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Application of ultrasound treatment in chicken gizzards tenderization: Effects on muscle fiber and connective tissue

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ABSTRACT

The tenderizing effect of different ultrasound treatments on the characteristics of muscle fibers and connective tissue of chicken gizzard was investigated. It could be concluded that the shear force and muscle fiber diameter of the sample treated with ultrasound for 500 W/30 min were decreased by 27.1% and 26.2%, respectively, while the myofibril fragmentation index (MFI) was increased by 238.1% than the control. More importantly, the contents of hydroxylysine pyridinoline and lysine pyridinoline of the samples treated with ultrasound for 500 W/30 min were 23.1% and 40.5% lower than those of the control. Tenderizing effect of 500 W/30 min sample on thermal stability was verified from the decrease in transition temperature (Tmax) (10.7%) and enthalpy (ΔH) (21.7%) of collage compared with the control. In general, proper ultrasound treatment could effectively improve the tenderness of gizzard, and 500 W/30 min had the best tenderization effect. Therefore, the treatment of ultrasound was considered as a promising and efficient technique in meat processing, especially for the meat tenderization.

1. Introduction

The chicken gizzard is part of the digestive system of the chicken, also known as the muscular stomach, which makes up 2.5 percent of the chicken carcass [1]. According to "Compendium of Materia Medica (Ming Dynasty, 16th century)", chicken gizzard contains high content of protein and iron, can be used as raw materials for the production of drugs (*Gigeriae galli endothelium corneum*), and has the effects of digestion, antinausea and defecation [2]. In China, the annual production of chicken gizzards has reached 0.27 Mt in 2020, according to OECD-FAO [3], chicken gizzard is expected to increase by 1 Mt by 2028. Chicken gizzard is one of the edible by-products in the poultry industry around the world. It is an economical source of protein and other nutrients since it is cheaper compared to chicken meat. Additionally, consumption of chicken gizzard is thought to be beneficial for human health since gizzards contain lower total saturated fatty acid levels and higher total monosaturated fatty acid [4]. Chicken gizzards are usually eaten directly after sauced and stir-fried to improve the utilization rate of by-products and reduce environmental pollution [5].

The chicken gizzard is the digestive organ of the chicken, due to the lack of teeth, it needs strong muscles as the driving force to grind and digest with the folds in the gizzard. In general, the greater the exercise load, the harder the muscle [6]. The reason for this phenomenon is that proteins in such muscles are prone to covalent cross-linking during high-intensity exercise, which forms strong and dense connective tissue that supports and protects muscle fibers from damage by external processing conditions (such as cooking), leading to muscle hardening, which is not conducive to chewing and digestion [7]. Therefore, proper softening of connective tissue and moderate decomposition of muscle fibers can effectively improve the tenderness of muscle. So far, relatively few studies on gizzards have been conducted systematically. Since the basic composition of gizzard is similar to that of muscle, the tenderness of gizzard can be improved by means of muscle tenderization.

At present, the commonly used and effective physical tenderization method is ultrasound treatment, as a non-thermal processing physical processing method, has been widely used in beef [8], pork [9] and rabbit [10] tenderization. For example, Yeung and Huang [11] treated pig loin with high intensity ultrasound, and the shear force of the samples treated with ultrasound was reduced by 12.1% compared with that of the samples without ultrasound. Chang, Wang, Tang, and Zhou [12] found that ultrasound treatment of beef *semitendinosus* for 30 min could partially damage the endomysium, reduce the thickness of the perimysium, weaken the covalent cross-linking of collagen, and reduce the mechanical strength of connective tissue, which effectively improved

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the tenderness of beef *semitendinosus*. However, there have been few reports to improve the tenderness of chicken gizzard by ultrasound treatment.

Ultrasound is an efficient, safe and non-toxic physical tenderizing technology, which can transform electrical energy into mechanical energy [13]. Mechanical energy is transmitted through ultrasound medium, producing mechanical effect, thermal effect and cavitation effect [14,15]. The cavitation effect is the main cause of physicochemical reaction of muscle, and it can increase tenderness by destroying the weak interactions between molecules (such as hydrogen bonds and van der Waals forces), resulting in the destruction of muscle tissue (muscle fiber fracture) [16]. Meanwhile, the micro-jet and micro-turbulence generated by ultrasound cavitation effect can cause cracks in the endomysium and the perimysium, destroy the physical structure of collagen fiber, increase the degradation of muscle enzymes, reduce the cross-linking degree of pyridine in collagen, and also play a positive role in the improvement of muscle tenderness [17]. The objective of the present study was to survey the mechanism of ultrasound treatments on the tenderness of chicken gizzard by the performance of muscle fiber and connective tissue properties. In addition, the optimal ultrasound condition was selected to provide theoretical support and technical guidance for the wide application of chicken gizzard in the food industry.

2. Materials and methods

2.1. Chemicals

Ethylene diamine tetraacetic acid (EDTA), sodium azide, KH_2PO_4, K_2HPO_4, $MgCl_2$ and sirius red were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were purchased from Wantai Biomedicals Inc. (Harbin, China) and at least analytical grade.

2.2. Sample preparation

The 2.0 kg chicken gizzards (12 h after being slaughtered, about 2.0 kg per Arbor Acres chicken raised for 50 days and about 30 g per chicken gizzards) were purchased from the Haoyouduo supermarket (Harbin, Heilongjiang) and quickly transported to the laboratory with an ice bag (the temperature of the gizzards was kept at about 4 °C). The yellow skin and fat on the surface of the chicken gizzards were removed, washed with clean water and placed in a refrigerator at 4 °C. The individual chicken gizzard was placed in separate zipper bag and fully immersed in distilled water for ultrasound treatment (30 kHz). An ultrasonic bath equipment (Nanjing Xianou Co., Ltd., Nanjing, China) was used for the ultrasound treatments. The preliminary experimental results showed that the relatively good tenderization conditions were as follows: the ultrasound power was 500 W/600 W (Fig. SI, supplementary), the ultrasound time was 20 min/30 min (Fig. S2, supplementary) and the ultrasound intermittent mode was 3 s/3 s (Fig. S3, supplementary). The ultrasonic intensities were measured according to the method of Malik and Saini [18], and the actual ultrasonic intensities of 500 W and 600 W were 2.09 and 2.46 W/cm^2, respectively. Therefore, the samples were treated with different ultrasound treatments (500 W/20 min, 500 W/30 min, 600 W/20 min, 600 W/30 min, 3 s/3 s), in order to select the best combination of ultrasound tenderization. The samples in the control group were not treated with ultrasound.

2.3. Microstructure

The microstructure of the chicken gizzards was observed using a scanning electron microscope (SEM) (S-3400 N; Hitachi, Tokyo, Japan). The method for determining the microstructure was referred to Li et al. [19]. The samples were trimmed by a double-sided blade 0.5 × 0.5 × 0.5 cm^3 and fixed with glutaraldehyde in phosphate buffer (pH = 7.2) for 2 h. The samples were flushed for 1 h and eluted with alcohol gradient. The dried samples were placed in conductive double-sided adhesive tape and coated using a gold–palladium alloy coater (Bal-Tec Co., Manchester, NH, USA). The acceleration voltage of the SEM was 5 kV. Magnification was 2000 x.

2.4. Shear force

The method for determining the Warner-Bratzler shear force was referred to Wheeler, Shackelford, and Koohmaraie [20] with a slight modification. 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 °C was reached at the geometric center. The shear force of the sample (2.00 × 1.50 × 1.50 cm^3) was measured using a texture analyzer (Stable Micro System, TA: XT2I, UK). The pre-test speed was 5.00 mm/s and the test speed was set at 2.00 mm/s. The arm of force was 30.0 kg. Distance was 1 cm. The time interval for the blade to measure the same sample was 5.00 s (two presses). Trigger force was 20.0 g.

2.5. Myofibril fragmentation index

The MFI of the samples was determined and the analysis of the MFI according to the methodologies described by Culler, Parrish Jr, Smith, and Cross [21]. Muscle tissue was pulverized in liquid nitrogen, and 0.5 g of powdered tissue was homogenized for 1 min in 30 mL 25 mmol/L phosphate buffer (0.1 mol/L potassium chloride, 1 mmol/L ethylenediaminetetraacetic acid, pH 7.0). The suspension was filtered to remove connective tissue, and residue was washed with 10 mL 25 mmol/L phosphate buffer. Then filtrate was centrifuged at 1000 × g for 15 min at 4 °C, the precipitate was resuspended in 10 mL phosphate buffer and centrifuged again. This step was repeated twice more and the pellet was suspended in buffer solution. The protein concentration was diluted to 0.5 mg/mL and measured spectrophotometrically at 540 nm (UV 6100, Jinan Hepu Instrument Equipment Co., LTD, Shandong, China). MFI was calculated by multiplying readings with 200.

2.6. Histological structure

The tissues (0.5 × 0.5 × 0.5 cm^3) were put into TSJ-Q automatic closed tissue dehydrator (Zhongwei Electronic Instrument Co., Jiangsu, China) and BMJ-III embedding machine (Zhongwei Electronic Instrument Co., Jiangsu, China) in sequence, and cut into 10-μm thick slices along the longitudinal section. The histological structure was evaluated in accordance with Flint and Pickering [22]. Images were obtained by the Olympus CX33 microscope (Jinan Jiwei Medical Instrument Co., LTD., Shandong, China). Magnification was 4 x. After histological structure observation, muscle fiber diameter and perimysium thickness were measured and calculated using ImageJ software.

2.7. Mechanical strength

The mechanical strength of connective tissue was determined by the method of Nishimura, Hattori, and Takahashi [23] using a texture analyzer (Stable Micro System, TA: XT2I, UK). Samples were cut into pieces of 1.0 cm × 1.0 cm × 1.5 cm^3 and the mechanical strength of acrylamide embedded connective tissue was measured by texture analyzer. The pre-test speed was 5.00 mm/s and the test speed was set at 2.00 mm/s. The arm of force was 30.0 kg. Distance was 5.00 mm.

2.8. Hydroxylysine pyridinoline and lysine pyridinoline

The gizzard was weighed and chopped, and the sample was crushed with 4 times volume of PBS (0.01 mol/L, pH = 7.4) by a homogenizer (Lue Shen Instrument Equipment, Shanghai, China). The homogenate was centrifuged at 2500 r/min for 20 min, and the supernatant was taken for testing. Standard holes and sample holes were respectively set on the plate. Standard holes were filled with standard substances of

different concentrations (50 μL). Sample holes were filled with 40 μL of sample diluent, and then 10 μL of sample to be tested. Add enzyme-labeled reagents (100 μL) to the standard holes and sample holes respectively, then seal the plate, and incubate at 37 °C for 60 min. After repeated washing 5 times, added 50 μL of chromogenic agent A and B respectively to each hole. After developed the color at 37 °C for 15 min (protected from light), and immediately measured the OD value of each hole at 450 nm.

2.9. Collagen content and heat solubility

To estimate the total collagen content, approximately 300 mg of freeze-dried samples were randomly selected and hydrolysed in 10 mL of 6 mol/L HCl for 16 h at 110 °C. The hydroxyproline content was measured in the method described by Wang et al. [24]. The amount of hydroxyproline was determined and converted to the collagen content with a factor 7.25.

Freeze-dried samples (approximately 300 mg) were suspended and homogenised with 3 mL of Ringer's solution. The homogenates were heated in a water bath (77 °C, 70 min) and centrifuged (6000×*g*, 20 min). The supernatant solution was decanted, and the pellet was suspended in the same solution and recentrifuged. The two supernatants were combined, and the amount of soluble collagen was determined as described above. The heat solubility of collagen was expressed as a percentage of the total amount of collagen.

2.10. Thermal stability

The extraction of connective tissue collagen and the determination of its thermal stability were slightly modified according to the method of Li, Zhou, and Xu [25]. The endothermal transition temperature of connective tissue collagen was measured using a calorimeter (DSC 7; Perkin Elmer, Waltham, MA, USA). Temperature calibration was run using the Indium thermogram. The samples (20 mg) were accurately weighed into aluminum pans and sealed. The samples were scanned at 2 °C/min over the range of 20–90 °C using liquid nitrogen as the cooling medium. The maximum transition temperature was estimated from the thermogram using the software Pyris Manager Series.

2.11. Statistical analysis

Datas were analysed using a mixed model according to the analytical method of Biffin, Smith, Bush, Collins, and Hopkins [26], in combination with the research contents of our study. The fixed terms were the ultrasound conditions, and each replication involved a random term in this model. The experiment was repeated three times, three times in parallel. Statistical significance ($P < 0.05$) was determined using IBM SPSS statistics 22.0 to perform the Duncan test. Graphs were generated by using SigmaPlot 12.5 and OriginPro 8.5.

3. Results and discussion

3.1. Microstructure

Myofibers coated with perimysium and endomysium connective tissues are slender cylindrical cells, and each myofiber is composed of perimysium, myoplasm, cell nucleus, and a large number of myofibrils [27]. The microstructure of muscle fibers can be observed by SEM, and the arrangement and integrity of muscle fibers can directly reflect the texture of the sample, so as to determine its tenderness [10]. The microstructure of chicken gizzard muscle fibers after different ultrasound treatments is shown in Fig. 1. The muscle fibers of the control arranged tightly and orderly. When subjected to ultrasonic processing as a supplementary means, the structural integrity of the myofibers was obviously damaged, and myofibrils were completely exposed. Among them, the effect of 500 W/30 min ultrasound treatment on the integrity of muscle fibers was the most obvious. Most of the myofibrils were fractured and fragmented significantly as reflected in the poor, disordered cell edge. Ultrasonic cavitation made chicken gizzard subjected to rapid molecular vibration, myofiber structure fracture and separation [27]. As a result, the complete tissue structure was fast decomposed, and myofibrillar fragmentation was strengthened (Consistent with MFI results). Furthermore, roughness is also a widely-used index to describe the surface status of the specimens. The physical weakening of muscle structure and cavitation caused by ultrasound influenced the roughness. The roughness of meat may be related to degree of occlusion. High roughness means high degree of occlusion, which is important for consumers when eating the meat [28].

3.2. Shear force

Warner-Bratzler shear force is usually used to determine the degree of muscle fiber fracture, which can directly reflect the tenderness of muscle, and tenderness is the most important quality characteristic of muscle [20]. The shear force of the control was 7.54 N, and the shear force of all samples decreased after ultrasound treatment. Among them, the sample after ultrasound treatment with 500 W power for 30 min had the least shear force, which was reduced by 27.1% compared with the control (Fig. 1). This result fully indicated that ultrasound treatment was effective in improving the tenderness of chicken gizzard. This phenomenon might be because the strong cavitation effect of ultrasound treatment could promote the softening of muscle tissue and the fracture of muscle fiber, leading to the decrease in the hardness of the sample [29]. Xiong et al [30] found that the shear force of ultrasound (frequency: 20 KHz, intermittent mode: 5 s/5s, power: 300 W, time: 50 min) assisted sodium bicarbonate curing (USC) of chicken breast meat was

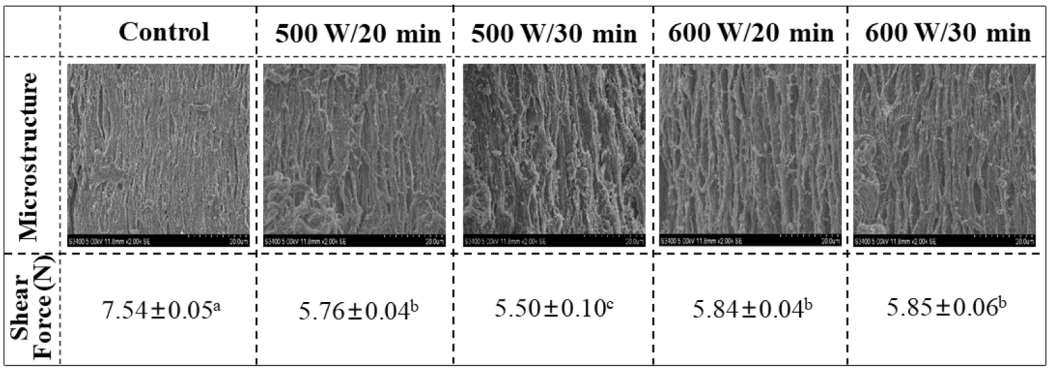


Fig. 1. Effect of different ultrasound treatments on the microstructure (magnification: 2000) and shear force of chicken gizzards. The means at the same index with different lowercase letters (a–c) differ significantly ($P < 0.05$).

significantly lower than sodium bicarbonate curing (SC) sample. The reason why the USC chicken showed lower shear forces than that of the SC chicken is that ultrasound could destroy lysosomes to release cathepsin, which could weaken the muscle fiber structure and ultimately lead to a decrease in shear force. Together with this research, acoustic cavitation of ultrasound waves can change physicochemical characteristics, processing and curing by contraction and expansion in the meat tissue. In addition, the reason for the higher frequency and power of ultrasound treatment in this research is that chicken gizzards are harder than chicken breast meat. Therefore, appropriate conditions should be selected when using ultrasound to improve the tenderness of meat and meat products.

3.3. Myofibril fragmentation index

MFI is an index to measure the average length of myofibrils, and it is positively correlated with the destruction degree of the internal structure of muscle fibers and skeletal proteins [31]. The MFI of the control was 12.6, and the MFI of all samples increased after ultrasound treatment. This is because ultrasound can break down the network structure of protein, the strength of protein reticular structure decreased, especially the coarse filaments in the membranes constituting muscle fiber bundles were easier to dissolve which led to the increase of MFI values [30].

It was worth noting that the MFI of the samples treated with 500 W ultrasound (20 min and 30 min) were higher than those of the samples treated with 600 W ultrasound (20 min and 30 min), and the MFI of 500 W/30 min sample was the highest. Carrillo-Lopez, Huerta-Jimenez, Garcia-Galicia, and Alarcon-Rojo [32] also showed that excessive ultrasound treatment had no positive effect on improving the tenderness of porcine *longissimus dorsi* muscle. The reason for this phenomenon is that moderate ultrasound treatment promotes the increase in muscle fiber space, so that the contents released by cells can more fully decompose muscle fibers into segments of different sizes, leading to the increase in MFI and the improvement of muscle tenderness [33].

3.4. Muscle fiber diameter

Muscle fiber diameter is one of the important factors affecting muscle tenderness, and the smaller the muscle fiber diameter, the tenderer the muscle [34]. As shown in Fig. 2, the muscle fiber diameter of the control was 43.5 μm, and the muscle fiber diameter of all samples significantly decreased after ultrasound treatment ($P < 0.05$). The vibration generated by the ultrasound wave itself can cause physical damage to the muscle tissue through the destruction of molecular interactions (such as hydrogen bonds and van der waals forces). Therefore, the integrity of Z-line might be damaged through ultrasound, leading to the fracture of muscle fibers. In addition, the microjet and high shear stress generated by ultrasonic cavitation effect will make the M−line disappear, the I-band fracture and the H-zone swelling, leading to the decrease in the diameter of muscle fiber [35]. This result could also be well explained by the muscle microstructure (Fig. 1).

When the ultrasound power was 500 W, the muscle fiber diameter of the sample treated with ultrasound for 30 min was 7.00% smaller than that treated with ultrasound for 20 min. When the ultrasound power increased to 600 W, the muscle fiber diameter of the sample increased with the extension of ultrasound time (from 20 min to 30 min). Especially, the muscle fiber diameter of the sample treated with ultrasound for 500 W/30 min was the smallest, which was 32.1 μm. Moderate ultrasound treatment can destroy the structure of myofibrils, cause the cells to release calpain, weaken the cross-linking between proteins, and decrease the diameter of muscle fibers. Excessive ultrasound treatment may damage the protease to a certain extent, and can not achieve the best effect of cleavage of muscle fibers [36].

3.5. Perimysium thickness

There are three main structures of connective tissue: epimysium

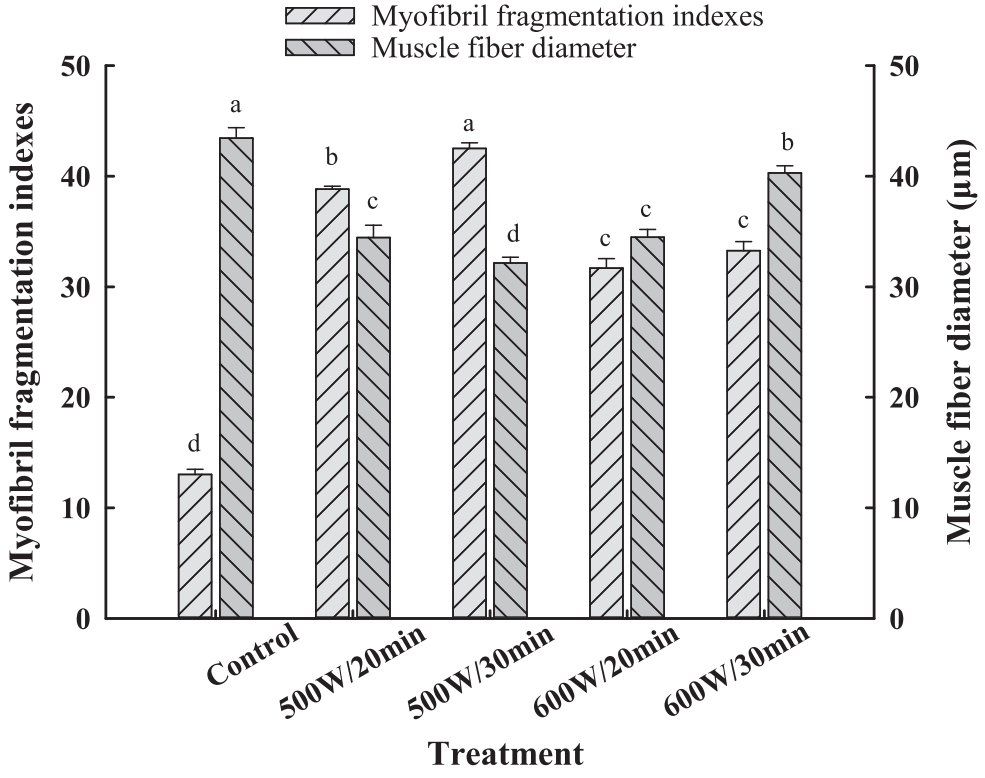


Fig. 2. Effect of different ultrasound treatments on the myofibril fragmentation indexes and muscle fiber diameter of chicken gizzards. The means at the same index with different lowercase letters (a–d) differ significantly ($P < 0.05$).

(wrapped on the outside of intact muscle mass), perimysium (wrapped on the outside of the muscle bundle) and endomysium (existed between muscle fibers). During the segmentation and trimming of muscle, the epimysium is often removed, and the remaining intramuscular connective tissue mainly refers to the endomysium and perimysium. Furthermore, the perimysium accounts for 90% of the total intramuscular connective tissue, and it is divided into primary perimysium and secondary perimysium [37].

Effects of different ultrasound treatments on the perimysium thickness of chicken gizzard are shown in Fig. 3. Compared with the control, the primary perimysium and secondary perimysium thickness of chicken gizzard were decreased after ultrasound treatment. It was worth noting that the primary and secondary perimysium thickness of the samples treated with ultrasound for 500 W/30 min reached the minimum values (23.49 μm and 104.54 μm), which were reduced by 22.8% and 19.7% compared with the control. The micro-streaming and turbulence resulting from ultrasound promotes the degradation of protein and polysaccharides in the perimysium to a greater extent, and weaken the structure of intramuscular connective tissue [38]. The microjet generated by ultrasound can also accelerate the rupture of muscle cells and the contraction of sarcomere, leading to the gradual dissociation of muscle fibers, which is manifested as the decrease in the thickness of the perimysium [39].

3.6. Mechanical strength

The mechanical strength of connective tissue not only depends on the size and arrangement of collagen fibers, but also depends on the cross-linking of collagen protein molecules. Moreover, the change in the mechanical strength of connective tissue can directly affect the tenderness of muscle [40]. The mechanical strength of the samples treated with ultrasound treatment was lower than that of the control, and the mechanical strength of the sample treated by ultrasound treatment for 30 min at 500 W power was the lowest (2.64 N), which was 63.6% lower than that of the control. The cavitation effect generated by ultrasound can destroy the integrity of the perimysium and endomysium, resulting in the rupture of the connection between collagen fibers and the weakening of the intramuscular connective tissue structure, and to explain this phenomenon at a deeper level, the cross-linking between collagen protein molecules used to stabilize the mechanical strength is broken to a certain extent [41].

However, when the ultrasound power was 600 W (whether the ultrasound time was 20 min or 30 min), the mechanical strength of the samples was higher than that of the sample treated with 500 W for 30 min. This result was in good agreement with the variation trend of shear force (Fig. 1), which further confirmed the improvement effect of moderate ultrasound treatment (500 W/30 min) on muscle tenderness. Similar results were obtained by Chang et al. [12] in studying the effects of ultrasound treatment on beef connective tissue. The mechanical strength of the beef sample sonicated for 50 min was significantly different ($P < 0.01$) from that of the control sample. This result confirmed that the weakening of connective tissue by high-power ultrasound is primarily responsible for the tenderization of meat.

3.7. Collagen covalent cross-linking

Muscle tenderness is closely related to the spatial structure and the interaction force of collagen [42]. The change in muscle tenderness is largely due to the conversion of collagen from disulfide based intermolecular cross-linking to lysine and hydroxylysine based intramolecular (pyridine cross-linking) during maturation. The content of hydroxylysine pyridinoline and lysine pyridinoline are the direct indicators reflecting the degree of pyridinoline cross-linking [43].

As shown in Fig. 4, the contents of hydroxylysine pyridinoline and lysine pyridinoline of the samples treated with 500 W ultrasound for 30 min were the lowest (10.76 ng/g and 11.59 ng/g), 23.1% and 40.5% lower than those of the control, respectively, but there was no significant difference between the other treatment groups ($P > 0.05$). The histological structure could also indirectly reflect that the cross-linking degree of collagen might be destroyed to the maximum extent under this ultrasonic condition, which led to the weakening of the network structure of connective tissue (Fig. 4). This phenomenon indicated that ultrasound treatment with appropriate intensity could destroy the spatial structure of collagen, reduce the contents of hydroxylysine pyridinoline and lysine pyridinoline of collagen (reduce the intramolecular cross-linking degree of collagen), and finally improve the tenderness of muscle.

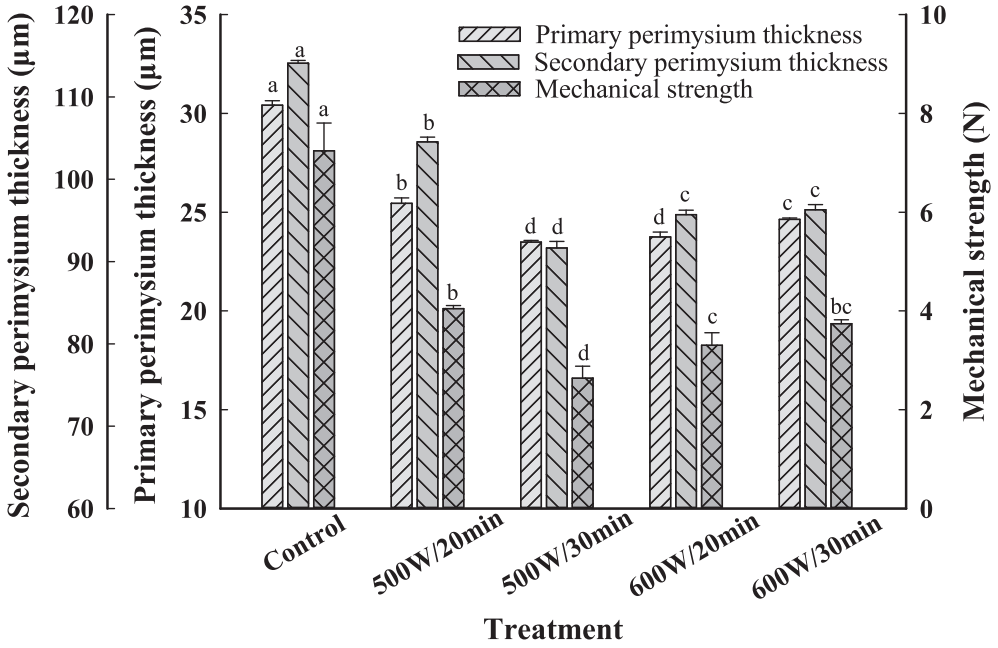


Fig. 3. Effect of different ultrasound treatments on the perimysium thickness and mechanical strength of connective tissue of chicken gizzards. The means at the same index with different lowercase letters (a–d) differ significantly ($P < 0.05$).

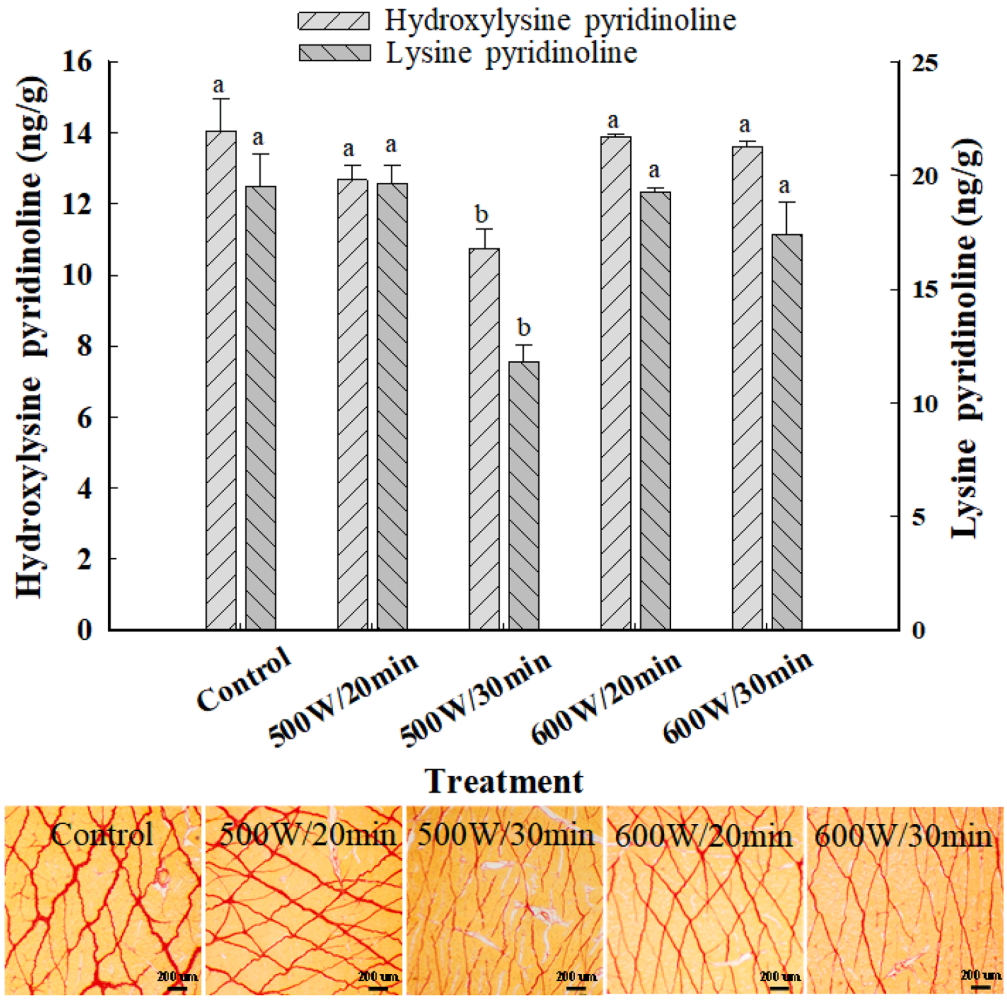


Fig. 4. Effect of different ultrasound treatments on the histological structure (4 ×), the content of hydroxylysine pyridinoline and lysine pyridinoline of chicken gizzards. The means at the same index with different lowercase letters (a, b) differ significantly ($P < 0.05$).

3.8. Collagen content

Collagen content and thermal solubility, apart from covalent cross-linking, are also important factors affecting muscle tenderness [44]. In general, the solubility of collagen is positively correlated with tenderness, based on the weakening of cross-linking [45]. Effects of different ultrasound treatments on the content of soluble collagen and insoluble collagen, and thermal solubility of collagen were shown in Table 1.

The contents of soluble collagen, insoluble collagen and total collagen of the control were 0.68%, 4.38% and 5.06%, respectively. After ultrasound treatment, the content of soluble collagen increased, while the contents of insoluble collagen and total collagen decreased. The amount of these three substances correlated well with tenderness and sensory characteristics (flavor and juiciness). The total collagen, insoluble collagen and soluble collagen were negatively associated with tenderness. The insoluble collagen was positively associated with juiciness and the soluble collagen negatively with juiciness. The insoluble collagen and soluble collagen were negatively associated with the flavor [46]. Compared with the control, the soluble collagen content of the sample treated with ultrasound for 500 W/30 min increased by 170.6%, the content of insoluble collagen and total collagen decreased by 37.0% and 9.09%. The results showed that proper ultrasonic treatment could improve the tenderness, flavor and juiciness of chicken gizzard. This phenomenon may be caused by the cavitation effect produced by ultrasound which destroys the complete structure of mitochondria and lysosomes, and releases some endogenous proteases, leading to the increase in soluble collagen content and the decrease in insoluble collagen content. However, in terms of total collagen content, Zou et al. [47] obtained contrary results in their study on ultrasound-assisted extraction of turtle calipash collagen, which might be due to the

Table 1
Effect of different ultrasound treatments on the content of soluble collagen, insoluble collagen, total collagen, the heat solubility and thermal stability of collagen.

Project	Control	500 W		600 W	
		20 min	30 min	20 min	30 min
Soluble collagen (%)	0.68 ± 0.63^{c}	1.40 ± 0.96^{b}	1.84 ± 0.36^{a}	1.48 ± 0.96^{b}	1.52 ± 0.96^{b}
Insoluble collagen (%)	4.38 ± 0.96^{a}	3.48 ± 0.16^{b}	2.76 ± 0.63^{c}	3.35 ± 0.36^{b}	3.45 ± 0.63^{b}
Total collagen (%)	5.06 ± 0.73^{a}	4.87 ± 0.73^{bc}	4.60 ± 0.36^{d}	4.83 ± 0.11^{c}	4.97 ± 0.36^{ab}
Heat solubility (%)	13.5 ± 1.3^{c}	28.7 ± 2.3^{b}	39.9 ± 1.0^{a}	30.6 ± 1.4^{b}	30.5 ± 1.7^{b}
T_{max} (°C)	47.5 ± 0.3^{a}	45.2 ± 0.2^{b}	43.3 ± 0.4^{c}	47.2 ± 0.3^{a}	47.0 ± 0.2^{a}
ΔH (J/g)	0.701 ± 0.025^{a}	0.615 ± 0.008^{b}	0.549 ± 0.005^{c}	0.690 ± 0.013^{a}	0.674 ± 0.010^{a}

Values in the table represent mean values ± standard deviations of at least triplicate determinations. The means in the same row with different lowercase letters (a-d) differ significantly ($P < 0.05$).

different ultrasound power and frequency.

The heating solubility of the control was 13.5%, and the heating solubility of all samples improved after ultrasound treatment. It was worth noting that the heating solubility of collagen was the largest (39.9%) when the ultrasound condition was 500 W/30 min. Studies had shown that there was a negative correlation between the heating solubility of collagen and the shear force of muscle [24]. In this study, the samples treated with 500 W/30 min ultrasound had the lowest shear force and the highest heating solubility, which jointly indicated that the muscle had the best tenderness under this condition.

3.9. Collagen thermal stability

Thermal stability of collagen of chicken gizzard is indicated by the transition temperature (*T*max) and enthalpy (*ΔH*) of DSC. *T*max represents the temperature of thermal denaturation of collagen, meanwhile *ΔH* represents the amount of energy to thermal denaturation of collagen [7]. The *T*max and *ΔH* of collagen treated with different ultrasound treatments are shown in Table 1. It could be seen that there was only one *T*max for all samples, which was about 47.0 °C. The *T*max of the control was 48.5 °C, and the *T*max of all ultrasound samples was lower than that of the control, indicating that the thermal stability of the samples decreased after ultrasound treatment. It was a remarkable fact that the 500 W/30 min sample had the lowest *T*max of 43.3 °C. Chang et al. [12] treated the bovine *semitendinosus* muscle with a power of 1500 W (40 kHz) for 30 min, and the *T*max of collagen was the minimum. However, the *T*max of collagen showed an increasing trend with the further ultrasonic treatment, which was similar to the results in this study.

ΔH showed a similar trend to *T*max, and the sample treated with ultrasound for 500 W/30 min had the lowest *ΔH*. This is because proper ultrasound treatment produces strong cavitation effect, which destroys the hydrogen bond that maintains the triple helix structure of collagen and reduces its thermal stability [48]. However, excessive ultrasound treatment might not play the same role in the destruction of collagen structure and covalent cross-linking, as could be seen from the results of hydroxylysine pyridinoline and lysine pyridinoline (Fig. 4).

4. Conclusions

Ultrasound treatment had positive effect on improving the tenderness of chicken gizzard. Ultrasound treatment could effectively destroy the integrity of the structure observed by SEM, and improve the degree of myofibril fragmentation. In addition, ultrasound treatment could also effectively reduce the covalent cross-linking degree and thermal stability of collagen, and improve the tenderness of muscle by weakening the mechanical strength of connective tissue. In general, 500 W/30 min ultrasound treatment had the best tenderization effect. Therefore, the treatment of ultrasound was considered as a promising and efficient technique in meat processing, especially for the meat tenderization.

CRediT authorship contribution statement

Xin Du: Methodology, Validation, Formal analysis, Writing – original draft. **Haijing Li:** Investigation. **Maheshati Nuerjiang:** Conceptualization, Resources. **Shuo Shi:** Investigation. **Baohua Kong:** Conceptualization, Software, Data curation. **Qian Liu:** Software, Investigation. **Xiufang Xia:** Conceptualization, Writing–original draft, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2021.105786.

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