Food Science of Animal Products

Research Article

High-intensity ultrasound of chicken myofibrillar protein under reduced-salt conditions: effect on emulsifying and interfacial properties

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Abstract: In this study, the effects of high-intensity ultrasound (HIU, frequency 20 kHz, amplitude 60%, power 450 W) for 0, 6, and 9 min on the emulsifying properties of chicken myofibrillar protein (MP) under different NaCl concentrations (0.2, 0.3, 0.4, and 0.5 mol/L) were investigated. The results showed that the increasing NaCl concentration and prolonged HIU treatment time significantly enhanced the emulsifying activity, the emulsifying stability and storage stability of MP emulsion were increased significantly ($P < 0.05$), and the particle size of MP emulsion were decreased significantly ($P < 0.05$). HIU pretreatment decreased the interfacial tension, and increased the apparent viscosity and the absolute zeta-potential of reduced-NaCl MP emulsions. Compared with the non-HIU group at 0.5 mol/L NaCl, the emulsions stability was significantly improved when the HIU time was extended to 9 min under 0.3 mol/L NaCl ($P < 0.05$). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis result of interfacial MP showed that the band strength of myosin heavy chain and actin significantly increased after 9 min-HIU treatment at both 0.3 and 0.5 mol/L NaCl. Circular dichroism analysis showed that the random coil relative content of interfacial MP was decreased significantly ($P < 0.05$), and the relative content of α-helix and β-sheet were increased ($P < 0.05$) after HIU. Extended HIU time could partially alternative the effect of NaCl on the emulsification properties of MP, and improve the stability of the interfacial protein structure and MP emulsion in reduced-salt conditions, which facilitated the processing of lower salt emulsion-based meat products.

Keywords: chicken myofibrillar protein; ultrasound; reduced-salt; emulsifying stability; interfacial properties; secondary structures

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1 Introduction

Many scientific reports have demonstrated that higher salt results in an increased risk of cardiovascular disease[1]. The World Health Organization (WHO) recommends limiting intake of salt to less than 5 g/day in adults[2]. The salt content of processed meat products is usually 2–3 g/100 g (0.47–0.68 mol/L)[3], and 15%–25% to the total salt intake in daily diet, which is a potential hazard to consumers' health[4]. As a result, more consumers, meat scientists, and manufacturers concern the amount of salt added into products or the availability of excellent quality meat processing options with reduced NaCl content[5–7]. Salt functions in the meat processing to improve product quality by promoting extraction of meat proteins and hydration (solubilizing proteins), and improve juiciness, adhesion, and cohesiveness[8–10]. In addition, salt content has been concerned and reported to affect the self-assembly, morphology and molecular structure of myofibrillar protein (MP)[11]. A higher concentration of NaCl (> 0.3 mol/L) significantly enhance the solubility of meat protein and improve the functionality during meat processing, however, due to the charge shielding of salt ions under high salt conditions the meat protein-prepared emulsions are still physically unstable systems that tend to aggregate and flocculate[12–13]. The lower-salt conditions (0.6–1.4 g/100 g or 0.10–0.24 mol/L) increase the degree of self-assembly of meat proteins, which reduces the solubility and emulsification properties[14], the task of maintaining the water and fat binding capacity of reduced-salt meat products is a great challenge[15]. When seeking to improve quality and acceptability of reduced-salt meat products, processing focused strategy can be designed to increase protein solubilization efficiency at reduced-salt concentrations[16–17].

Ultrasound technology as an alternative processing, showed a good potential for use in the production of meat emulsions[18–20], which has been shown to effectively induced physicochemical and structural properties of muscle proteins[4,21–23]. High-intensity ultrasound (HIU, 10–1 000 W/cm^2) produces a cavitation phenomenon to be able to alter the structural properties and the functional properties of muscle proteins at the molecular level, improving the taste and quality of processed meat products while reducing processing time and production costs[24–26]. MP is major protein in muscle tissue and have a key role in the textural development, water-fat combination of meat products[27]. MP was also considered as a novel and desirable encapsulation material or a delivery of the emulsion system[28–29]. It has amphiphilic character and ability as an emulsifier to form interfacial films to create stable O/W emulsions. Interestingly, our study reported that HIU (20 kHz, 450 W) for 6 min could enhance the emulsifying characteristics of MP under common salt condition (0.6 mol/L)[12].

Guo et al.[6] and Jiang et al.[30] found that ultrasound technology can be used as a new tool to reduce phosphate and salt content of meat products. Based on the foundation of our previous studies, is it possible to further inhibit the self-assembly behavior of proteins by moderately prolonging the duration of HIU treatment, and thus optimize the weakening effect of reducing salts on MPs to achieve the partial replacement of salts by HIU. Additionally, limited research exists regarding the impact of HIU on the emulsification characteristics of MP under reduced-salt conditions, and there is a lack of systematic comparative analysis of the effects of HIU on the emulsification properties and oil-water interfacial adsorption properties of MP under different salt conditions. Therefore, learning about the effect of the emulsifying properties of HIU treatment MP under lower salt level is of great scientific interest for developing and promoting healthy and development of appealing reduced-salt products utilizing HIU as a substitute for salt functionalities.

This study aimed to investigate the impact of HIU (frequency 20 kHz; power 450 W; duration 0, 6, 9 min) on the emulsifying properties of MP in different salt concentrations (0.2, 0.3, 0.4, and 0.5 mol/L), including emulsion stability and rheological properties of MP emulsions, and elucidate the potential mechanisms contributing to emulsion stability through physicochemical properties, microstructure and interfacial properties (interfacial tension and adsorbed proteins composition) and interfacial structure of MP-stabilized emulsion, to provide basic information and application potential on how the application of HIU improve the overall stability of MP emulsions in reduced salt condition.

2 Materials and methods

2.1 Materials

The chicken breast meat (*pectoralis major* muscle, 24–36 h postmortem) and Jinlongyu soybean oil (Yihai Kerry Food Co., Ltd., Shanghai, China) were purchased from Dazhang supermarket (Zhengzhou, China). The stain Nile red was supplied by Aladdin Biochemistry and Technology Co., Ltd. (Shanghai, China). All chemicals used in the experiment were of analytical grade or better.

2.2 Extraction of MP

MP was extracted using the procedures of our earlier research[31], the final concentration of MP was determined[32] and stored at 4 °C, utilized in the succeeding tests during 48 h.

2.3 HIU treatment of the MP suspensions

The MP suspensions were adjusted to a protein concentration of 20 mg/mL with the addition of phosphate buffer (10 mmol/L K_2HPO_4/KH_2PO_4, pH 7.0, 4 °C). NaCl was added to the suspensions and adjusted at a concentration of 0.2, 0.3, 0.4, and 0.5 mol/L, respectively. All the samples were stirred and dispersed with the help of magnetic stirrer (C-MAG HS4, IKA, Germany) at 4 °C for approximately 15 min before application of HIU treatment.

HIU was carried out following our previously reported method[12]. Place about 70 mL of MP suspension in a 100 mL beaker, HIU treatment was performed using an ultrasonic generator (VC 750, Sonics & Materials, Inc., USA) at a frequency of 20 kHz (60% amplitude and 450 W), with the ultrasound probe (13 mm in diameter) penetrating to a depth of 15 mm below the liquid surface, and was treated sequentially for 0, 6, and 9 min in sequence (ultrasound mode of on-time for 2 s, off-time for 4 s). The temperature of the samples did not exceed 12 °C during ultrasound. HIU treatment produced acoustic intensities of (29.74 ± 2.83) W/cm², as determined by the calorimetric method outlined by Jambrak et al.[33]. All the sample preparations (for each NaCl concentration) and HIU treatment (for each ultrasound time) were conducted in triplicate. Following the HIU treatment, the samples were stored at 4 °C for subsequent analysis.

2.4 Preparation of emulsions

The oil-in-water emulsion was prepared as previously described by our earlier research[12,31]. HIU treatment MP samples under different NaCl levels as the emulsifiers, a part of the obtained emulsions was immediately prepared for evaluating the storage ability of emulsion.

2.5 Determination of emulsion activity index (EAI) and emulsion stability index (ESI) of MP

The EAI and ESI of HIU treatment MP samples emulsion with different NaCl levels were assessed using the approach described in our earlier research[12].

2.6 Storage stability analysis of the emulsions

According to our previous study[12], images of emulsions stored for 0, 12, 24, 36, 48, 60, and 72 h were taken with a cell phone camera (iPhone 6, Apple, USA).

2.7 Confocal laser scanning microscopy (CLSM) observation

The microstructure of freshly prepared MP-stabilized emulsions were determined using a CLSM as previously described by Li et al.[12]. The emulsion sample (1.0 mL) were stained with 45 μL of Nile red. After that, a CLSM (Leica TCS SP5, Heidelberg, Germany) was used to observe the emulsion droplets (excitation wavelength was 488 nm) at room temperature with a 10× oil immersion objective[34].

2.8 Determination of particle size and zeta-potential

The emulsions were diluted 500-fold with the phosphate buffer. Particle distribution and size of the samples were measured by the dynamic light scattering using a laser particle sizer (Zetasizer Nano-ZS 90, Malvern Instruments, Malvern, England)[35].

Diluted the emulsion 50 times with deionized water and mixed well, then, the zeta-potential of emulsion droplets was determined by the laser particle sizer.

2.9 Rheological measurements of emulsions

The steady shear rate (apparent viscosity) rheological behavior of the emulsions at 0.1–500 s^{-1} was determined using a dynamic rheometer (DHR-1, TA Instruments Inc., USA) by the method of Zhao et al.[35].

2.10 Determination of characteristics of interfacial proteins

2.10.1 Interfacial tension

Refer to the method of O'sullivan et al.[36] and modify it slightly. Place a glass dish containing 20 g of water phase (0.1 g/100 mL MP solution) into the instrument and the platinum plate was immersed in, then, 50 g oil is transferred into the water phase to form an interface between the water phase and oil phase. The interfacial

tension was measured using the automatic interfacial tensiometer (K100, Krussand, German) the whole test was held at 25 °C for 3 600 s.

2.10.2 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

According to our previous study[12] with slight modification. The obtained top layers were carefully resuspended in the phosphate buffers (1:4, *V*/*V*) containing the corresponding NaCl concentration. Then, the top layers containing the adsorbed proteins were purified and collected using the centrifugation (10 000 × *g* for 30 min, 4 °C) and filtration. Finally, they were further used for SDS-PAGE to identify the adsorbed proteins composition. Each sample was loaded on the gels and electrophoresed according to the steps of Laemmli[37].

2.10.3 Secondary structures of adsorbed proteins in the emulsified layer

Before further use for SDS-PAGE (section 2.10.2), the filtered emulsified layer containing the adsorbed proteins were subjected to circular dichroism (CD) spectroscopy using a Chirascan instrument (Chirascan V100, Applied Photophysics, Leatherhead UK) as described by Saleem et al.[38] and Zou et al.[26]. The adsorbed proteins concentration of the samples was determined by Biuret method[32] and further diluted to 0.10 mg/mL by the phosphate buffer containing the corresponding NaCl concentration. Then the sample was placed in a quartz cell (1 mm path length). The scanning range was 260–190 nm and the resolution was set at 1 nm. After scanning, CDNN software (CEVA Logistics, Inc., USA) was utilized to determine the relative content of secondary structure of the adsorbed proteins.

2.11 Statistical analysis

Three independent experiments were carried out on different occasions. Each measurement was performed at least three times. All data were expressed as the mean ± standard deviation. Analysis of variance (ANOVA) and Duncan's multiple range test were applied to compare significant differences ($P < 0.05$) among groups using SPSS 21.0 software (IBM, USA).

3 Results and discussion

3.1 EAI and ESI

As shown in Figure 1A, the EAI of untreated MP showed a significant increase ($P < 0.05$) from 8.92 to 20.37 m^2/g as NaCl level rise from 0.2 to 0.5 mol/L. HIU showed a significant increased ($P < 0.05$) the EAI of MP at any given NaCl concentrations. After HIU treatment, MP demonstrated an enhanced capacity for adsorption at the oil-water interface. The EAI of the samples (0.2 mol/L NaCl) treated with HIU for 9 min was significantly higher ($P < 0.05$) than that of the untreated samples (0.3 mol/L NaCl), moreover, they were not different significantly from the untreated sample containing 0.4 mol/L NaCl. A comparable effect was noted with HIU treatment for 9 min at a concentration of 0.3 mol/L NaCl compared with the untreated samples containing 0.5 mol/L NaCl, even significantly higher than the untreated 0.4 mol/L NaCl ($P < 0.05$). The HIU treatment MP at reduced NaCl concentrations had comparable EAI to the samples containing higher NaCl level. When the NaCl concentration of untreated MP was raised from 0.2 to 0.3 mol/L, there was no significant increase in ESI. However, a significant increase in ESI ($P < 0.05$) was observed when the NaCl level rose from 0.3 to 0.5 mol/L (Figure 1B). At all the NaCl levels, the ESI further increased compared to the untreated MP with increasing HIU time from 6 to 9 min. The ESI of MP in 0.2 mol/L NaCl treated with HIU of 9 min was higher significantly than that in 0.4 mol/L NaCl without receiving the HIU treatment ($P < 0.05$). The MP in 0.3 mol/L NaCl treated with HIU of 9 min had much higher ESI than the untreated MP in the 0.4 and 0.5 mol/L NaCl. HIU treatment had a more pronounced effect to achieve ESI improvement at lower NaCl concentrations. Our previous study demonstrated that HIU treatment for 6 min improved the emulsifying properties of MP suspension containing 0.6 mol/L NaCl[12]. This finding was adopted in the reduced NaCl condition. The improvement in the EAI and ESI of HIU treatment MP was probably associated with the unfolded structure and dissociation of MP aggregates[39]. HIU could disrupt myofibrils and promote to the higher protein extraction at lower NaCl concentrations, moreover, HIU treatment had the higher protein hydrophobicity[40], contributing to the protein adsorption to the oil-water interface. These findings indicate that HIU treatment may serve as a partial alternative to NaCl addition for enhancing the MP emulsification.

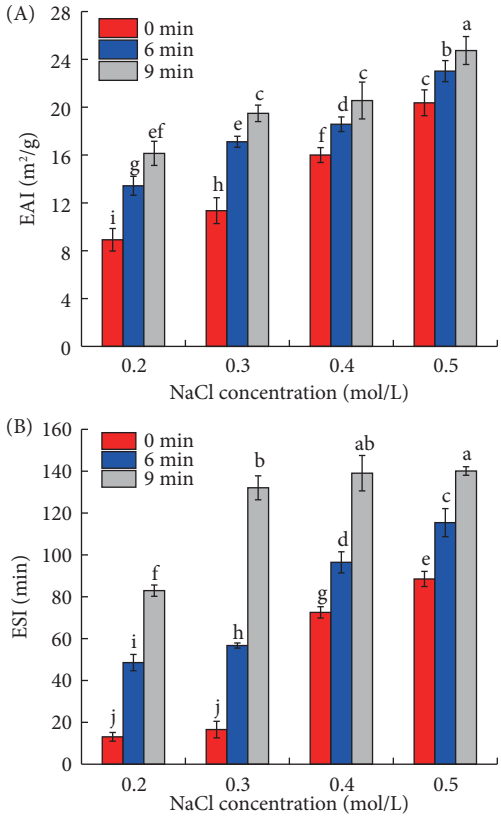

Figure 1 The (A) EAI and (B) ESI of HIU-treated (0, 6, and 9 min) MP under different NaCl concentrations. Different lowercase letters indicate significant differences ($P < 0.05$).

3.2 Photos and microscopy of emulsions

Figure 2 showed the image recordings of MP stabilized emulsions after 0, 12, 24, 36, 48, 60, 72, and 84 h of storage under different processing conditions. Untreated MP emulsions at 0.2 and 0.3 mol/L NaCl exhibited a similar creaming process. They

displayed the phase separation within 24 h of storage. As the NaCl level increased from 0.3 to 0.5 mol/L, untreated MP emulsions had better stability. Untreated MP emulsions at 0.4 and 0.5 mol/L NaCl displayed phase separation until being stored at 72 and 84 h, respectively. When compared to untreated MP groups, HIU treatment MP groups had better emulsion stability even at reduced NaCl concentration. No apparent phase separation was observed at 12, 24, 48, or 60 h for the emulsion stabilized by the MP in 0.2 mol/L NaCl treated with an HIU time of 9 min. In addition, compared to the untreated MP in 0.5 mol/L NaCl, the emulsions stabilized by the MP in 0.3 mol/L NaCl treated with an HIU time of 9 min displayed better emulsion stability after being stored at 84 h. The improvement in the storage stability for the HIU treatment MP stabilized emulsions are consistent with those in above ESI.

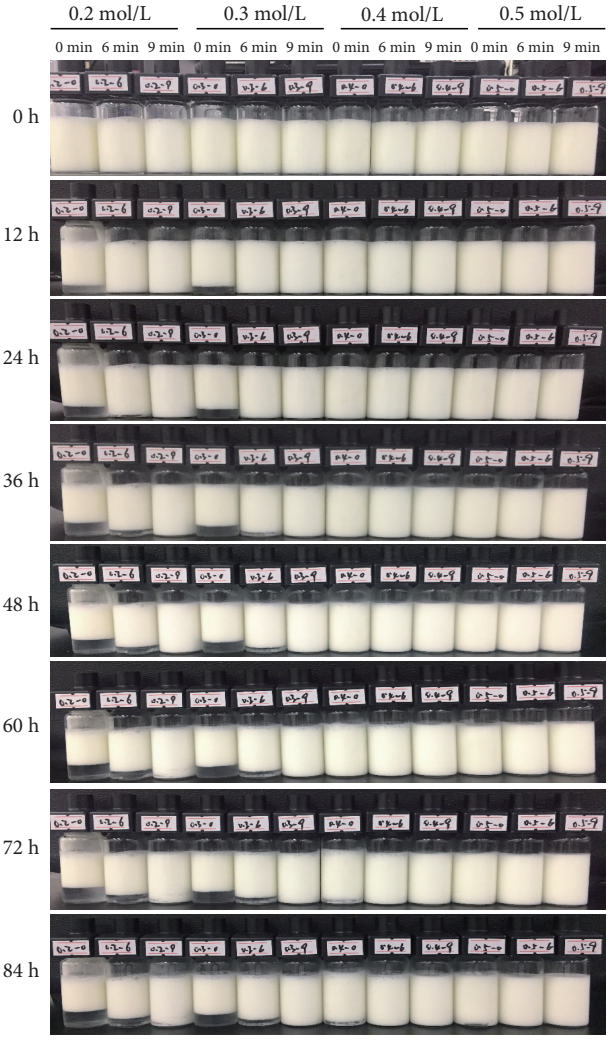

Figure 2 The photos of emulsions stabilized with HIU-treated (0, 6, and 9 min) MP under different NaCl concentrations (0.2, 0.3, 0.4, and 0.5 mol/L) during the storage at 4 °C for 0, 12, 24, 36, 48, 72, and 84 h, respectively.

3.3 The microstructure of emulsions

As shown in Figure 3, the relatively fewer and larger droplets were observed in the emulsion stabilized by the untreated MP in 0.2 mol/L NaCl, indicating the lower emulsion stability[41]. As NaCl concentration increased from 0.2 to 0.5 mol/L, the droplet size was progressively decreased in the untreated MP stabilized emulsions. For all the HIU treatment MP at given any NaCl levels, the droplets were obviously decreased with increasing the HIU time from 0 to 9 min. The 9 min of HIU treatment MP stabilized emulsions had smaller and more homogeneously droplets at 0.2 and 0.3 mol/L NaCl level, suggesting that HIU treatment MP at lower NaCl conditions for 9 min improve emulsion stability. The ultrasonic cavitation effects generated the dissociation and unfolding of MP[21,38], favor the rapid protein movement and increased MP coated on the oil droplets, leading to the decreased droplet size and higher emulsion stability[12].

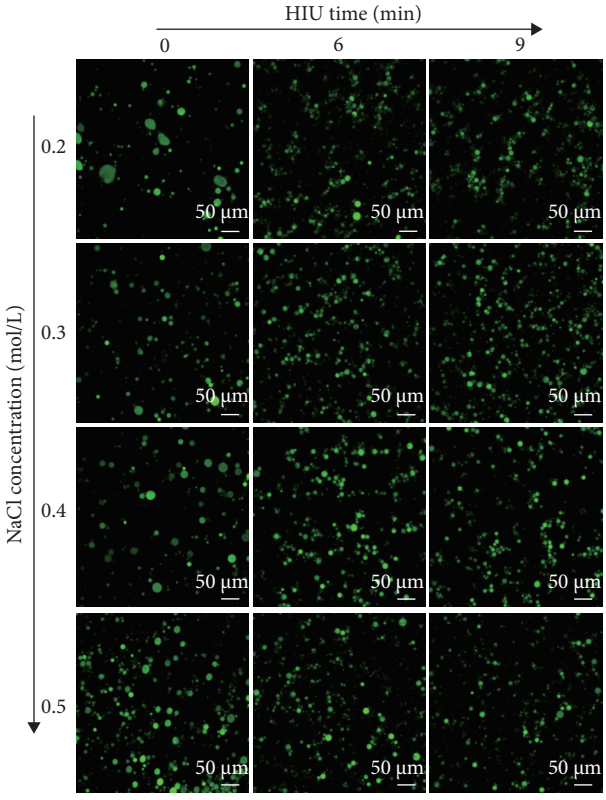

Figure 3 CLSM images of HIU-treated (0, 6, and 9 min) MP stabilized emulsions under different NaCl concentrations (0.2, 0.3, 0.4, and 0.5 mol/L). MP-soybean oil emulsion droplets were observed under 488 nm (argon laser).

3.4 Particle size distribution and zeta-potential of the emulsions

Figure 4 shows the particle size distribution of MP emulsions under different treatment conditions. The emulsions prepared by untreated MP at all four NaCl concentrations exhibited a multimodal distribution, while the particle size distribution of HIU treatment MP stabilized emulsions had less peaks and became narrow, with an apparent shift in the distribution peak to the left. Especially as the time of HIU treatment MP reached 9 min, the particle size of emulsion droplets decreased and more emulsion droplets moved to the smaller particle size range (0.1–1.0 μm). This may be due to the smaller particle size of MP resulting from HIU effect, which enhanced the protein-oil interactions and consequently reduced the droplet size in the emulsion[12].

As shown in Table 1, the $D_{4,3}$ for the untreated MP stabilized emulsions were decreased significantly when the NaCl level

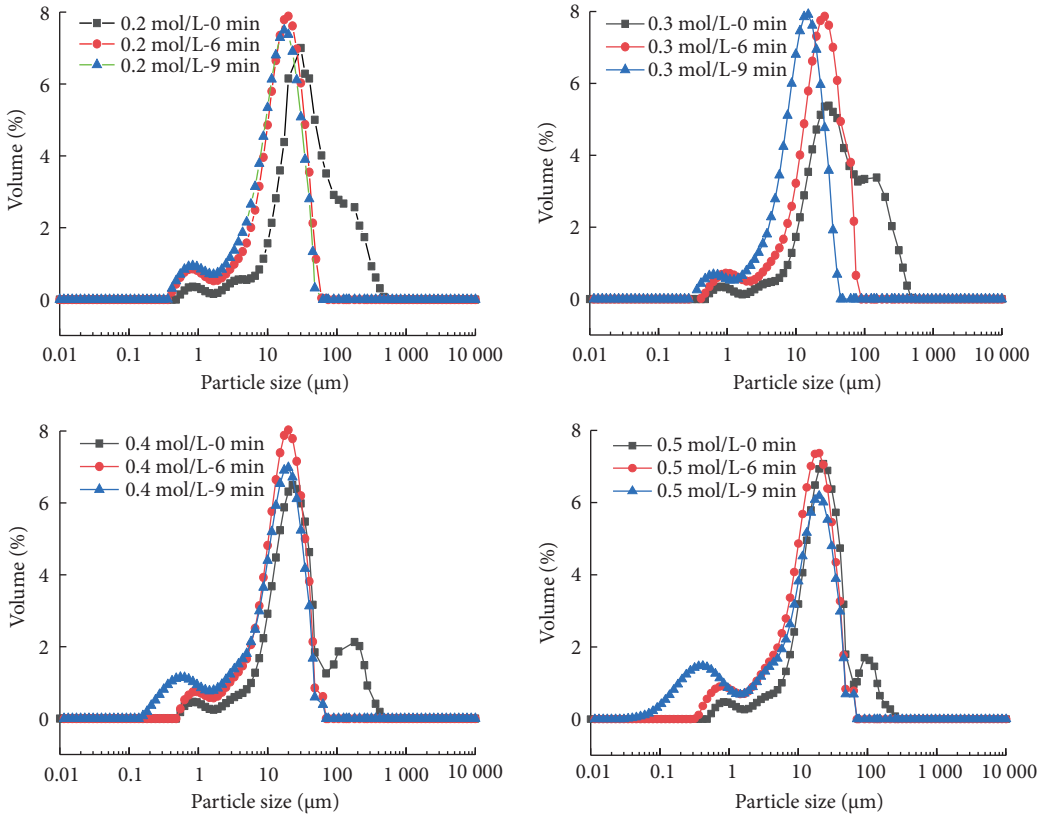

Figure 4 The particle size distribution of HIU-treated (0, 6, and 9 min) MP stabilized emulsions under different NaCl concentrations (0.2, 0.3, 0.4, and 0.5 mol/L).

Table 1 Particle size ($D_{4,3}$) and zeta-potential of emulsions by the HIU-treated (0, 6, and 9 min) MP under different NaCl concentrations (0.2, 0.3, 0.4, and 0.5 mol/L).

NaCl concentration (mol/L)	$D_{4,3}$ (μm)			Absolute zeta-potential (mV)		
	0 min	6 min	9 min	0 min	6 min	9 min
0.2	67.18 ± 1.04^{Aa}	16.71 ± 0.24^{Ba}	16.25 ± 0.21^{Ca}	27.30 ± 0.20^{Ca}	28.33 ± 0.90^{Ba}	31.73 ± 0.06^{Aa}
0.3	56.17 ± 0.67^{Ab}	16.68 ± 0.13^{Bab}	14.50 ± 0.34^{Cb}	24.17 ± 0.46^{Cb}	25.87 ± 0.55^{Bb}	27.60 ± 0.72^{Ab}
0.4	46.74 ± 1.45^{Ac}	16.56 ± 0.11^{Bb}	14.38 ± 0.41^{Cc}	22.80 ± 0.36^{Cc}	26.27 ± 0.67^{Ab}	25.30 ± 0.70^{Bc}
0.5	38.97 ± 0.46^{Ad}	15.41 ± 0.81^{Bc}	13.30 ± 0.23^{Cd}	21.10 ± 0.70^{Cc}	22.93 ± 1.03^{Bc}	23.07 ± 0.11^{Ad}

Note: Different capital letters in the same row indicate significant differences between different HIU times for the same NaCl concentration ($P < 0.05$); Different lowercase letters in the same column indicate significant differences between different NaCl concentrations for the same HIU time ($P < 0.05$).

increased ($P < 0.05$). When the NaCl content increased from 0.2 to 0.5 mol/L, the $D_{4,3}$ of untreated MP-stabilized emulsions was reduced significantly ($P < 0.05$) from 67.18 to 38.97 μm (a decrease of approximately 41.99%). The $D_{4,3}$ of emulsions of HIU treatment MP was significantly decreased ($P < 0.05$) at all four NaCl levels. In comparison to the non-HIU group, HIU for 9 min reduced $D_{4,3}$ of the emulsions by over 65%. In addition, the $D_{4,3}$ of reduced-salt HIU MP emulsions is much lower than that of normal salt/high salt emulsions without HIU treatment. These data reveal the effectiveness of HIU treatment MP to displace NaCl to some extent and promote the generation of small droplets of MP emulsion, which is in agreement with the CLSM observations.

Zeta-potential represented the near surface charge or surface charge of a suspended particle. The electrostatic repulsion is determined by the charge on the surface of the emulsion[42]. Increasing NaCl concentration enhanced the electrostatic repulsion between oil droplets, which generally improved emulsion stability. The emulsions exhibited reduced negative charges with increasing concentration of NaCl (Table 1). This was probably attributed to the reduced thickness of the electrical double layer, caused by an increase in the ionic strength of the medium[40]. The absolute zeta-potential of HIU treatment MP stabilized emulsions were significantly higher ($P < 0.05$) compared to the untreated samples (Table 1). MP aggregates were disrupted and exposed more charged groups, which was induced by the cavitation of HIU[23,26]. The MP are more inclined to adsorb to the oil-water interface and lead to stronger mutual repulsion between the protein membranes surrounding the oil droplets. These results were consistent with EAI and ESI.

3.5 Rheological property

Figure 5 shows the apparent viscosity of untreated and HIU treatment MP stabilized emulsions at different NaCl concentrations. With the increase of shear frequency, the viscosity

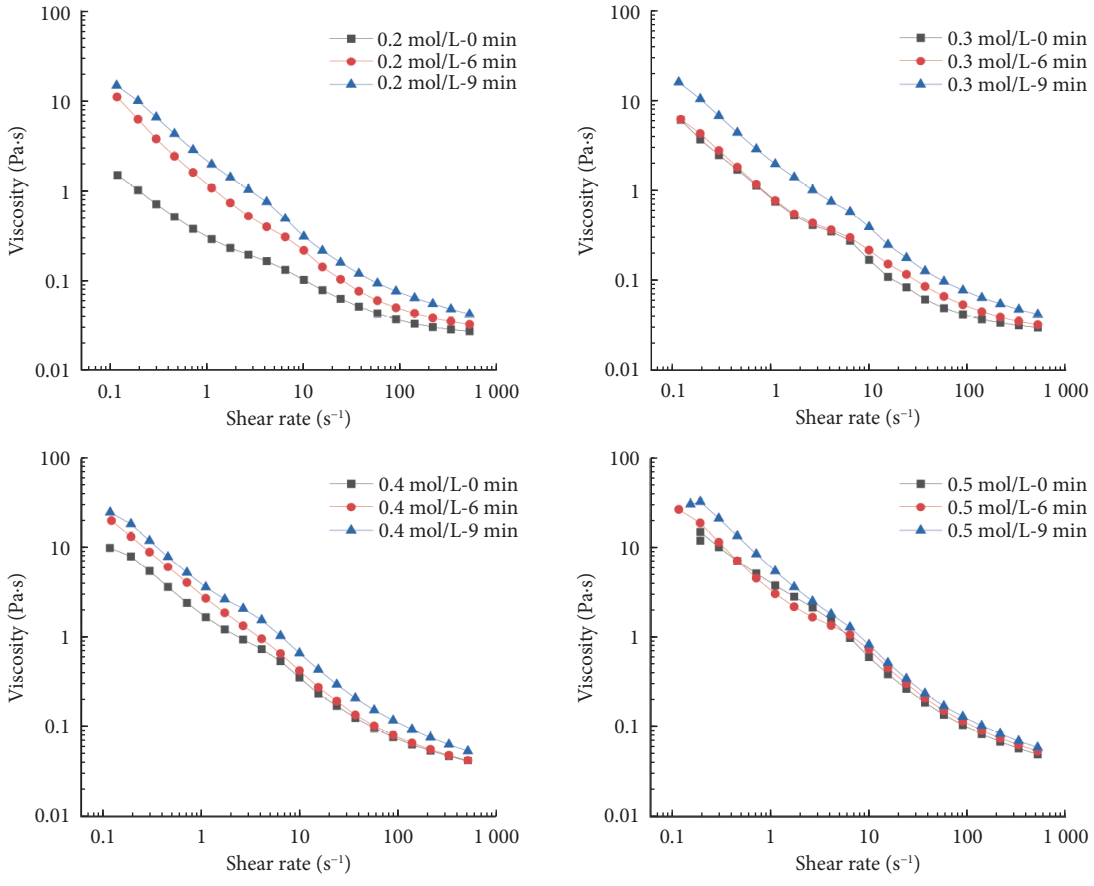

Figure 5 The apparent viscosity of HIU-treated (0, 6, and 9 min) MP stabilized emulsions under different NaCl concentrations (0.2, 0.3, 0.4, and 0.5 mol/L).

of all samples decreased continuously, showing shear thinning behavior[43]. In addition, under the condition of the same NaCl concentration, the viscosity of MP emulsion increased with the increase of ultrasonic time. Under the condition of the same ultrasonic time, as the concentration of NaCl increased, the apparent viscosity of the emulsion increased accordingly. However, there was no significant difference between the apparent viscosity of MP emulsion non-HIU treatment and MP stabilized emulsions for 6 min under the condition of 0.3 mol/L NaCl. There was no significant difference in apparent viscosity between the emulsions of HIU treatment MP (6 and 9 min) and non-HIU treatment under 0.5 mol/L NaCl. However, it can still be shown that both HIU treatment and the addition of NaCl make MP emulsions have higher apparent viscosity. The result may be related to the reduction of particle size of MP. HIU treatment MP formed smaller emulsion droplet size, and the decreased droplets will reduce the spacing between droplets, resulting in increased contact area, enhanced hydrodynamic interaction and improved viscosity[44]. In addition, higher ionic strength will promote the solubility of MP, resulting in more MP adsorbed on the oil-water interface, thus improving the viscosity of the emulsion[45]. Tang et al.[46] also confirmed in their study that ionic strength can enhance protein interaction in continuous phase and improve the apparent viscosity of MP emulsions.

3.6 Interfacial tension

The oil-water interfacial tension between HIU treatment MP and soybean oil was studied with the 0.3 and 0.5 mol/L NaCl. Figure 6 shows that the interfacial tension of all systems changed continually as a function of time. The MP emulsions have an initial rapid decrease in interfacial tension, followed by a leveling off, which may be related to the different stages of protein adsorption[47]. On the whole, ionic strength and HIU time had great influence on the interfacial tension of MP. On the one hand, in the absence of HIU treatment MP stabilized emulsions, the interfacial tension of samples with 0.3 mol/L NaCl was higher than that of samples with 0.5 mol/L NaCl, which was consistent with the result of EAI. On the other hand, the interfacial tension of all systems decreased with time, and it is noteworthy that the interfacial tension of HIU treatment MP stabilized emulsions were significantly lower than that of the untreated MP emulsions, regardless of whether the emulsions were treated with 0.3 or 0.5 mol/L NaCl. These results proved that HIU treatment MP had a stronger interfacial adsorption capacity than that of the native MP. Furthermore, the reduction of MP-oil interfacial tension induced by HIU was better at low ionic strength (0.3 mol/L NaCl), compared to 0.5 mol/L NaCl. O'sullivan et al.[36] had reported the HIU treatment pea protein isolate (PPI) aggregates were more significant than untreated PPI in terms of both size and hydrophobicity, resulting in a significant decrease in initial interfacial tension. A similar situation occurred with bovine gelatin, PPI and ovalbumin[48]. The faster adsorption rate of proteins which had smaller particle size and greater hydrophobicity will be to the oil-water interface, the more the maximum adsorption capacity of monolayer near saturation at the oil-water interface is, so the oil-water interface

tension can be reduced to a greater extent[36]. After HIU treatment, MP exhibited increased surface hydrophobicity and reduced particle size[12]. Comparable findings were reported by Zou et al.[26] and Amiri et al.[49].

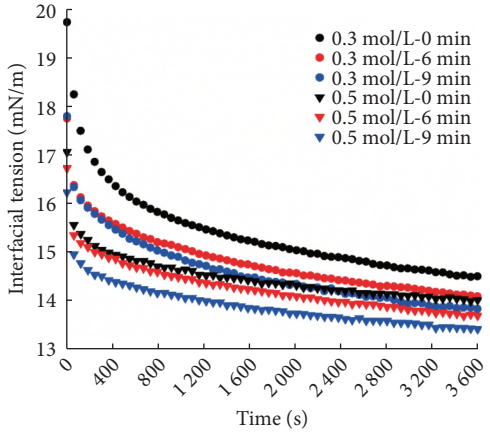

Figure 6 The interfacial tension of HIU-treated (0, 6, and 9 min) MP stabilized emulsions under different NaCl concentrations (0.2, 0.3, 0.4, and 0.5 mol/L).

3.7 SDS-PAGE

As shown in Figure 7, the mainly adsorbed protein components were myosin heavy chain and actin. When the NaCl content increased from 0.3 to 0.5 mol/L, the adsorbed content of myosin and actin increased significantly. Significantly higher adsorbed protein content (higher intensity of spectral bands) was observed in the HIU treatment group compared with untreated MP, which suggested that the use of HIU enhance the absorption of MP on the surface of oil droplets. Furthermore, the emulsion adsorbed myosin and actin content of MP treated with HIU for 9 min at 0.3 mol/L NaCl was higher than that of the untreated group at 0.5 mol/L NaCl. Myosin or actin are the main protein that constitutes the interfacial protein membrane or adsorbed protein surrounding oil droplets[50]. Under higher salt conditions, actin has a weaker adsorption capacity to emulsion droplets compared to myosin. Since actin exists as a monomeric globular molecule under reduced NaCl content conditions, its solubility in water and dilute salt solutions is better[51], which is more conducive to adsorption to the oil-water interface to participate in interfacial protein film formation, in contrast, at reduced NaCl content (< 0.3 mol/L), myosin monomers can be assembled by regular stacking and extension through electrostatic interactions in their tails to form high molecular weight myosin thick filament structures with specific structures, leading to reduced solubility of myosin, which was unfavorable for myosin mass adsorption at the oil-water interface[12,52–53]. The variation in the solubility of these two proteins resulted in differences in their participation in the adsorption of the emulsion layer at different NaCl concentrations. After the MP treated with HIU, HIU treatment generated enormous shear stress and turbulence through the cavitation effect that may disrupt the structure of the myosin thick filaments and modify actin, leading to the solubilization of more myosin[40,54], which enhanced the emulsification capacity of myosin and actin. Therefore, HIU can effectively enhance the adsorption capacity of MP at the oil-water interface under reduced-salt concentration.

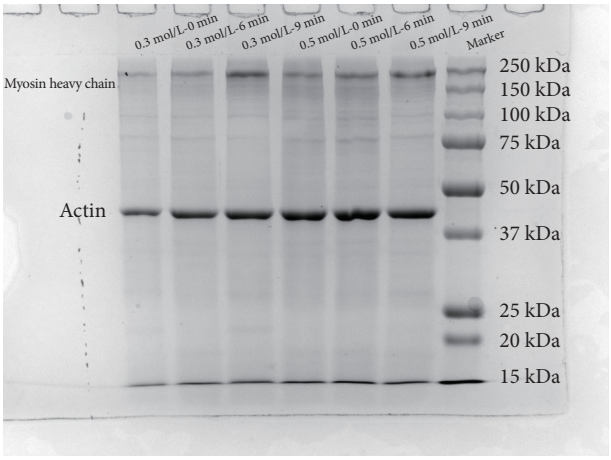

Figure 7 The SDS-PAGE of HIU-treated (0, 6, and 9 min) MP stabilized emulsions under different NaCl concentrations (0.3 and 0.5 mol/L).

3.8 Secondary structure of emulsion interface protein

At 0.3 and 0.5 mol/L NaCl concentrations, the CD spectra of different HIU treatment MP which absorbed at the oil-water interface was shown in Figure 8, in the mass, all samples showed the negative peaks near 208 and 222 nm, indicating that the α-helix structure dominates the conformation, and the degree of negative peaks at these two wavelengths was significantly enhanced by ultrasonic treatment. Besides, there was also a significant change of the CD spectrum in the β-sheet region near 192 nm, which was a positive rise near 192 nm.

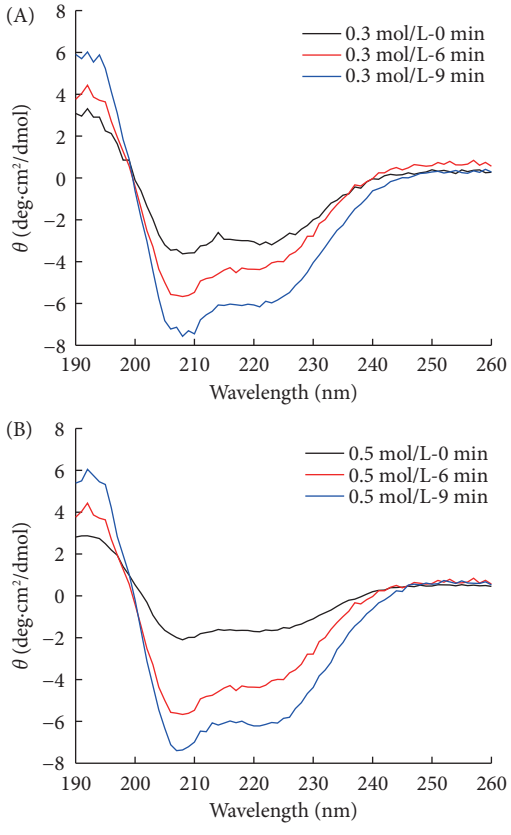

Figure 8 The CD spectrogram of emulsions interfacial adsorbed protein of HIU-treated (0, 6, and 9 min) MP under different NaCl concentrations. (A) 0.3 mol/L; (B) 0.5 mol/L.

Table 2 The relative content of secondary structures of interfacial protein of emulsions stabilized by the HIU-treated (0, 6, and 9 min) MP under different NaCl concentrations (0.3 and 0.5 mol/L).

NaCl concentration (mol/L)	HIU time (min)	Relative content (%)			
		α-Helix	β-Sheet	β-Turn	Random coil
0.3	0	23.04 ± 0.48^{e}	23.47 ± 0.31^{d}	16.94 ± 0.38^{ab}	37.27 ± 0.19^{b}
	6	23.99 ± 0.44^{d}	25.18 ± 0.53^{c}	17.07 ± 0.69^{a}	33.86 ± 0.31^{c}
	9	25.03 ± 0.13^{b}	27.19 ± 0.11^{a}	17.06 ± 0.71^{a}	30.71 ± 0.26^{e}
0.5	0	21.66 ± 0.04^{f}	22.36 ± 0.43^{e}	16.59 ± 0.43^{b}	39.39 ± 0.18^{a}
	6	24.39 ± 0.12^{c}	26.63 ± 0.24^{b}	17.07 ± 0.33^{a}	33.06 ± 0.22^{d}
	9	26.01 ± 0.18^{a}	27.31 ± 0.38^{a}	17.13 ± 0.21^{a}	29.45 ± 0.14^{f}

Note: Different lowercase letters in the same column indicate significant differences ($P < 0.05$).

In order to study this change in depth, we calculated and quantitatively estimated the relative content of each secondary structure of MP, as shown in Table 2. First, salt level had a significant effect on secondary structure. As salt concentration of MP were increased, the α-helix and β-sheet were reduced. Because native MP cannot be fully dissolved to form large protein polymers at lower NaCl content (0.3 mol/L NaCl). These polymers adsorbed between the oil-water interface and still maintain a highly ordered structure. Second, according to the results for α-helix and β-sheet at identical salt concentrations, HIU treatment MP for 6 and 9 min showed a significant improvement ($P < 0.05$) compared to the control group. Not only that, the relative content of α-helix and β-sheet were higher in the samples treated by HIU treatment for 9 min at the 0.3 mol/L NaCl compared to the samples treated by HIU for 6 min at the 0.5 mol/L NaCl. Finally, compared with the non-HIU treated MP, the random coil of MP treated by HIU was significantly ($P < 0.05$) reduced at 0.3 and 0.5 mol/L NaCl levels. The reduced relative content of random coil might be conversion to the structure of α-helix and β-sheet. These changes might be the reason of the increased of α-helix and β-sheet. This is consistent with the findings reported by Li et al.[55], who found that the physical stability of soybean oil-myosin emulsions and the α-helix and β-sheet relative content of myosin at soybean oil-water were improved by *L*-arginine and *L*-lysine. Zhang et al.[56] reported that the dramatic increase of the majority of β-sheet in protein aggregates implied that interfacial MP interact with each other to form hard gel-like interfacial membranes. Therefore, the ordered structure (α-helix and β-sheet) of interfacial proteins is critical to emulsion stability. These organized structures in the protein are more conducive to the formation of a dense and rigid protein film. Furthermore, the increased in α-helix structures established better interactions with the oil phase. Interfacial protein-protein interactions were primarily β-sheet structures[57]. MP was decomposed into smaller protein aggregates and dissociated into more myosin monomers through cavitation by HIU. During emulsification, these myosin monomers could be adsorbed on the oil-water interface more quickly, forming an ordered interfacial protein film, enhanced protein-oil and protein-protein interactions at the oil-water interface, and reduced oil-water interfacial tension. These results are consistent with those of interfacial tension and interfacial protein adsorption.

4 Conclusion

Under reduced salt conditions, MP emulsions had larger particle size and poorer emulsification stability. With increasing salt content, the emulsification stability of MP increased, emulsion particle size was decreased and storage stability were improved. Compared with the non-HIU MP under normal and high salt conditions, HIU inhibited the self-assembly of MPs under reduced-salt conditions and increased the solubility and zeta-potential, enhanced the emulsification stability and storage stability, reduced the particle size of MP emulsion, promoted the adsorption of MPs at the oil-water interface, and changed the interfacial emulsification layer from a disordered to an ordered structure. These structural changes facilitated the formation of a dense protein film at the oil-water interface of MP, which enhanced the emulsification ability of MP under reduced-salt conditions. This study demonstrated that HIU treatment could be an effective substitute for NaCl addition to improve the stability of MP emulsions under reduced-salt conditions, providing a theoretical basis for further developing the reduced-salt emulsified meat products.

Conflict of interest

The authors have no conflict of interest to declare.

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Authors contribution

Linmeng Wang: Investigation, data curation, writing-original draft, writing-review editing, formal analysis. **Yanfang Zhou:** Data curation, formal analysis, writing-original draft, visualization. **Qing Yang:** Investigation, visualization. **Lei Fu:** Investigation, methodology, data curation, writing-original draft, visualization. **Ke Li:** Conceptualization, methodology, validation, writing-original draft, writing-review editing, funding acquisition. **Yanhong Bai:** Conceptualization, supervision.

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