because this parameter is commonly used to evaluate myofibril degradation and fragmentation (Lametsch et al., 2007). Because high MFI values are commonly associated with a more intense fragmentation of the myofibrillar structure, the increased MFI observed in NORM+HIU fillets could indicate a partial degradation of muscle microstructure as a result of mechanical exposure to ultrasonic waves. Møller et al. (1973) and Culler et al. (1978) previously reported that myofibril degradation, which is responsible for more than 50% of the variations in muscle toughness, is a useful predictor of meat tenderness. Within this scenario, the increased degree of myofibril fragmentation after HIU application could help explaining the lower compression forces observed in NORM+HIU samples (Table 1), as also reported in previous studies carried out on meat from chicken (Cao et al., 2021), goose (Li et al., 2018), pig (Yeung and Huang, 2017), and beef cattle (Kang et al., 2017).

Unexpectedly, despite the lower compression forces detected in WB+HIU samples (Figure 1, Table 1), WB fillets did not show any increase in MFI values following the ultrasonication treatment, suggesting that the softening observed in WB meat after HIU may not be due to increased fragmentation of myofibrils. Previous studies evidenced greater MFI values in WB meat compared with unaffected fillets (Sun et al., 2018; Hasegawa et al., 2020), likely because of the remarkably altered muscular structure and increased collagen content of WB meats as a result of preharvest myofiber degeneration (Sihvo et al., 2014). In addition, a study carried out by Hasegawa et al. (2020) suggests that the greater myofibril fragmentation usually observed in WB meat may be also ascribable to a significant weakening of the myofibrillar structure because of enhanced endogenous protease activity. Within this scenario, it could be hypothesized that HIU might not have altered WB myofibrils integrity to the same extent it did in NORM fillets because the myofibrillar structure of WB muscles is usually already damaged and weakened because of the occurrence of myopathic conditions.

To better understand the eventual effect of HIU on myofibril structure, the expression level of desmin was also investigated because several studies suggested this protein as a potential marker of the proteolytic processes affecting the myofibrillar structure (Wang et al., 2018; Chen et al., 2021). However, regardless of the occurrence of WB, no differences in the relative intensity of the desmin band were observed when comparing treated and untreated samples, suggesting that HIU did not remarkably alter muscle microstructure. This finding may likely be ascribed to the short time interval between the application of HIU and the execution of analyses that may have not permitted the activation of endogenous proteolytic enzymes, suggesting that a longer storage of the treated samples before analyses would have allowed a better detection of eventual effects.

As concerns meat quality traits and technological properties, the application of HIU did not affect the muscular pH of either NORM or WB fillets, corroborating the results of previous studies carried out on chicken meat (Got et al., 1999; Stadnik et al., 2008). In contrast, according to what was previously found by Royintarat et al. (2020), HIU caused a distinct color change in NORM chicken breast meat, as shown by the higher lightness and yellowness values. However, it is worth noting that such HIU-induced color changes were negligible and probably cannot be detected by the human eye.

Regarding WHC, the ultrasonication process did not affect the ability of meat to retain water both during the refrigerated storage and the cooking process regardless of the occurrence of WB abnormality. Results from the available literature concerning meat WHC following HIU are often quite contrasting because of differences in the ultrasonication conditions and nature of the samples in each study. However, most authors agree that meat WHC progressively worsens with increasing intensity or duration of sonication, not so much because of the effect of cavitation but rather because of the increase in the temperature of the medium inside the treatment chamber, which may trigger protein denaturation (Li et al., 2015; Alarcon-Rojo et al., 2019). In this study, the ultrasonication chamber was equipped with a cooling unit that allowed meat

samples to be processed at refrigerated temperatures, likely avoiding heat-induced protein denaturation. That may likely explain the discrepancies found when comparing the obtained results with those found in previous studies.

In order to better explore the effect of HIU on chicken breast meat WHC, TD-NMR technique has been applied to measure water distribution and mobility within the muscle tissue. The results of TD-NMR confirmed that HIU did not affect meat WHC. Thus, in light of the overall outcomes obtained within this study, it could be hypothesized that ultrasonication performed at 25 kHz, 17.8 W/cm^2 for 40 min at refrigerated temperatures neither improved nor worsened water binding properties of chicken breast meat, likely because the aforementioned processing conditions were unable to induce electrical charge changes in muscle proteins and/or trigger protein denaturation processes, mechanisms that are believed to be responsible for variations in meat WHC following HIU (Zhang et al., 2017; Cao et al., 2021).

Conclusions

HIU efficiently improved the texture by reducing the hardness of raw chicken breast meat without negatively affecting the main meat quality characteristics and technological properties, regardless of the occurrence of WB condition. Overall results obtained within this study suggested that the softening effect induced by HIU was not due to a water redistribution within the muscle compartments nor to a heat-induced protein denaturation effect because meat samples were ultrasonicated at refrigerated temperatures. Rather, it could be hypothesized that the reduced compression forces observed in unaffected meats following HIU might be due to structural changes such as the physical degradation of myofibrillar structures caused by the cavitation process. However, although HIU remarkably mitigated WB meat hardness, the results of MFI and western blot analyses seemed not to fully support this hypothesis. In this scenario, it could be speculated that the myodegenerative lesions typically occurring in WB muscles (i.e., damaged and weakened myofibrillar structures as well as the massive deposition of interstitial connective tissue) may have partially masked the mechanical effects of ultrasonic waves on muscle cells' structures, making the elucidation of the mechanisms that lead to WB meat softening particularly complex. Therefore, further studies should be performed to better elucidate whether the softening of WB muscle following HIU should be truly imputable to the physical damage of myofibrillar protein structures rather than the fragmentation of collagenous macromolecules as well as to determine if it is maintained also after meat processing and cooking operations.

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Supplementary Material

Representative image of a nitrocellulose membrane incubated with polyclonal rabbit anti-desmin primary antibody after performing the final detection with enhanced chemiluminescence.