Evaluation of ultrasound-assisted L-histidine marination on beef M. semitendinosus: Insight into meat quality and actomyosin properties

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ABSTRACT

This paper aimed to evaluate the effects of ultrasound-assisted L-histidine marination (UMH) on meat quality and actomyosin properties of beef M. semitendinosus. Our results found that UMH treatment effectively avoided excessive liquid withdrawal, and disrupted myofibril integrity by modifying the water distribution and weakening connection of actin-myosin with increased muscle pH. The ultrasound-treated sample provided more opportunity for the filtration of L-histidine to intervene the isoelectric point and conformation of muscle protein. The activated caspase-3 and changes of ATPase activity in UMH-treated meat accelerated the postmortem ageing, and L-histidine might competitively inhibit the actin-myosin binding by the imidazole group. UMH decreased the surface hydrophobicity by shielding hydrophobic area and unfolding the actomyosin structure. In addition, the increased actomyosin solubility with smaller particle size enhanced the SH content for better cross-linking of myosin tail, and formation of heat-set gelling protein structure. Therefore, UMH treatment manifested the potential to improve beef quality.

1. Introduction

From the perspective of consumers, meat products with desirable palatability are beneficial to pay a premium and to purchase again [1]. Given the rich nutrition, better flavor, and low fat intake, beef is increasingly preferred by the athletic youth, but not suitable for the elderly due to its harder chewiness. Thus, numerous strategies concerning mechanical intervention and proteolysis regulation have been successfully applied to improve beef tenderness [2,3]. Studies have shown that postmortem ageing time, especially in the early stage, significantly affects meat tenderness [4], and beef generally takes 1–3 weeks to mature [5]. In short, the ageing tenderization is mainly induced by multiple mechanisms including the apoptosis, calpain system, and the mediated-glycolysis enzymes [6]. However, the optimized tenderness based on ageing tenderization is usually at the expense of long ageing time, and high energy consumption. Thus, effective methods applied to accelerate the beef ageing with improved tenderness are of importance based on economic and energy-saving considerations.

Successful applications of basic amino acids (e.g., L-arginine and L-lysine) have attracted increasing attention for their good performance in compensating sensory defects of non-chloride salt-substituted meat products [7,8]. Among them, L-histidine could increase myosin solubility by the trans-formative secondary structure and intervention of filaments self-assembly process by its imidazole ring [9,10], and develop the formation of myosin gel network [11]. It is well known that the proteolysis of key structural proteins (e.g., actin and titin) and the changes of sarcomere are chiefly responsible for the developed meat tenderness during postmortem ageing [6]. After slaughter, actomyosin (AM) complex will be formed to prevent sliding of actin and myosin filaments, resulting in the development of rigidity. This phenomenon is mainly ascribed to the ATP consumption, which is mediated by the impaired Ca 2+ -accumulating ability in muscle metabolism, causing the sarcomere contraction and increased hardness [12]. In other words, the AM dissociation, to some degree, favors the developed tenderness. Given the efficiency of these amino acids on myosin, and myosin being a major muscle protein of actomyosin, it is essential to further investigate their
possible mechanism on AM for promotion of beef ageing, L-arginine has been found to display a more effective tenderizing effect than L-lysine due to the promotion of AM dissociation accompanied by the decreased Mg$^{2+}$-ATPase activity [13,14]. However, few literature focus on the effects of L-histidine on accelerating the beef ageing time by regulating the physicochemical properties of actomyosin.

The rupture of myofibrils also accelerates the AM dissociation during ageing process, and several reports have indicated that proper ultrasonication was conductive to the unfolding of actomyosin structure and the reduction in particle size, inducing more exposure of free-sulphhydryl to change the gel properties [15,16]. As an auxiliary strategy for meat tenderization, it possessed an important role in the effective diffusion of additives [17]. Our previous study has revealed the ultrasound-assisted L-histidine marination (UMH) accelerated the beef ageing process by enhancing the caspase-3 activity of AM and sarcomere lengthening [18]. Accordingly, the current study mainly focused on how these changes induced by synergistic treatment (ultrasound plus L-histidine marination) influenced the beef M. semitendinosus (ST) quality and AM properties.

2. Material and methods

2.1. Materials and reagents

The ST muscles were obtained from steers (25 months old, 560 ± 15 kg body weight), which were raised under the same feeding and management conditions at Wanghengfa animal farm (Jiangning district, Nanjing). These steers were harvested with standard commercial procedures and on the basis of inspection guidelines in China. After slaughter, ST muscles were rapidly transported to the lab within 1.0 h (4 °C), sliced into blocks and frozen at −80 °C for usage, and these muscles remained frozen and were used up within 3 months. In current study, L-histidine (purity of 99.5%) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China), and other reagents used were analytical grade and obtained from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China).

Fig. 1. Ultrasound treatment (A) and H&E staining of tissue with transverse section of myofibril. Scale bar = 200 μm.
2.2. Sample treatment

After defrosting the ST muscles at 4 °C for 12 h, they were cut into rectangular blocks weighing approximately 30 ± 1 g (50 mm × 20 mm × 10 mm). An ultrasonic processor (SCIENTZ-IID, Ningbo Xinzhi Ultrasonic Technology Co., Ltd., China) equipped with 12 mm diameter probe was inserted (15 mm depth) into the beaker and cooled with the addition of circulating cooling water (seen in Fig. 1A). The parameters of ultrasonic treatment were set at 300 W (20 kHz, 15.6 W/cm²) for 5 min with a pulse of 2 s on and 3 s off. The ultrasonic intensity measured by calorimetry was shown in Table S1. The optimized concentration (1.5 g/L) of L-histidine solution used in this investigation was chosen based on our previous study [18], and all the samples were treated in low temperature (4–10 °C). To alleviate the oxidation effect and free radicals on meat induced by ultrasound, a nanoparticle size potentiometer (NICOMP-Z3000, PSS, America), with the aforementioned principle. The obtained precipitant was fully suspended in 15 mL of extracting buffer, and then filtered through 80 mesh paraformaldehyde, and the manufactured paraffin slices were dewaxed

2.3. Water-holding capacity

2.3.1. Cooking loss

The fresh ST muscles were wiped with filter papers before being placed in a retort pouch. A digital thermometer (CENTER-309, Taiwan) was inserted into the center of the meat along the fiber direction. The sample was then immersed in a thermostat water bath (80 °C) until its core temperature reached 71 °C [18], and circulating water was used to cool the sample. Then, the meat after being wiped the excess water from surface was weighted, and cooking loss was calculated by the Eq. (1).

\[ \text{Cooling loss} = \frac{m_1 - m_2}{m_1} \times 100\% \]  

Where \( m_1 \) and \( m_2 \) represented the weight before and after cooking, respectively.

2.3.2. Centrifugation loss

The tissue (10 g) wrapped with three layers of filter papers was placed in a 100 mL of centrifuge tube for centrifugation at 10,000 × g (4 °C, 5 min) [21]. The centrifugation loss was expressed as the ratio of losing weight and raw weight of tissue.

2.4. Texture

The cooked meats were cut into cubes (10 × 10 × 10 mm³), and the textural parameters were measured at room temperature by a texture analyzer (TMS-TOUCH, FTC, American) [21]. The testing procedures were as follows: test rate = 120 mm/min, deformation = 50%, trigger force = 5 g.

2.5. Hematoxylin and eosin (H&E) staining

Samples were cut into cubes (5 × 5 × 5 mm³) and fixed with 4% paraformaldehyde, and the manufactured paraffin slices were dewaxed and stained with H&E [1]. After dehydration, the histological images were obtained for analysis by optical microscopy (Eclipse E100, Nikon, Japan).

2.6. Low-field nuclear magnetic resonance (LF-NMR) proton relaxation

A total of 2 g sample cut from the inner meat was placed in a cylindrical glass tube (d = 15 mm) with a resonant frequency of 21.0 MHz by an LF-NMR analyzer (MesoMR23, Newsmy, Suzhou) [1]. The transverse relaxation time (T₂) was measured using a Carr-Purcell-Meiboom-Gill (CPMG) with following parameters: TR = 12,000 ms, SW = 200 kHz, NS = 16, and NECH = 12,000. The resulting attenuation curve was subjected to an inversion operation with MultiExp Inv Analysis software (Niumag Electric Corporation, Suzhou, China).

2.7. Myofibril fragmentation index (MFI)

MFI was determined by our previous study with minor modification [22]. Two gram minced meat was weighed and added with 20 mL of extracting buffer (0.1 M KCl, 8.8 mM KH₂PO₄, 11.2 mM K₂HPO₄, 1.0 mM NaCl, 1.0 mM MgCl₂, 1.0 mM EGTA, pH 7.0, 4 °C). The mixture was homogenized (Model T25, IKA, Germany) in ice bath (12,000 r/min, 30 s, 2 times), and then centrifuged at 12,000 g for 15 min at 4 °C. The crude precipitant was treated again using the same buffer and following the aforementioned principle. The obtained precipitant was fully suspended in 15 mL of extracting buffer, and then filtered through 80 mesh of medical absorbent gauze (three layers) to remove insoluble connective tissue. The protein concentration of filtrate was determined and adjusted to 0.5 mg/mL by Coomassie Brilliant Blue Kit. MFI value were calculated according to the absorbance at 540 nm determined by microplate spectrophotometer multiplying 200.

2.8. Extraction of beef actomyosin (BAM) and its SDS-PAGE

The extraction of beef actomyosin (BAM) was performed following the method of Zhou et al. [16] with minor modification. Two grams of beef ST was mixed with 25 mL of Weber-Edsall solution (0.6 M KCl, 0.04 M NaHCO₃, 0.01 M Na₂CO₃, pH 7.2) and homogenized (Model T25, IKA, Germany) at 12,000 r/min for 30 s with interval of 60 s in an ice-water bath repeated for 2 times. The obtained homogenate in tube was shaken at 200 r/min at 4 °C for 24 h. The extract was filtered through two layers of medical absorbent gauze to obtain the filtrate. The KCl concentration of filtrate was adjusted to 0.2 M with chilled (0–4 °C) DI water. The obtained solution was shaken again for 1 h, and then centrifuged (UnicenMR, Herolab, Germany) at 15,000 × g at 4 °C for 20 min. The precipitate dissolved in appropriate amount of KCl-Tris solution (0.6 M KCl, 0.02 M Tris-HCl, pH 7.2) was BAM. The SDS-PAGE patterns of actomyosin complex (1 mg/mL) was obtained following the procedure of our previous study [21].

2.9. Ca²⁺/Mg²⁺-ATPase activities of BAM

The ATPase activities of actomyosin samples (4 mg/mL) were determined following the procedure of Li et al. [23]. The Ca²⁺/Mg²⁺-ATPase activity of actomyosin was calculated as the amount of inorganic phosphorus produced per milligram of protein per minute at 25 °C (U/mg Pro).

2.10. Particle size and solubility of BAM

The particle size of BAM solution (1 mg/mL) were determined using a nanoparticle size potentiometer (NICOMP-23000, PSS, America), with buffer solution (0.6 M KCl, 0.02 M Tris-HCl, pH 7.0) as a dispersion medium [16]. For the determination of protein solubility, 5 mL of BAM suspensions (2 mg/mL) were centrifuged at 10,000 × g at 4 °C for 20 min. Then, the protein concentration of supernatant (Cs) was measured at 562 nm by the BCA protein assay kit, and the ratio of Cs to 2.0 mg/mL was the AM solubility.
2.11. Surface hydrophobicity

The surface hydrophobicity of BAM solution was determined following the method of Zhu et al. [24] with slight modification. Briefly, 1 mL of BAM suspension (5 mg/mL) was mixed with 200 μL of bromophenol blue solution (BPB) (1 mg/mL), and the buffer solution was as reference. All samples were shaken for 10 min at dark environment and then centrifuged at 8,000 rpm for 10 min. After centrifugation, the obtained supernatant was diluted 10 times with buffer and then measured at 595 nm (i.e. $A_{\text{sample}}$). The sample with the same treatment in the absence of protein was used as the control (i.e. $A_0$). The surface hydrophobicity of BAM was characterized by BPB bound content and calculated by the Eq. (2).

$$\text{BPBound}_{\mu g} = \frac{200 \mu g \times (A_0 - A_{\text{sample}})}{A_0} \quad (2)$$

2.12. Fluorescence spectroscopy

The fluorescence spectroscopy of BAM suspension (0.5 mg/mL) was measured with the LS-55 spectrophotometer (Perkin Elmer, Washington, USA) in a 1.0 mm path length cell [1]. Samples were excited at 280 nm, and the intrinsic fluorescence spectra was obtained at the emission wavelength from 300 to 500 nm with a speed of 1,200 nm/min. In current study, the slit widths for excitation and emission were both 3.5 nm.

2.13. Total sulfhydryl group (T-SH) content

The T-SH content of BAM was determined by mixing 0.5 mL of actomyosin (2.5 mg/mL) with 4.5 mL of 20 mM Tris-HCl buffer (containing 10 mM EDTA-2Na, 8 M urea, 2% SDS, pH 6.8), followed by adding 0.5 mL of Ellman’s reagent (0.1% DNTB, 0.2 M Tris-HCl, pH 6.8) [25]. The mixture was incubated at 40 °C water bath for 25 min, and then the absorbance was measured at 412 nm when the mixture was cooled to room temperature. T-SH content was obtained by the measurement kit provided by Nanjing Chemical Reagent Co., Ltd. (Nanjing, China).

2.14. Rheology

The storage modulus ($G’$) of BAM suspension (35 mg/mL) was evaluated within the linear viscoelastic region using a rotational rheometer (MCR301, Anton-Paar, Austria, with 1.0 mm gap and 50° parallel plate) followed the methods of Shi et al. [21] with minor modification. The measurement was conducted in the strain of 2% and the constant frequency of 0.1 Hz. The swept gradient temperature program included 3 steps. 1) Samples were heated from 80 to 80 °C at the speed of 1 °C/min, 2) samples were kept at 80 °C for 3 min, 3) samples were cooled from 80 to 20 °C at the speed of 5 °C/min.

2.15. Statistical analysis

All experiments were performed in at least triplicate samples, and data was presented as the mean ± standard deviation (SD). Analysis of variance (ANOVA) in SPSS 24.0 was applied to compare the differences among the samples at the significant level of $P < 0.05$, which was evaluated by Tukey’s test.

3. Results and discussion

3.1. Water-holding capacity (WHC) and texture changes

Normally, excessive liquid loss during meat processing is adverse to the economical profit and oral palatability [26]. Table 1 shows the WHC and textural parameters among the different groups. Except the Control or DW group, all the other groups manifested higher WHC ($P < 0.05$), especially in the meat marinated with ultrasound-assisted $\gamma$-histidine (UMH group). The higher cooking loss in the DW or Control group was mainly attributed to greater myofibril rigidity, which induced thermo-irreversible denaturation of muscle proteins [27]. This indicated that both ultrasonication and $\gamma$-histidine marination effectively avoided excessive liquid withdrawal during the heating and centrifugation process. Ultrasound treatment could destroy the muscle tissue and loose the connection of myofibrils by exerting strong pressure and cavitation-generated micro-jet [1]. This effect provided more opportunities for the exchange of marinated solution and internal media in tissue, to some degree, increasing the muscle pH and solution infiltration. Therefore, the $\gamma$-histidine marinated meat manifested increased pH value due to the lack of obvious damage by external mechanical force [1]. Similar to that the basic group of its side chain was protonated by the water in samples [18], some reports have revealed that the meats with higher pH accelerated the tenderization process [28], and released the salt-soluble proteins (SSPs) onto the tissue surface when in combination with ultrasonication [29]. The solution-trapping capacity of myofibrils was thus enhanced due to the water-retaining and cohesive-enhancing behaviors mediated by SSPs [30], and the ultrasound-modified gel structure with higher electrostatic repulsion. Zhang et al. [31] also found the electrostatic interaction between the acidic residues of protein molecule and the imidazolyl group of $\gamma$-histidine, as well as the formation of $\gamma$-histidine-mediated hydrogen bonds with other groups in protein molecules. The UMH thus exhibited the highest water-holding capacity, which was vital for ensuring the consumed product with optimized tenderness and juiciness.

The TPA parameters of cooked meat are highly correlated with the tenderness perception and consumer palatability. Vandenberghe-Desccamps et al. [32] pointed that an improved meat tenderness and juiciness facilitated the formation of texture perception and oral comfort when consumption. As presented in Table 1, the degree of decreased hardness between Control and DW groups was markedly lower than that of Control and other groups. This phenomenon can arise from the slight softening of tissue surface mediated by neutral DI water marination, and the lack of obvious damage by external mechanical force [1]. Similar to another reported basic amino acids, i.e. $\gamma$-lysine [33], the meat only marinated with $\gamma$-histidine solution (MH group) also decreased in the hardness, which might be mainly ascribed to the good water retention. Additionally, $\gamma$-histidine was found to positively regulate the protein phosphorylation correlated with muscle contraction [34], which might change the heat-set behavior and further affect the cooked meat texture. In addition, ultrasonication disrupted the myofibril integrity and induced the degradation of structural proteins, rendering the meat with decreased shear force [35]. Therefore, the synergistic treatment (UMH group) manifested the lower hardness, which was coincident with the performance in developed tenderness in our previous published study [18]. In the present study, the increased springiness indirectly reflected that the formation of firm gel network for grasping water, and the decreased chewiness was conductive to the consumption for the elderly.

| Table 1 | Variance of water loss and texture profile parameters of meat samples. |
|---------|------------------------------------------------------------------|
|         | Control     | DW         | UDH        | MH         | UMH        |
| Centrifugation loss/% | 5.40 ± 0.29 $^a$ | 2.14 ± 0.14 $^b$ | 2.17 ± 0.24 $^b$ | 1.40 ± 0.10 $^b$ |
| Cooking loss/% | 38.53 ± 0.45 $^a$ | 23.46 ± 1.23 $^b$ | 22.36 ± 0.42 $^a$ | 16.16 ± 0.65 $^b$ |
| Hardness/N | 58.14 ± 0.25 $^a$ | 27.99 ± 0.22 $^b$ | 27.88 ± 0.20 $^b$ | 15.27 ± 0.22 $^b$ |
| Springiness/mm | 4.51 ± 0.65 $^a$ | 4.64 ± 0.06 $^b$ | 5.37 ± 0.22 $^b$ | 6.52 ± 0.22 $^b$ |
| Chewiness/mj | 148.20 ± 0.61 $^a$ | 133.40 ± 0.22 $^a$ | 49.30 ± 0.20 $^a$ | 68.20 ± 0.20 $^b$ | 42.10 ± 0.80 $^b$ |

Different letters in the same line indicate significant differences ($P < 0.05$).
3.2. Tissue microstructure and water distribution properties

Tissue histological observation can effectively characterize the differences in microstructure. As displayed in Fig. 1B, the meats treated with ultrasound or L-histidine marination exhibited more wider myofibril space and extensive myofiber swelling. It has been widely reported that the ultrasound-mediated cavitation and mechanical effects could loosen the tightly-connected myofibrils and generate erosion on tissue surface [1,36]. In our previous report, the altered structures of sarcomere, especially around the I-band and the Z-line, further provided the ultra-structural evidence [18]. Moreover, proper ultrasonication could bury the acidic groups to increase pH through the change of protein conformation [37]. This change made myofibrillar protein in tissue further deviate from the isoelectric point, generating space between myofilaments to sufficiently retain water. After ultrasonication, the decreased strength of myofibrils created the convenient channels for L-histidine solution to function its swelling effect. Thus, the more obvious space and cavities between myofibers were observed.

The information concerning the water state (distribution and mobility) in tissue can be characterized by transverse relaxation time (T<sub>2</sub>). Generally, the peak point migrating to lower relaxation time is correlated with the weak water mobility, and the broadening of the peak means that the corresponding composition is complicated [38]. In the current study, three relaxation populations of water ranged from 0.1 ms to 1000 ms were identified (T<sub>2b</sub>, T<sub>21</sub> and T<sub>22</sub> represented the bound, immobilized and free water, respectively). As illustrated in Fig. 2, the ultrasound-treated meats (UDW and UMH groups) exhibited the decrease in T<sub>21</sub> relaxation time (47.14 ± 0.61 and 42.97 ± 0.53, respectively), and T<sub>21</sub> was usually identified as myofibrillar water and water within structure, which was related to myofibrillar swelling [39]. Therefore, the faster relaxation times induced by ultrasound might effectively avoid the extensive mobility of immobilized water by changing the protein structure in meat. In other words, more tightly trapped water of meat was retained, accompanied by the lower fluid losses in beef M. semitendinosus. Furthermore, the meat marinated with L-histidine also shifted to lower T<sub>21</sub> (45.01 ± 0.49) compared with that of Control and DW groups (49.37 ± 0.32 and 49.37 ± 0.44, respectively). Synergetic effects based on ultrasound and L-histidine infiltration (i.e. UMH group) collectively contributed to the lowest T<sub>21</sub> relaxation time. According to the corresponding ratio of three peak area, both the P<sub>21</sub> and P<sub>22</sub> in UDW group were higher than that of Control group. It might be that the deionized (DI) water infiltrated in ultrasound-mediated myofibrils partially existed in the form of free water, but mainly grasped by the stronger network of ultrasound-modified myofibrillar protein. For the DW group, the decreased P<sub>22</sub> and increased P<sub>21</sub> could arise from tissue surface softening for the little free water withdrawal from tissue, which induced by neutral DI water. Different from DI water marination, L-histidine itself could significantly affect the water distribution, suggesting that appropriate L-histidine marination facilitated more water being retained into the MP network. This result was in accordance with the fact that the L-histidine increased the soluble protein content of meat by changing protein conformation. Guo et al. [40] also reported that L-histidine promoted the unfolding of MPs to improve the protein adsorption and stability for grasping water. Ultrasound-assisted L-histidine treatment was thus an effective way to increase the meat WHC by changing the moisture distribution and mobility.

3.3. Myofibril fragmentation index (MFI)

MFI is usually used to evaluate the myofibril integrity during post-mortem ageing, and positive correlation has been demonstrated between MFI and meat tenderness [1]. As shown in Fig. 3, significant differences in MFI value were observed among the examined samples. The increased MFI value may be caused by the MPs degradation (e.g., troponin-T) and/or AM dissociation, indicating that these treatments might promote the rupture of I-band and the breakage of intermyofibrillar linkages [22]. In our previous study, both ultrasound and L-histidine marination were indeed observed to disrupt myofibril ultrastructure with obscure Z-line and I-band [18]. MFI also reflects the extent of postmortem proteolysis or muscle cytoskeletal and associated proteins [41]. In the early postmortem, caspase-3 is activated to hydrolyze MP and participate in ageing tenderization due to the apoptosis development [42]. It also includes cysteine residues in the active sites, and the L-histidine imidazolyl group plays an important role in the polarization activation of the cysteine side chain [43]. Our previous study indicated that both MH and UMH treatments induced higher caspase-3 activity [18], which was conductive to the degradation of myofibrils and development of cell apoptosis [42]. The calpain system was thus activated to increase the degree of the myofibril fragmentation, and finally to improve meat tenderness. Additionally, the higher pH value in L-histidine-treated meat, to some extent, maintained the calpain activity for continuous tenderization.

3.4. SDS-PAGE analysis and Ca<sup>2+</sup>/Mg<sup>2+</sup>-ATPase activities of BAM

In postmortem muscle, the actin-myosin binding results in muscle contraction and meat toughening, whereas the dissociation of the actomyosin complex will, to a great extent, contribute to tenderization [15]. Therefore, it is essential to evaluate the dissociation degree of

![Fig. 2. Effects of different treatments on LF-NMR relaxation times and corresponding peak ratio of beef M. semitendinosus. There are significant differences in different letter representations on graphs (P < 0.05), the same below.](image-url)

![Fig. 3. Effects of different treatments on the myofibril fragmentation index of actomyosin.](image-url)
actomyosin under different treatments. **Fig. 4A** and **Fig. 4B** present the actomyosin profile and the quantitative results of free actin, respectively. According to the BAM electrophoresis profiles shown in **Fig. 4A**, the ultrasound- or L-histidine-treated group did not induce major changes in comparison with the Control group. This phenomenon indicated that no obvious effects on primary structure of BAM after ultrasonication and L-histidine marination, and similar results were observed by the Xu et al. [44]. As illustrated in **Fig. 4B**, the gray values of actin in UDW, MH and UMH groups increased by 17.5%, 21.7% and 34.9%, respectively compared with the Control group \((p < 0.05)\). This indicated that both ultrasound and L-histidine marination had remarkable effect on weakening the actin-myosin connection. On the one hand, ultrasonication destroyed the myofiber structure and induced the release of cell contents concerning calpain [45]. Jiang et al. [46] revealed that the activated calpain could hydrolyze the structural proteins accompanied by sarcomere lengthening through the weaker actin-myosin connection. Moreover, the increased caspase-3 activity previously reported in both L-histidine and ultrasound groups had direct proteolytic activity against calpastatin for improved tenderness. On the other hand, the higher pH value in L-histidine-marinated meat further promoted the dissociation of actomyosin. Therefore, UMH treatment played the synergetic effects on loosening the actin-myosin connection.

Usually, \(\text{Ca}^{2+}\)-ATPase and \(\text{Mg}^{2+}\)-ATPase activity indicate the structural integrity of S-1 unit of myosin head and the dissociated degree of actomyosin, respectively [21]. As displayed in **Fig. 4C**, UMH group manifested the highest \(\text{Ca}^{2+}\)-ATPase activity, but lowest \(\text{Mg}^{2+}\)-ATPase activity of actomyosin \((p < 0.05)\). The higher \(\text{Ca}^{2+}\)-ATPase activity indicated the occurrence of fast metabolism in muscle, and ultrasonication and L-histidine marination shortened the postmortem ageing period. The higher integrity of internal S-1 unit might be speculated that L-histidine acted as a protective role to prevent actin binding to myosin head in the actin-binding cleft. In addition, when actin binds to myosin, the activity of \(\text{Mg}^{2+}\)-ATPase will increase by several orders of magnitude [47]. In the current study, ultrasound treatment disrupted the stability of thin and think filaments, thus inhibiting the actin-myosin interaction accompanied by the increased dissociation. The decreased \(\text{Mg}^{2+}\)-ATPase activity in MH group might be associated with the interactions between the imidazole group of L-histidine and the aromatic residues of myosin.

**Fig. 4.** Effects of different treatments on the AM profile (A), quantitative analysis diagram of actin (B), and \(\text{Ca}^{2+}/\text{Mg}^{2+}\)-ATPase activities (C) of beef actomyosin.
These changes were in accordance with the results of actomyosin profile and the optimized tenderness in UMH group.

3.5. Particle size and solubility of BAM

The change of particle size is mainly caused by cross-linking and aggregation in protein molecules, and Table 2 exhibits the size variance of BAM under different treatments. It was found that UDW, MH and UMH groups obviously decreased the actomyosin size compared with that of DW and Control groups \((P < 0.05)\). Cavitation effect and microjet caused by ultrasound could interrupt the non-covalent bond between actomyosin, enabling the steady dispersion of protein in solution and inducing the formation of smaller protein structure. The increased pH values of meat induced by ultrasound and \(\lambda\)-histidine also took place an important role in disrupting electrostatic interaction, and thus reduced the particle size. Our previous reported evidence observed both ultrasound and \(\lambda\)-histidine treatments weakened the denaturation and aggregation of actomyosin molecules as the increasing temperature \([18]\), which was closely related to the stability of actomyosin caused by smaller size. These results indicated that the depolymerization of actomyosin complex and inhibition of covalent or non-covalent cross-linking between protein molecules for smaller particle size could be realized under the combination of ultrasound and \(\lambda\)-histidine marination. In general, protein denaturation was closely correlated with the protein dissolution behavior, and lower solubility was often accompanied by the destruction of protein structure and function \([25]\). Among all the groups, both UDW and UMH groups had higher actomyosin solubility \((P < 0.05)\), which might be attributed to the unfolding of actomyosin structure and whole damage in connection between actin and myosin \([16]\). Owing to higher pH value in the MH-treated meats, the electrostatic interactions in myosin were disrupted by \(\lambda\)-histidine through electrostatic binding with the exposed negatively-charged amino acid residues. More specifically, the functional groups of \(\lambda\)-histidine might bind to the carbonyl oxygen atoms of the exposed aromatic residues of myosin as reported by Gao et al. \([48]\). In terms of UMH group, on the one hand, ultrasound induced the preliminary dissociation of actomyosin by the physical effect, which destroyed the interaction of natural aggregation of proteins \([46]\). On the other hand, the infiltrated basic \(\lambda\)-histidine solution further improved solubility through attacking the loose myosin filament \(\text{(i.e. suppressing myosin aggregation)}\) and avoiding the inhibition of filaments self-assembly process by the imidazole ring of \(\lambda\)-histidine \([9,11]\). Accordingly, ultrasound-assisted \(\lambda\)-histidine treatment produced synergistic effect on improving actomyosin solubility.

3.6. Surface hydrophobicity and endogenous fluorescence analysis of BAM

Inter-molecular covalent cross-linking and hydrophobic interactions are proposed as the crucial driver of the protein aggregation behavior. With regard to actomyosin, the initial inter-molecular covalent cross-linking is mainly mediated by the sulphhydril-disulphide (S-S) interchange and -SH oxidation in the cysteine enrichment region of myosin \([49]\). As shown in Fig. 5A, the actomyosin in meat marinated with \(\lambda\)-histidine exhibited lower surface hydrophobicity compared with that of Control and DW groups \((P < 0.05)\). Due to the increased pH in \(\lambda\)-histidine-marinated meat, and the charge difference between the \(\lambda\)-histidine and myosin, \(\lambda\)-histidine might change the electrostatic property of myosin by electrostatic binding with the exposed negatively charged amino acid residues. This result might disarrange hydrophobic surfaces to weaken the hydrophobic interactions \([48]\), and destroy the intramolecular and intramolecular ionic bonds maintaining the structural stability of BAM molecules \([18]\), ultimately suppressing the fierce aggregation of BAM. The unfolding of the spiral tail of actomyosin and the intervention of \(\lambda\)-histidine changed the secondary structure of actomyosin, inducing the depolymerization of myosin. It might be attributed that the imidazole ring of \(\lambda\)-histidine competitively combined with the actin-binding sites of myosin head region, leading to the decrease in surface hydrophobicity. Additionally, ultrasoundication, to a certain extent, contributed to the decrease of \(\alpha\)-helix content and the unfolding of protein tertiary structure, shielded hydrophobic areas \([16]\).

The fluorescence results of tryptophan are also closely correlated with surface hydrophobicity, and the decrease of fluorescence intensity indicates that tryptophan residues have changed. Fig. 5B shows the micro-environmental changes of tryptophan residues, and the red shift was observed in UDW, MH and UMH groups. This meant that the tryptophan residues in actomyosin tended to hydrophilic environment \([50]\), which was in consistence with the decreased surface hydrophobicity and the improved protein solubility. The non-covalent bonds between protein molecules might be damaged by the turbulence and micro-jet produced by ultrasound. \(\lambda\)-histidine destroyed the negative residue of myosin, resulting in the decrease of myosin \(\alpha\)-helix structure.

![Fig. 5. Surface hydrophobicity (A) and endogenous tryptophan fluorescence spectra (B) of actomyosin.](image)

Control and DW groups \((P < 0.05)\). Due to the increased pH in \(\lambda\)-histidine-marinated meat, and the charge difference between the \(\lambda\)-histidine and myosin, \(\lambda\)-histidine might change the electrostatic property of myosin by electrostatic binding with the exposed negatively charged amino acid residues. This result might disarrange hydrophobic surfaces to weaken the hydrophobic interactions \([48]\), and destroy the intramolecular and intramolecular ionic bonds maintaining the structural stability of BAM molecules \([18]\), ultimately suppressing the fierce aggregation of BAM. The unfolding of the spiral tail of actomyosin and the intervention of \(\lambda\)-histidine changed the secondary structure of actomyosin, inducing the depolymerization of myosin. It might be attributed that the imidazole ring of \(\lambda\)-histidine competitively combined with the actin-binding sites of myosin head region, leading to the decrease in surface hydrophobicity. Additionally, ultrasoundication, to a certain extent, contributed to the decrease of \(\alpha\)-helix content and the unfolding of protein tertiary structure, shielded hydrophobic areas \([16]\).

The fluorescence results of tryptophan are also closely correlated with surface hydrophobicity, and the decrease of fluorescence intensity indicates that tryptophan residues have changed. Fig. 5B shows the micro-environmental changes of tryptophan residues, and the red shift was observed in UDW, MH and UMH groups. This meant that the tryptophan residues in actomyosin tended to hydrophilic environment \([50]\), which was in consistence with the decreased surface hydrophobicity and the improved protein solubility. The non-covalent bonds between protein molecules might be damaged by the turbulence and micro-jet produced by ultrasound. \(\lambda\)-histidine destroyed the negative residue of myosin, resulting in the decrease of myosin \(\alpha\)-helix structure.

### Table 2

| Protein size (nm) | Control | DW | UDW | MH | UMH |
|------------------|---------|----|-----|----|-----|
| ± 17.20          | ± 28.28 | ± 30.22 | ± 22.58 | ± 23.57 |
| Protein solubility (%) | 0.32<sup>a</sup> | 0.17<sup>a</sup> | 0.17<sup>b</sup> | 0.28<sup>b</sup> | 0.32<sup>b</sup> |
| T-SH content (µmol/gprot) | 5.50<sup>a</sup> | 6.53<sup>a</sup> | 7.87<sup>a</sup> | 7.20<sup>a</sup> | 8.97<sup>a</sup> |
content [10]. The addition of L-histidine made the tail of myosin elongate, which destroyed the charge balance of the charged residue peptide in the tail of myosin, thus inhibiting the aggregation of myosin and improving the solubility [51]. The imidazole ring in L-histidine destroyed the original equilibrium structure of myosin in some way, changed the distribution of charged residues on the surface of myosin, and depolymerized myosin [9]. Additionally, L-histidine can be embedded into the tail of myosin by electrostatic attraction, forming an “overhead layer” around hydrophobic groups, shielding the interaction between hydrophobic groups [11]. Accordingly, the actomyosin in UMH group tended to the hydrophilic environment, and was thus accompanied with higher solubility.

3.7. Total sulfhydryl (T-SH) content and rheological analysis

The sulfhydryl group is considered as the most functional group in protein, which is related to Ca^{2+}-ATPase activity [52]. In the present study, both ultrasonication and L-histidine increased the T-SH content, which was consistent with the fact that the Ca^{2+}-ATPase activities in MH and UMH groups were increased. It might be attributed to the disintegration of the filamentous myosin polymer caused by the ultrasound-mediated mechanical force, and the subsequent release of the buried SH groups [53]. The increased content of T-SH might be due to that ultrasound-mediated cavitation and L-histidine intervention changed the protein conformation with unstable structure and exposed the di-sulfide bonds [54], and this was closely related to the smaller particle size.

The changes in storage modulus (G’) of all groups during heating and cooling process (20–80 °C) are presented in Fig. 6A. In the current study, after ultrasonication or L-histidine addition, an improved final G’ values of actomyosin during heating were observed in Fig. 6B (P < 0.05). The increased final G’ values indicated the formation of homogenous and high-intensity protein gel network structures [55]. Besides, the smaller protein size further induced better alignment of protein chains [1]. These changes led to the enhanced water binding by promoting the hydration of hydrophilic groups on the surface of protein with the surrounding water. Generally, the formation of initial peak characterizing myosin in actomyosin would appear at 42–52 °C, which is correlated with the head connection of myosin molecules and subsequent partial unfolding of myosin tail [56]. Interestingly, the actomyosin extracted from ultrasound-treated and L-histidine-treated meat displayed the decrease in initial denatured temperature of myosin, especially in UMH groups. This result might be the weaker aggregation of myosin head induced by ultrasound and L-histidine, which was in accordance with the performance in turbidity of actomyosin [18]. Moreover, the reduction of myosin head connections of myosin provided more opportunity for subsequent tail unfolding and cross-linking, to some degree, contributing to acceleration of myosin gel and water retention capacity [57]. UMH group showed the increased gel firmness after cooling compared that of other groups (Fig. 6C), further indicating that the interaction between protein chains for grasping water became stronger with the decreasing temperature (80–20 °C).

4. Conclusions

The current study revealed that the ultrasound-assisted L-histidine marination (UMH) enhanced the water retention capacity of beef M. semitendinosus accompanied by optimized tenderness, and changed the physicochemical properties of actomyosin. UMH not only weakened myofibril strength, but also increased the muscle pH for quicker post-mortem ageing. The filtrated L-histidine in myofibril competitively inhibited the actin-myosin binding by the imidazole group, and further intervened the hydrophobic area and structure of actomyosin. This combination strategy increased protein solubility with smaller size enhanced the SH content for better cross-linking of myosin tail and formation of water-trapped structure. Therefore, we concluded that ultrasound combined with L-histidine marination can be regarded as a promising method to improve beef quality.

Fig. 6. Storage modulus (G’) of actomyosin during heating (B) and cooling (C) process.
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.2022.105987.

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