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Enhanced gelling properties of myofibrillar protein by ultrasound-assisted thermal-induced gelation process: Give an insight into the mechanism

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ABSTRACT

Effects of the incorporation of ultrasound with varied intensities (0–800 W) into the thermal-induced gelation process on the gelling properties of myofibrillar protein (MP) were explored. In comparison with single heating, ultrasound-assisted heating (<600 W) led to significant increases in gel strength (up to 17.9%) and water holding capacity (up to 32.7%). Moreover, moderate ultrasound treatment was conducive to the fabrication of compact and homogenous gel networks with small pores, which could effectively impair the fluidity of water and allow redundant water to be entrapped within the gel network. Electrophoresis revealed that the incorporation of ultrasound into the gelation process facilitated more proteins to get involved in the development of gel network. With the intensified ultrasound power, α-helix in the gels lowered pronouncedly with a simultaneous increment of β-sheet, β-turn, and random coil. Furthermore, hydrophobic interactions and disulfide bonds were reinforced by the ultrasound treatment, which was in support of the construction of preeminent MP gels.

1. Introduction

Multitudinous processed meat products, including bolognas and frankfurters, can be categorized as emulsion-type products, which are heterogeneous composite systems embedded with protein-coated oil droplets. The texture-forming properties of such products critically depend on the gelling capacity of meat proteins upon heating, especially myofibrillar protein (MP), which is the dominant gel-forming contributor [1].

In order to achieve desirable gelling properties of MP, miscellaneous exogenic additives like microbial transglutaminase and polysaccharides, and/or physical strategies such as high-pressure, microwave, and high-intensity ultrasound, have been extensively explored [2,3]. Especially, ultrasound, as an emerging and environmental-friendly nonthermal technology, has drawn considerable interest in the food industry, relying on its microscopic cavitation effects that generate vigorous physical energies (e.g., microjet, shear force, shock wave, and turbulence). In meat processing, ultrasound has been triumphantly introduced to assist in freezing, thawing, salting, frying, fermentation, and sterilization of meat products [4–6]. High-intensity ultrasound can accelerate the freezing or thawing rate and mass transfer, as well as promote the generation of volatiles. Furthermore, it has been demonstrated that the cavitation effects of ultrasound can induce the conformational unfolding of muscle protein to expose more active sites to the surface, thereby facilitating the puissant interactions between proteins, which are conducive to the formation of high-quality elastic gels [7,8]. It should be noted that ultrasound is generally employed to pretreat proteins prior to the heat-induced gelation process; however, there is little information available on the effects of the involvement of ultrasound in the gelation process on the final gel characteristics.

Thermal-induced gelation is a multistage thermodynamic process (suwari, modori, and gel setting stage) that involves the structural unfolding of MP molecules to expose buried hydrophobic domains and other functional groups, accompanied by progressive protein–protein association and further aggregation, eventually contributing to the construction of a highly viscoelastic gel network capable of entrapping water and immobilizing fat globules [9,10]. The latter processes usually involve the participation of hydrogen bonds, ionic bonds, van der Waals forces, hydrophobic interactions, and covalent bonds such as disulfide linkages [11]. During the gelation process, thermal specifications, for instance, conductive medium, heating velocity, persistent period, and temperature, play crucial roles in the gelling performance of MP, thereby affecting the quality characteristics of final meat products. In meat industries, the most common and traditional way of heating is performed in a water bath employing water as a conductive medium. This method has many drawbacks, for instance, slow heating rate and

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long duration in the gel-cracking zone (modori stage), resulting in gel deterioration [3]. Recently, He and coworkers [12] found that in contrast with the traditional water bath heating, the introduction of ultrasound-assisted water bath heating during the suwari and gel setting stages could contribute to the fabrication of more compact gel microstructure with diminutive holes, and result in manifest improvements of the textural properties and water retention of surimi gels. An analogous phenomenon has also been observed in composite surimi gels prepared by ultrasound-supplemented heating [13]. Nevertheless, the underlying mechanism is still not well understood.

Therefore, the objective of this study was to investigate the impacts of ultrasound involvement during the thermal-induced gelation process on the gel characteristics of MP filled with emulsion oils. Furthermore, the mechanism for the increased gel strength of MP on account of ultrasound-assisted heating was deeply explored. This work would provide valuable knowledge on the utilization of ultrasound-assisted heating to produce superior meat products.

2. Materials and methods

2.1. Materials

Fresh porcine muscle (*Longissimus lumborum*) was collected from Auchan supermarket (Yangzhou, China). Individual muscle section (approximately 70 g) was vacuum-packaged and stored in a −20 °C refrigerator until further utilization within 3 months. All chemicals of at least analytical grade were acquired from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) or Sangon Biotech Co., Ltd. (Shanghai, China).

2.2. Extraction of myofibrillar protein (MP)

Frozen muscle sections were tempered at 4 °C for 12 h and then used for MP preparation with an isolation buffer (10 mM sodium phosphate, 0.1 M NaCl, 2 mM $MgCl_2$, and 1 mM EGTA, pH7.0) with reference to Park, Xiong, and Alderton [14]. The protein concentrations were quantified by the Biuret method using bovine serum albumin as standard. The isolated MP was stored at 4 °C and used up within 48 h.

2.3. Preparation of MP-emulsion composite sols and gels

MP emulsion was prepared at 4 °C by mixing MP suspension (10 mg/mL, pH6.25) with olive oil (20%, w/w) via a high-speed homogenizer (IKA T18, IKA-Werke GmbH & Co. KG, Staufen, Germany) at 10,000 r/min for 60 s. The freshly prepared emulsion was instantly incorporated into the MP suspension (pH 6.25) to obtain a composite sol with a final concentration of 20 mg/mL protein and 10% (w/w) oil. Afterwards, the composite sol was transferred into a glass vial (5 cm height × 2.2 cm diameter), covered with aluminum foil, and then heated at 70 °C for 20 min in a THC-1000SF ultrasound generator-equipped water bath (Jining Tianhua Ultrasonic Electronic Instrument Co., Ltd., Jining, China) with ultrasound power outputs of 0, 200 W, 400 W, 600 W, and 800 W at a constant frequency of 20 kHz, respectively. Finally, the gels were promptly chilled in ice water for 20 min and stored at 4 °C overnight.

2.4. Gel strength

After equilibration at 25 °C for 30 min, the set of gels was penetrated by a P/6 cylindrical probe (12 mm in diameter) at a pre-test speed of 5.0 mm/s, a test speed of 1.0 mm/s, and a post-test speed of 5.0 mm/s using a TMS-Pro texture analyzer (Food Technology Corporation, Sterling, VA, USA). Gel strength (N) was defined as rupture strength × rupture distance.

2.5. Water holding capacity

All composite gels (5 g) were centrifuged at 10,000 *g* and 4 °C for 10 min. Subsequently, the supernatant was completely expelled, and the precipitate was weighted. WHC (%) was indicated as the weight percentage of the precipitate to the original gel.

2.6. Low-field NMR

To evaluate the distribution and mobility of water molecules within the gel, low-field nuclear magnetic resonance (NMR, Niumag Electric Corporation, Shanghai, China) measurements were performed on the basis of the protocol illustrated by Zhang et al. [15]. Approximately 5 g composite gels were put into cylindrical glass tubes with a diameter of 15 mm and then placed in the NMR probe. Spin-spin relaxation time (T_2) was determined by Carr–Purcell–Meiboom–Gill (CPMG) sequence under a resonance frequency of 22.6 MHz and a τ-value (time between 90° and 180° pulses) of 200 μs at 32 °C. Data were collected from 15,000 echoes after 16 scan repetitions. The CPMG curve achieved was fitted with multi-exponents using a Multi-Exp Inv Analysis software (Niumag Electric Corp., Shanghai, China).

2.7. Scanning electron microscopy (SEM)

The microstructural analysis of composite gels was executed on a Gemini 300 SEM (Zeiss, Oberkochen, Germany). Small cubes (approximately 5 × 5 × 1 mm^3) were excised from the intact gels and then immobilized by 2.5% formalin solution in 0.2 M phosphate buffer (PBS, pH 7.2) for 24 h. Thereafter, the gel cubes were rinsed 3 times using 0.2 M PBS (pH 7.2), followed by a dehydration process with a series of gradient ethanol. After freeze-drying, the gel was mounted on a bronze stub and sputtered with gold. Finally, the gel was imaged under an accelerating voltage of 5 kV and a magnification of 200 ×.

2.8. Electrophoresis

The gels were subjected to centrifugation at 10,000 *g* for 20 min, and changes of protein composition in the resulting supernatants (the expressed liquid) were analyzed by SDS–PAGE with 4% polyacrylamide stacking gel and 12.5% polyacrylamide resolving gel. Electrophoresis was run at an initial voltage of 90 V for 0.5 h and a subsequent voltage of 120 V for another 1 h. Eventually, the gels were stained by 0.025% Coomassie brilliant blue G250 in 10% acetic acid for 1 h and then destained with 5% methanol and 7.5% acetic acid.

2.9. Raman spectra

Raman analysis of MP-emulsion composite gels was performed on a micro-confocal laser Raman spectrometer (Renishaw-inVia, Renishaw Co., Ltd., UK) at a laser excitation wavelength of 532 nm and a laser energy of 20 mV. Raman spectra were recorded 30 times in the range of 600 cm^{-1} to 3200 cm^{-1} at 25 °C with an exposure time of 15 s. The relative portions of secondary structures were quantitatively computed according to the procedure addressed by Alix, Pedanou, and Berjot [16].

2.10. Chemical forces

The chemical forces that stabilized the gels were assessed in view of the divergent solubility of proteins in selective solvents that cleave specific bonds according to Wu et al. [17]. The solvents used included 0.6 M NaCl (S_1), 0.6 M NaCl + 1.5 M urea (S_2), 0.6 M NaCl + 8 M urea (S_3), and 0.6 M NaCl + 8 M urea + 0.5 M β-mercaptoethanol (S_4). The composite gel (1 g) was homogenized with 9 mL S_1 by a high-speed homogenizer (IKA T18, IKA-Werke GmbH & Co. KG, Staufen, Germany) for 1 min. The homogenate was continuously stirred at 4 °C for 60 min, and then subjected to centrifugation at 10,000 *g* for 20 min. The

supernatant was retained, whereas the precipitate was sequentially homogenized with S_2, S_3, and S_4 followed by centrifugation as the above procedure, respectively, and the corresponding supernatants were retained as well. Whereafter, the protein concentration in different supernatants was determined using the Biuret Method. The protein solubility in S_1, S_2, S_3, and S_4, expressed as the percentage of soluble protein to total protein, represents ionic bonds, hydrogen bonds, hydrophobic interactions, and disulfide bonds, respectively.

2.11. Statistical analysis

All experiments were performed at least in triplicate or sextuplicate (for textural analysis) and repeated at least three times ($n \geq 3$), each with a fresh batch of MP. The significant differences ($P < 0.05$) between means were identified by Duncan's multiple range test with SPSS 22.0 software (SPSS Statistical Software Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Gel strength and water holding capacity (WHC)

Gel strength is a vital index that dictates the overall acceptability of muscle products. The impacts of ultrasound-supplemented heating on the gel strength of MP composites were estimated by a penetration test. Compared with single heating (0 W), the incorporation of ultrasound during the gelling process contributed to an evident gain (up to 17.9%) in the gel strength (Fig. 1A). The gel strength encountered a gradual enhancement with increasing ultrasound power from 0 W to 600 W but evidently descended as the ultrasound power reached to 800 W. An analogous phenomenon has been observed in chicken MP pretreated with pulsed ultrasound prior to gelation [18]. During the ultrasonication process, the transient cavitation bubbles were first generated and then rapidly enlarged. Within several acoustic cycles, the bubble collapses violently when the bubble size reached a critical value, resulting in intense physical effects (high pressure) and mechanical effects (hydrodynamic shear forces and turbulent flow). The total acoustic energy exerted in the cavitation zone were sufficient to disrupt the non-covalent and covalent bonds, leading to the structural unfolding of proteins and the enhanced interactions between protein molecules during the thermal-induced gelling process [12,19]. Additionally, it was claimed that ultrasound could ameliorate the dissolution of MP when interrupting the highly ordered filamentous structure of myosin [20], which could facilitate the inter- and intramolecular interactions of proteins, thereby contributing to the improved gel strength. However, excessive ultrasound power might impede the ordered protein–protein interactions, which was not instrumental in the formation of rigid and fracture-resistant gels [10].

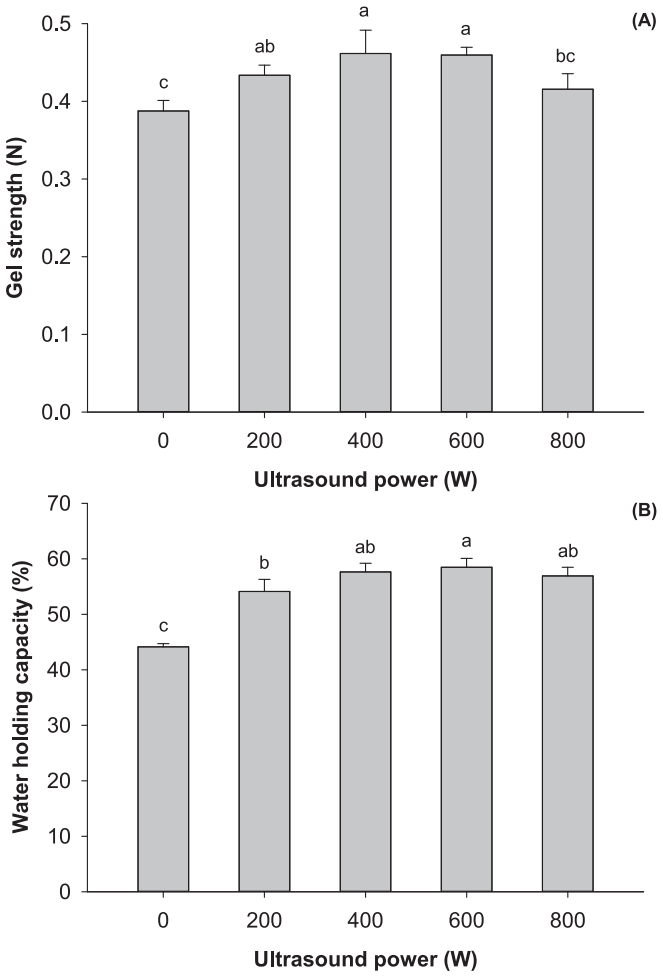

Fig. 1. Gel strength (A) and water holding capacity (B) of MP composite gels prepared by ultrasound-assisted heating (0–800 W). Means without common lowercase letters differ significantly ($P < 0.05$).

WHC, which reflects the binding force of the gel network structure to water, is closely associated with the palatability characteristics (e.g., appearance, juiciness, and tenderness) of meat products [21]. A well-structured gel can sufficiently capture and immobilize superfluous water in the three-dimensional gel network. As depicted in Fig. 1B, a notable increase of WHC ($P < 0.05$) occurred in composite gels induced by ultrasound-assisted heating. With the reinforcement of ultrasound powder from 0 W to 600 W, the WHC of MP composite gels augmented from 44.1% to 58.5%, in cooperation with the variations of gel strength (Fig. 1A). It is plausible that the involvement of ultrasound during gelation was in favor of the formation of a denser and ordered gel network, accordingly entrapping more excrescent water [12]; additionally, the exposed reactive groups in proteins could interact with more water molecules through hydrogen bonding, contributing to the enhanced WHC. It was noteworthy that a slight but insignificant diminution in WHC was observed at an ultrasound power of 800 W, possibly resulting from the destruction of orderly-packed gel networks and the formation of porous water channels. Likewise, Wang et al. [22] also noted that extravagant ultrasound treatment abated the WHC of gels prepared with MP extracted from chicken wooden breast.

3.2. Water distribution and mobility

Variations in water states within the composite gels were assessed

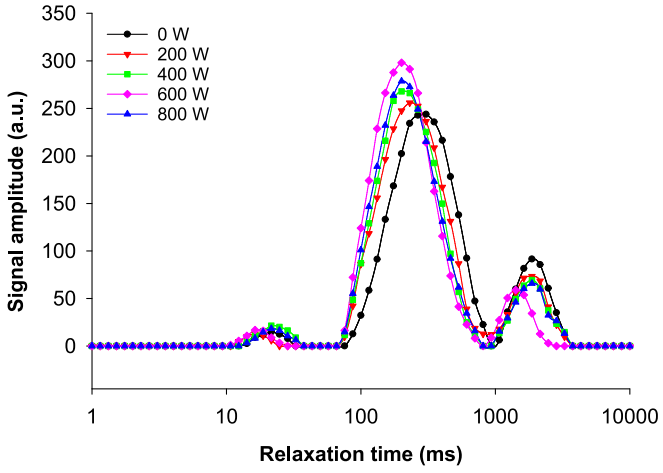

Fig. 2. Water distribution of MP composite gels prepared by ultrasound-assisted heating (0–800 W).

via the T_2 transverse relaxation times measured by low-field NMR. As presented in Fig. 2, the NMR curves of all MP composite gels were characterized by three distinct peaks in the relaxation time of 1–10,000 ms, corresponding to three states of water. Hereinto, T_{21} (10–30 ms) was associated with tightly bound water to protein structure, T_{22} (100–1000 ms) represented immobilized water entrapped within the protein structure and the three-dimensional gel network, whereas T_{23} (1000–3000 ms) indicated free water outside the myofibril lattice [23]. Typically, a shorter relaxation time (T_2) suggests lower water mobility and more vigorous interaction between water molecules and proteins in the gel structure [24]. It can be seen from Fig. 2 that ultrasound-assisted heating observably impacted the water distribution in composite gels, hinging on the ultrasound power. With the intensified ultrasound power from 0 W to 600 W, T_{21}, T_{22}, and T_{23} decreased significantly, from 22.6 ms to 17.1 ms, from 321 ms to 192 ms, and from 1711 ms to 1356 ms, respectively, insinuating that the corresponding water fluidity was impaired. It is generally believed that the alteration of water relaxation behavior is predominately due to the proton diffusion and chemical interchanges between water molecules and proteins [25]. Variations in inter- and intramolecular bonds within or between proteins induced by ultrasound-assisted heating affected the accessibility of protons, leading to variations in the relaxation velocity of water in close contact with proteins molecules [26]. Nevertheless, when the ultrasound power reached 800 W, an increase in T_{21} and T_{23} was witnessed.

Furthermore, the proportion of corresponding peak areas of three states of water was computed and expressed as P_{21}, P_{22}, and P_{23}. As depicted in Table 1, P_{22} accounted for 81–90%, suggesting immobilized water was the preponderant form of water in the gels. Upon ultrasound treatment, P_{22} augmented manifestly with the fortified ultrasound power from 200 W to 600 W where up to 11.1% increase in comparison with the control gel (0 W) was spotted. Simultaneously, P_{23} underwent a marked drop from 17.3% to 8.0%. This phenomenon indicated ultrasound-assisted heating could interdict the water fluidity in the gels as well as facilitate the transmutation of free water into immobile water, attesting more water was entrapped in the protein structure and ultimately immobilized. Liang and coworkers [27] also reported that ultrasound-assisted heating could weaken the water fluidity within the surimi-crabmeat gels. The results were consistent with the observations in WHC (Fig. 1). At higher ultrasound power (>600 W), the portion of immobilized water (P_{22}) declined markedly accompanied by a boost in free water (P_{23}), possibly resulting from the deterioration of gel network or the formation of cavities.

3.3. Microstructures of composite gels

Microstructural analysis was exploited to elucidate the divergences in the network structure of MP composite gels under distinct ultrasound treatments. As exhibited in Fig. 3, the control gel (0 W) without ultrasound treatment appeared a coarse and discontinuous three-dimensional gel network with large and heterogeneous pores. Upon ultrasonication, a compact and homogenous gel network with small, uniform pores, and filamentous structure were generated, notably at the ultrasound power of 600 W. In line with our results, Zhang et al. [28] also found that moderate ultrasound pretreatments resulted in the fabrication of a denser gel network with smaller pores in MP gels. It has been documented that the microstructure of heat-induced protein gels is closely associated with the relative velocity of protein unfolding and aggregation [29]. The introduction of ultrasound during the thermal-induced gelation process stimulated sufficient unfolding of myosin and actin before protein aggregation, conducing to the construction of a well-structured gel network. Moreover, the densely packed network favored the gel strength (Fig. 1A) and possessed enhanced competence to immobilize water (Fig. 1B and Fig. 2). Above 600 W, the gel cavities became larger and more heterogeneous, potentially owing to the unfolding rate and aggregation rate of proteins were disharmonious. The deterioration of the gel structure could partially explain the decrease in the gel strength and WHC (Fig. 1).

Table 1
Corresponding relaxation time (T_2) and peak area proportion (P) of MP composite gels prepared by ultrasound-assisted heating (0–800 W).

Treatment	T_{21} (ms)	T_{22} (ms)	T_{23} (ms)	P_{21} (%)	P_{22} (%)	P_{23} (%)
0 W	22.6 ± 1.9^{a}	321 ± 26^{a}	1711 ± 141^{ab}	1.72 ± 0.07^{d}	81.0 ± 0.8^{e}	17.3 ± 0.8^{a}
200 W	17.9 ± 1.4^{b}	242 ± 20^{b}	1967 ± 162^{a}	1.19 ± 0.01^{e}	86.6 ± 0.1^{c}	12.2 ± 0.1^{b}
400 W	19.7 ± 1.6^{ab}	192 ± 15^{bc}	1792 ± 141^{a}	2.73 ± 0.05^{a}	85.9 ± 0.2^{d}	11.4 ± 0.2^{c}
600 W	17.1 ± 1.4^{b}	192 ± 15^{bc}	1356 ± 107^{b}	2.01 ± 0.04^{c}	90.0 ± 0.2^{a}	8.0 ± 0.1^{e}
800 W	19.7 ± 1.6^{ab}	183 ± 15^{c}	1711 ± 141^{ab}	2.20 ± 0.09^{b}	87.1 ± 0.1^{b}	10.7 ± 0.1^{d}

Notes: Means within the same column without common lowercase letters differ significantly ($P < 0.05$).

3.4. Composition of non-participating proteins

The pellets (participating proteins) of centrifuged gel samples were firstly analyzed by SDS-PAGE, however, no significant changes were captured in gels with different treatments (results not shown). Therefore, to reveal the effect of ultrasound treatment on the formation of MP gels, the expressed liquid of centrifuged gel samples was further subjected to SDS-PAGE so as to identify the non-contributing MP polypeptides in the composite gels. As presented in Fig. 4, the non-participating proteins in the gel formation were mainly composed of low molecular weight proteins (20–50 kDa), particularly tropomyosin (a doublet in 34–36 kDa) and troponin I (about 20 kDa), in support of previous studies that proved tropomyosin and troponin marginally participated in the gel formation [30]. As expected, myosin heavy chain (MHC) and actin were virtually absent in the expressed liquid of all gels, further verifying their dominant role in gelation, in support with the findings by Gao et al. [31]. Upon ultrasonication, the overall SDS-PAGE patterns of composite gels were barely affected, however, the intensity of tropomyosin attenuated progressively with increasing ultrasound power from 0 to 800 W. Moreover, it should be noted that a tenuous band located at 22 kDa (Band 1), which could be assigned to a part of myosin light chain (MLC), vanished as the ultrasound intensity was above 400 W. The findings suggested that ultrasound can effectively promote more proteins to get involved in the construction of gel network structure. Under reducing condition (+β-mercaptoethanol), all bands were barely affected, except a band at 95 kDa ascribed to α-actinin were degraded.

3.5. Secondary structure

Raman spectra were implemented to evaluate the variations in the protein secondary structures of MP composite gels induced by ultrasound-supplemented heating, as shown in Fig. 5. Protein secondary structures were predominately identified from the Amide I band (1600–1700 cm^{-1}) that primarily involved C═O stretching and, to a lesser extent, C–N stretching, C_{α}–C–N bending, and N–H in-plane bending of peptide groups [32]. The bands at 1650–1658 cm^{-1}, 1665–1680 cm^{-1}, 1680–1690 cm^{-1}, and 1660–1665 cm^{-1} correspond to α-helix, β-sheet, β-turn, and random coil, respectively [33].

As depicted in Fig. 6, α-helix (71.4%) was the principal secondary structure presented in the control gel (0 W), echoing the reports by Cen et al. [34]. With the augmented ultrasound powder from 200 W to 800 W, α-helix declined gradually along with the increase of β-sheet, β-turn, and random coil, notably β-sheet, substantiating that the structural conversions from α-helix to other structures. Homologous phenomena have been viewed in the ultrasound-assisted surimi gels, evincing a diminution in the α-helix accompanied by a rise in β-sheet with the

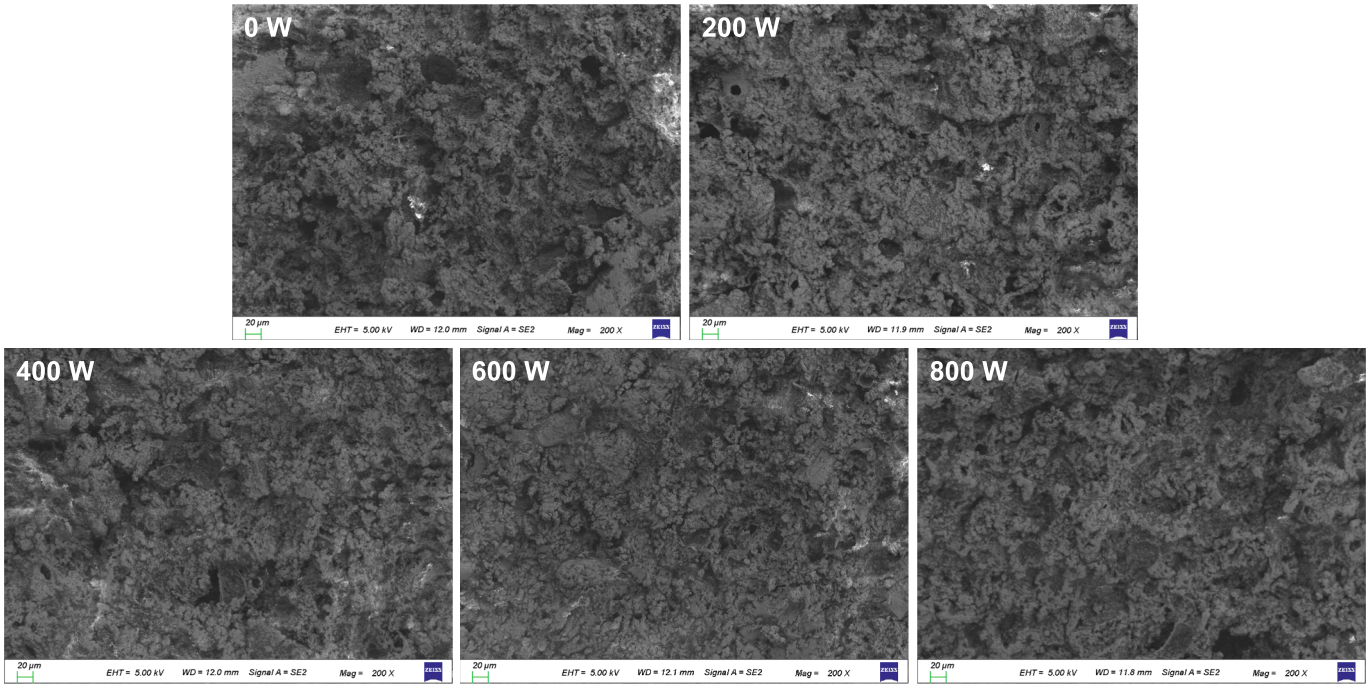

Fig. 3. Microstructure of MP composite gels prepared by ultrasound-assisted heating (0–800 W).

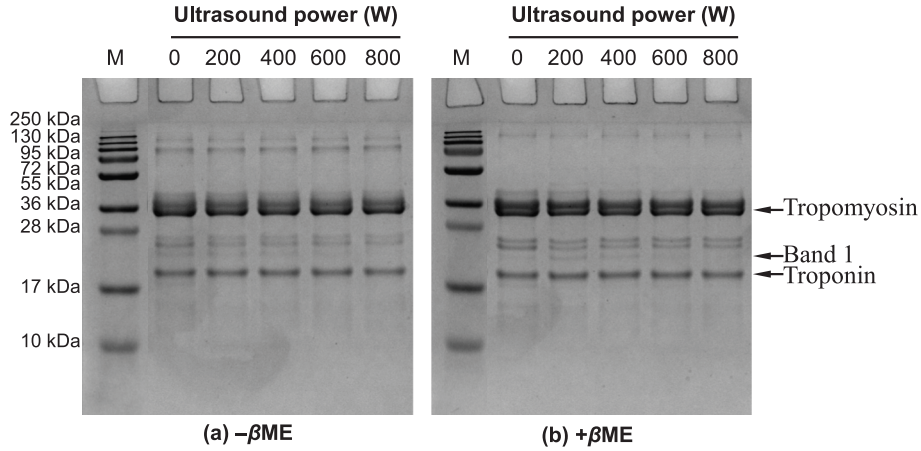

Fig. 4. SDS–PAGE of non-participating proteins in MP composite gels prepared by ultrasound-assisted heating (0–800 W). Samples were prepared in the absence (−βME) or presence (+βME) of 10% β-mercaptoethanol M: molecular weight standard.

incremented ultrasound intensity from 0.35 to 0.82 W/cm^2 [35]. The results could be because ultrasound treatment expedited the unfolding of α-helix and the production of β-sheet. A higher magnitude of β-sheet, which indicated a reinforcement in the intermolecular hydrogen bonding between peptide chains and an ordered rearrangement of protein molecules [36], has been proved to be conducive to the fabrication of a stronger gel network with competitive water retention capacity [13,27,37]. Authentically, gels induced by ultrasound-supplemented thermal treatment with a higher β-sheet exhibited better performance in terms of gel strength and WHC (Fig. 1).

3.6. Variations of local environment in gels

3.6.1. Tryptophan residues

Aromatic amino acid side chains present diverse representative Raman bands (760 cm^{-1}, 830 cm^{-1}, and 850 cm^{-1}), some of which are able to adumbrate the polarity of the microenvironment or the participation of hydrogen bonding [38]. For instance, changes in the normalized intensity of the Raman band at around 760 cm^{-1} (I760/I1003) ascribed to the stretching vibrations of the tryptophan ring can be adopted to assess the hydrophobicity of tryptophan residues and to mirror the hydrophobic interactions within the gels [39]. As depicted in Table 2, I760/I1003 increased remarkably from 0.32 to 0.91 when the ultrasound output was magnified from 0 W to 600 W, implying that more tryptophan residues were buried inside a hydrophobic circumstance [40]. Semblable findings have been obtained in previous studies on gels prepared from ultrasound-pretreated wooden breast MP [22]. The formation of protein aggregates via strong hydrophobic interactions during gelation might be responsible for the amplified I760/I1003 [41], which was beneficial to the fabrication of a finer gel structure, as revealed in Fig. 3. As the ultrasound intensity reached 800 W, I760/I1003 underwent a slight but not notable diminution, hinting that a small number of tryptophan residues originally buried in hydrophobic microenvironment were exposed to polar aqueous surroundings.

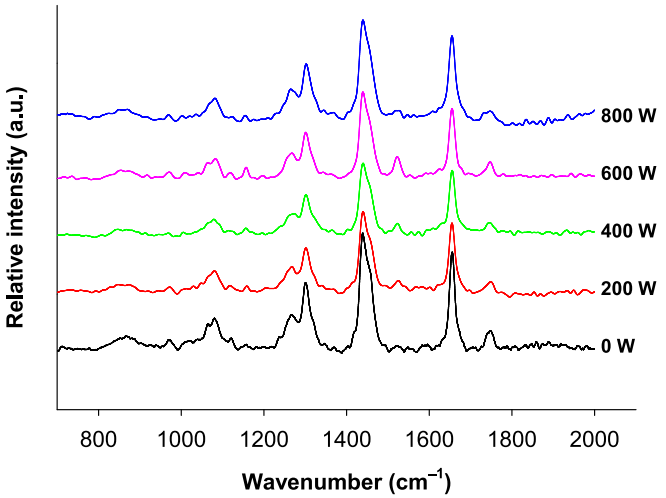

Fig. 5. Raman spectra (700–2000 cm^{-1}) of MP composite gels prepared by ultrasound-assisted heating (0–800 W).

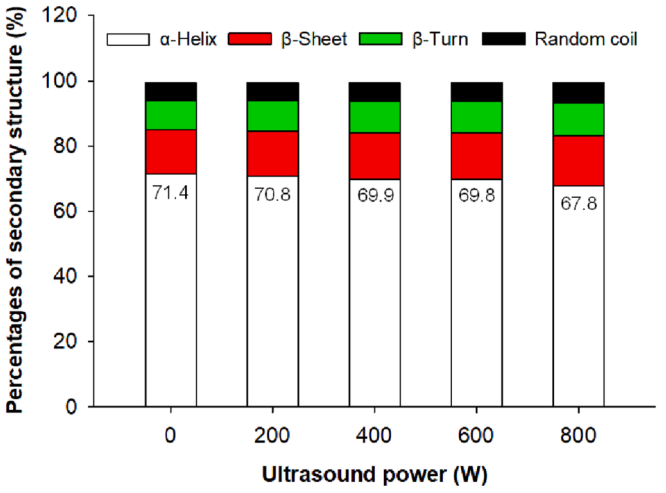

Fig. 6. Secondary structures of MP composite gels prepared by ultrasound-assisted heating (0–800 W).

Table 2
Normalized intensities of Raman bands at 760 cm^{-1} and 850/830 cm^{-1} of MP composite gels prepared by ultrasound-assisted heating (0–800 W).

Treatment	I760/I1003	I850/I830
0 W	0.32 ± 0.04^{d}	1.37 ± 0.01^{c}
200 W	0.49 ± 0.05^{c}	1.57 ± 0.07^{b}
400 W	0.79 ± 0.02^{b}	2.00 ± 0.13^{a}
600 W	0.91 ± 0.02^{a}	2.00 ± 0.04^{a}
800 W	0.87 ± 0.01^{ab}	1.53 ± 0.01^{b}

Notes: Means within the same column without common lowercase letters differ significantly ($P < 0.05$).

3.6.2. Tyrosine doublet bands

The doublets in the vicinity of 830 cm^{-1} and 850 cm^{-1} as a consequence of the *p*-substituted benzene ring vibrations of tyrosine are closely related to the microenvironment around tyrosine residues and the participation of phenolic hydroxyl groups in hydrogen bonding [42]. A high ratio (0.9–2.5) of I850/I830 manifests that more tyrosine residues are placed in aqueous or polar environments and can interact with water molecules via moderate or weak hydrogen bonding, serving as simultaneous hydrogen bond donors and receptors. On the contrary, tyrosine residues are encapsulated in hydrophobic environments and inclined to function as donors to consolidate internal hydrogen bonding if the I850/I830 proportion is lower [43]. The proportion of I850/I830 was between 1.36 and 2.00 with diverse ultrasound intensities, testifying that tyrosine residues prevailingly tended to be exposed to get involved in moderate-to-weak hydrogen bonding with water molecules. With the intensified ultrasound level from 0 W to 400 W, the I850/I830 ratio presented a progressively ascending tendency. Nevertheless, a high level of ultrasound power (800 W) gave rise to a decrease in the I850/I830 ratio. These changes confirmed that moderate ultrasound-assisted heating could give impetus to the formation of more hydrogen bonding, which was in favor of a finer gel structure to retain superfluous water (Fig. 1 and Table 1).

3.7. Chemical forces in the gels

Intermolecular chemical forces that maintained the gel network were determined to reveal the reinforcing functions of ultrasound-supplemented heating on the MP composite gels. As indicated in Fig. 7, compared with ionic and hydrogen bonds, hydrophobic interactions and disulfide bonds, especially disulfide bonds, contributed to a prominently larger proportion of chemical forces in all gels irrespective of ultrasonication, demonstrating the dominant role in stabilizing the gel structure. The findings were in consonance with a previous study by Wu et al. [17]. The incorporation of ultrasound into the heat-induced gelation process had little effect on the ionic and hydrogen bonds. It should be addressed that the variation trend of hydrogen bonds was not in good agreement with the observations in the Raman analysis (I850/I830). The inconsistent results may be due to the difference in the principles of the two determination methods. The hydrogen bond analysis according to the vibrations of tyrosine only represented the involvement of phenolic hydroxyl groups in hydrogen bonding. In contrast, the reagent method was based on the action of urea to disrupt all the hydrogen bonds presented in the gel. On the other hand, hydrophobic interactions in gels were conspicuously enhanced by the ultrasound treatments, apparently originating from more exposed hydrophobic groups and patches as a result of the unfolding of protein conformation and the disintegration of protein aggregates. The changes in hydrophobic interactions were in corroboration with the Raman spectra analysis (Table 2). Concomitantly, a pronounced increase in disulfide bonds was noted in gels induced by ultrasound-assisted heating relative to the control gels (0 W). This phenomenon could be

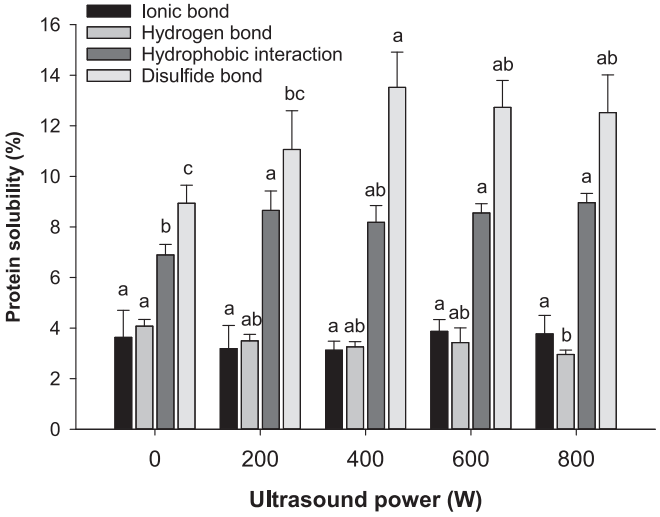

Fig. 7. Chemical forces involved in MP composite gels prepared by ultrasound-assisted heating (0–800 W). Means within the same chemical force without common lowercase letters differ significantly ($P < 0.05$).

attributable to more sulfhydryl groups originally occluded were exposed, and subsequently oxidized into disulfide bonds, potentially arising from the continuous attack of highly reactive free radicals (·OH and ·H) impelled by the cavitation effects of ultrasonication [44]. This phenomenon was in consonance with previous observations on the composite surimi gels subjected to ultrasound-assisted heating [13]. The exaltation of hydrogen bonds and disulfide bonds facilitated the cross-linking and aggregation of proteins, which were favorable to the formation of elastic gels with superior gel strength and WHC as vindicated in Fig. 1. It seemed that extravagant ultrasound intensity (>600 W) was detrimental to the construction of a crosslinked gel network, as evidenced by the slight reduction in disulfide bonds. This might partially explain the decrease in gel strength at a higher ultrasound power.

4. Conclusions

The assistance of moderate ultrasound (<600 W) during thermal-induced gelation markedly improved the gelling performance of MP, as corroborated by the enhanced gel strength and water retention capacity. Ultrasound-assisted heating enhanced protein–protein interactions and triggered a more compact and homogenous gel network. T_2 relaxation analysis demonstrated that ultrasound involvement could retard the water fluidity within the well-structured gel network and prompt the transformation of free water into immobilized water. Moreover, the incorporation of ultrasound gave an impetus to the conformational conversion of proteins from α-helix to β-sheet, which was in support of intermolecular interaction and gel network construction. Besides, the chemical forces that stabilized the gels were altered by the ultrasound treatment where remarkable reinforcements in hydrophobic interaction and disulfide bands were identified, conducing to the consolidation of gel strength. Collectively, the present study would be beneficial for the application of ultrasound as a potential tool to manufacture functional meat products with improved gel performance.

CRediT authorship contribution statement

Qingling Wang: Formal analysis, Funding acquisition, Writing – original draft. **Chen Gu:** Formal analysis, Data curation. **Ranran Wei:** Methodology, Investigation, Formal analysis. **Yi Luan:** Formal analysis. **Rui Liu:** Methodology, Validation. **Qingfeng Ge:** Validation. **Hai Yu:** Validation. **Mangang Wu:** Supervision, Funding acquisition, Project administration.

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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