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# Potential of high pressure homogenization to solubilize chicken breast myofibrillar proteins in water

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ABSTRACT

Myofibrillar proteins (MPs) of chicken breast were generally insoluble in water. The potential of high-pressure homogenization (HPH) to solubilize chicken breast MPs in water was tested. The effects of 0 psi (0.1 MPa), 10,000 psi (69 MPa), 15,000 psi (103 MPa) and 20,000 psi (138 MPa) for two passes HPH on solubility, protein profile, particle property, flow property and microstructure of MPs in water were investigated. HPH at 15,000 psi (103 MPa) could induce the suspension of MPs with small particle size species (sub-filament, oligomers or monomer structure) and high absolute zeta potential, thus enhancing the solubility, flow ability and stability without individual protein degradation. Reduction of particle size and strengthening of intermolecular electrostatic repulsion appeared to be the main reasons in solubilizing MPs in water treated with HPH. *Industrial relevance:* The qualitative characteristics of meat products are closely related to the solubility of meat proteins. Myofibrillar proteins (MPs), as major part of total muscle proteins, are generally considered to be insoluble in water. The results showed that high-pressure homogenization has potential application for solubilizing MPs in water to develop new meat-based products in the food industry.



## 1. Introduction

Meat is a nutrient-rich food that plays an important role as a supplementary protein source for humans. Compared with that of beans and whole wheat, meat protein distinguishes itself for its completeness in all the essential amino acids with no limiting amino acids along with high digestibility (Pereira & Vicente, 2013). However, meat has not been fully utilized to the same extent that milk or soybean products has been utilized due to the low solubility of myofibrillar proteins (MPs) that comprise approximately 50% of meat proteins in a low ionic strength solution or water. Also, meat proteins require a relatively high concentration of salt (>0.3 M NaCl or KCl) to solubilize them (Krishnamurthy et al., 1996). And meat products can possess a firm texture that makes it difficult for those with mastication and swallowing impairments to consume. If MPs could be solubilized in water or low-salt solutions, the study of myofibrillar proteins might be advanced to promote the innovation of meat products. For example, meat could be developed as a liquid diet for an Oral Nutritional Supplements (ONS) to elderly people and dysphagic patients with malnutrition (Nieuwenhuizen, Weenen, Rigby, & Hetherington, 2010; Tokifuji, Matsushima, Hachisuka, & Yoshioka, 2013).

Several studies have been conducted on the solubility of MPs in water. Essentially all of cod myofibrillar proteins were shown to become soluble when minced cod muscle was washed three times in water and then extracted with a sufficient volume of water (1/50:weight/volume) to reduce the ionic strength below 0.3 mM (Stefansson & Hultin, 1994). However, when chicken breast muscle was homogenized and washed in water, no solubilization of the myofibrillar proteins occurred even after lowering the ionic strength to values well below that achieved with fish muscle (Hultin, Feng, & Stanley, 1995). Washing with a NaCl solution (25 mM–150 mM) buffered with L-histidine (pH 7.0–7.5) and subsequently ultrasonication of swelling myofibrillar were necessary to accomplish the solubilization of chicken breast MPs in water (Ito, Tatsumi, Wakamatsu, Nishimura, & Hattori, 2003; Krishnamurthy et al., 1996). It was indicated that certain proteins were removed preferentially by (25 mM–150 mM) NaCl solution that had been adjusted to neutral pH to render the proteins of the final washed sediment soluble in water by ultrasonication (Ito et al., 2003; Krishnamurthy et al., 1996). This would inhibit the formation of myosin filaments contributing to the solubilization of myosin in a low ionic strength solutions (Chen et al., 2016; Hayakawa, Ito, Wakamatsu, Nishimura, & Hattori, 2009, 2010). The ultrasonication is essential for disruption of the highly-ordered structure of the myofibrils and solubilization. However, this procedure is not feasible for food production. The limitations are attributed to difficulties in controlling the relatively low ionic strength and neutral pH (Saeki & Inoue, 1997), complicated procedures (25 mM–150 mM NaCl solutions step washing) and uneven treatment by ultrasonication. A better procedure for dissolving chicken breast myofibrillar proteins in water is needed.

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High pressure homogenization (HPH) is a non-thermal processing technology capable of producing interesting functional properties to foods (Zamora & Guamis, 2015). It is a particular form of technique utilizing high pressures to make liquid materials by combining actions such as intense shear, impact force and cavitation passing through the homogenization valve (depends on the design and characteristics of each machine), resulting in the modification of biomacromolecules such as plant and milk proteins (Song, Zhou, Fu, Chen, & Wu, 2013; Sørensen et al., 2014), dietary fiber(Lin, Li, Lopez-Sanchez, & Li, 2015) and endogenous enzymes (Liu, Zhang, Liu, Xie, Tu, Liu et al., 2010). As a novel technology, HPH has been studied in food, cosmetic and pharmaceutical areas to fragment particles in dispersions or emulsions, to produce fine, stable or nano emulsions(Donsì, Annunziata, Sessa, & Ferrari, 2011; Kapoor, Pathak, Najmi, Aeri, & Panda, 2014; Qian & McClements, 2011), to modify the functional properties of fluids due to the particle size reduction(Leite, Augusto, & Cristianini, 2014; Van Buggenhout et al., 2015), to facilitate extraction of functional ingredients as well as to achieve inactivation of microorganisms, enzymes or even some viruses due to its ability to mechanically disrupt microbial cells (Donsì, Annunziata, & Ferrari, 2013; Dumay et al., 2013; Jing et al., 2016; Toro-Funes, Bosch-Fusté, Veciana-Nogués, & Vidal-Carou, 2014). When the processed fluid is forced through the very narrow HP-valve gap, particles or macromolecules can be ruptured by the mechanical associated forces (high pressure plus shearing effects) inducing a significant reduction of size down to the micron/submicron range. Comparing HPH with other homogenization processes, e.g., laminar or turbulent rotor-stator systems, jet-dispersers, membrane or ultrasonic systems, HPH delivers the highest potential energy of emulsification able to create the smallest size of particles below the micron level (Dumay et al., 2013). In addition, this technology is an effective continuous process which could be easily scaled-up. With the development of this technology, huge progress was made with the change of scale from the HPH devices at laboratory scale to the design, assembly, and testing of a full pilot plant-scale prototype in food industry (Zamora & Guamis, 2015). Therefore, HPH is a sustainable process that has potential application in a large number of liquid foods to produce innovative foods while retaining nutrients and using safe, quick, economical, and environmental friendly processes.

In the present study, we assumed that HPH might be a good alternative to accelerate the solubilization of chicken breast MPs in water due to the particle size reduction and physical modification effect. Therefore, the objective of the study was to test the potential of HPH on solubilizing chicken breast MPs in water without NaCl solution washing, ionic strength and pH adjustment steps. The effects of HPH pressures on solubility, protein profile, particle size distribution (PSD), zeta-potential, flow property and microstructure of MPs in water were investigated. To our knowledge, HPH has been poorly considered in meat products processing. Attempts addressed toward the solubilization of MPs in water by HPH would open up a promising area of research for development of a wide range of meat products and extend the application area of HPH technology in food industry.

## 2. Materials and methods

### 2.1. Materials

The frozen chicken breast used during this research was purchased from a local market (Sushi Food Co., Ltd., Nanjing, China).

### 2.2. Preparation of chicken breast myofibrils

The frozen chicken breast was thawed for about 12 h at 4 °C, washed chicken breast myofibrils were prepared by using a modified procedure according to previous reports (Krishnamurthy et al., 1996; Stefansson & Hultin, 1994). After removing connective and adipose tissues, muscles were ground three times in a chilled cutter (Grindomix GM 200, Retsch, Germany) for 10 s at the speed of 3000 rpm. The minced meat (100 g) was washed four times with cold (4 °C) deionized, distilled water. In each washing step the mince and water (1:10 W/V) were allowed to sit for 10 min after an initial homogenization (Ultraturrax T25, IKA, Staufen, Germany) of 2 min, and the sediment of each step was recovered by centrifugation at 18,000 *g* for 20 min at 4 °C. In the third washing step, the suspension before centrifugation was filtered through three layers of gauze to remove the connective tissue and lipid. After the final step washing and centrifugation, the collected sediment was termed washed myofibrils. The pHs of each washing step were 6.19 ± 0.06, 6.48 ± 0.08, 6.87 ± 0.04 and 6.94 ± 0.03, respectively. All procedures were carried out at 4 °C.

### 2.3. Preparation of MP dispersions in water by HPH

The stock myofibril suspensions were prepared by homogenizing washed myofibrils in cold (4 °C) deionized, distilled water at 8000 rpm for 2 min with an Ultraturrax (T25, IKA, Staufen, Germany) and adjusted to a concentration of 5 mg/mL (based on preliminary experiment) for further analysis and treatment.

HPH was carried out by using a high pressure homogenizer (Mini DeBee, Bee International, USA) equipped with a single pressure intensifier and a 75-μm opening Y-type diamond nozzle (Genizer™, Los Angeles, USA) in a modular homogenization cell. The geometry and schematic of this homogenizing cell were described in details elsewhere (Donsì, Sessa & Ferrari, 2011; Donsì et al., 2013). Myofibril dispersions (300 mL) with an inlet temperature of 4 °C were passed through the interaction cell at a constant pressure, 10,000 psi (69 MPa), 15,000 psi (103 MPa) and 20,000 psi (138 MPa) for two passes. For maintaining the outlet temperature blow 20 °C, a rapid cooling system was implemented in the homogenizer, by connecting a heat exchanger immediately downstream of the chamber. The obtained samples were immediately stored at 4 °C prior to further analysis. The non-pressurized MP dispersion was used as the control.

### 2.4. Solubility

The treated samples were centrifuged at 20,000 *g* for 20 min (Takai, Yoshizawa, Ejima, Arakawa, & Shiraki, 2013) at 4 °C (Beckman Coulter model Avanti J-26SXP, Beckman Instruments Inc., USA), and the obtained supernatant was defined as MPs solubilized in water. The solubility was expressed as percent of protein concentration in the supernatant with respect to that of MP suspension before centrifugation.

### 2.5. Protein profile

The protein profiles of HPH treated suspensions and soluble muscle proteins were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by previous study (Khiari, Pietrasik, Gaudette, & Betti, 2014). Ten μL of each sample (25 mg of proteins) and markers were loaded on a precast 4–20% ready gel (Bio-Rad Laboratories Inc., Hercules, CA, USA). The electrophoretic analysis was performed on a Bio-Rad Mini-PROTEAN II System Cell apparatus (Bio-Rad Laboratories Inc., Hercules, CA, USA) at a constant voltage of 120 V for 1 h. The stained gel was scanned by using Imager Scanner III (EU-88, Epson, Japan) and the densities of bands were analyzed by Quantity One software (Bio-Rad, Laboratories Inc., Benicia, CA, USA).

### 2.6. pH and conductivity measurements

pH was determined with a glass electrode and pH meter (Mettler-Toledo International Inc.) The ionic strength of protein solutions was estimated with a conductivity meter (ORION 3 STAR™, Thermal Scientific Inc.) as previous reported (Krishnamurthy et al., 1996). A standard

curve of the conductivity versus salt concentration was obtained over the range 0–2 mM NaCl.

### 2.7. Particle properties of myosin suspensions

#### 2.7.1. Dynamic light scattering (DLS) measurement of particle size distribution (PSD)

DLS measurement was performed according to Chen et al. (2016) with a slight modification by using Zetasizer Nano ZS 90 (Malvern Instruments, Worcestershire, UK) equipped with a 4 mW He-Neion laser ($\lambda = 633$ nm). The MP suspensions (0.5 mg/mL) in water treated by 0 psi (0.1 MPa), 10,000 psi (69 MPa), 15,000 psi (103 MPa) and 20,000 psi (138 MPa) HPH, respectively were placed in a 1-cm path-length quartz cuvette and subjected to DLS measurement with a detection angle of 90° at 25 ± 0.1 °C. Hydrodynamic diameters of MP particles were estimated from the auto-correlation function, using the Cumulants method (Chen et al., 2016), based on a single exponential fit of the auto-correlation function to obtain the mean particle size (*Z*-average diameter). The scattering particle size distributions based on the scattering intensity was monitored. The polydispersity index (PDI) value was determined as a measurement of the breadth of the size distribution.

#### 2.7.2. Measurement of zeta potential

The zeta potential was measured as reported previously (Chen et al., 2016) by laser Doppler electrophoresis using a Zetasizer Nano ZS 90 (Malvern Instrument Ltd., Malvern, Worcestershire, U.K.) equipped with a 4 mW He-Neion laser with an output of 633 nm. The zeta potential was calculated from the electrophoretic mobility using the Smoulokowski model.

### 2.8. Rheological measurements

Flow curves of samples were obtained using a rheometer (Physica MCR301, Anton Paar Corporation, Austria) fitted with parallel plate geometry (50-mm diameter) (Zhao et al., 2014). The measurements were carried out using a gap distance of 0.5 mm. The samples were equilibrated in parallel plates for 30 s prior to measurements to obtain a desirable temperature of 25 °C. Viscosity was then recorded as the shear rate that linearly increased from 1 $s^{-1}$ to 1000 $s^{-1}$. The experimental data were fitted by a power law constitutive equation: $\eta = K(\gamma)^{n-1}$, where $\eta$ is the apparent viscosity (Pa s); K is the consistency coefficient (Pa $s^n$); g is shear rate ($s^{-1}$); n is the flow behavior index (Liu, Wang, Li, & Zhang, 2015).

### 2.9. Atomic force microscopy (AFM) imaging and analysis

AFM images scanned in tapping mode were obtained according to previous report (Zhong et al., 2015) by using a PeakForce Tapping technology AFM (Dimension Icon, Bruker Corporation) equipped with a $Si_3N_4$ cantilevered scanner (SCANASYST-AIR Mode; resonant frequency, 70 kHz; spring constant, 0.4 N/m) under atmospheric pressure at room temperature (25 °C). The linear scanning rate was optimized at 1 Hz with scan resolution of 512 samples per line. The samples were continuously diluted into 0.05 mg/mL with ultrapure deionized water, and 5 μL MP aqueous solution was cast on freshly cleaved mica and was allowed to dry in ambient air for 20 min, then subjected to AFM analysis. All height images were treated with "flatten" function using Nanoscope Analysis software (version 1.40, Bruker Corporation) prior to analysis. The height differences and three-dimensional structures were analyzed using the "section" function and "3D image" function, respectively in the Nanoscope Analysis software.

### 2.10. Statistical analysis

All data given were mean ± SD (standard deviation) values of three or four independent experiments. The analyses of variances, means and SDs were analyzed with the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA). A $P < 0.05$ significance level was used to determine the differences between the treatments.

## 3. Results and discussion

### 3.1. Effects of HPH pressures on solubility of MPs in water

Without HPH treatment in Fig. 1A, it was expected that the solubility of MPs in water was rather low (2%). It was consistent with literature, highlighting the insolubility of MPs in water in the absence of unique processing procedures (Ito et al., 2003). However, it is interesting to observe that HPH (10,000–20,000 psi) treatment could significantly increase ($P < 0.05$) the solubility up to 60% (Fig. 1A). As pressure increases from 0 psi to 15,000 psi, a progressive increase ($P < 0.05$) of solubility (to 69%) was promoted by HPH treatment. Although MPs treated by washing with NaCl solutions buffered with L-histidine (pH 7.5) and subsequently ultrasonication presented a solubility of 80% in water (Ito et al., 2003), a high solubility (69%) and enhanced stability can also be achieved by HPH without NaCl solution washing, ionic strength and pH adjustment steps (Fig. 1). Due to the application of very high forces, HPH of higher pressure (20,000 psi) might cause an "over-processing" effect (Alvarez-Sabatel, de Marañón, & Arboleya, 2015) as a weakened

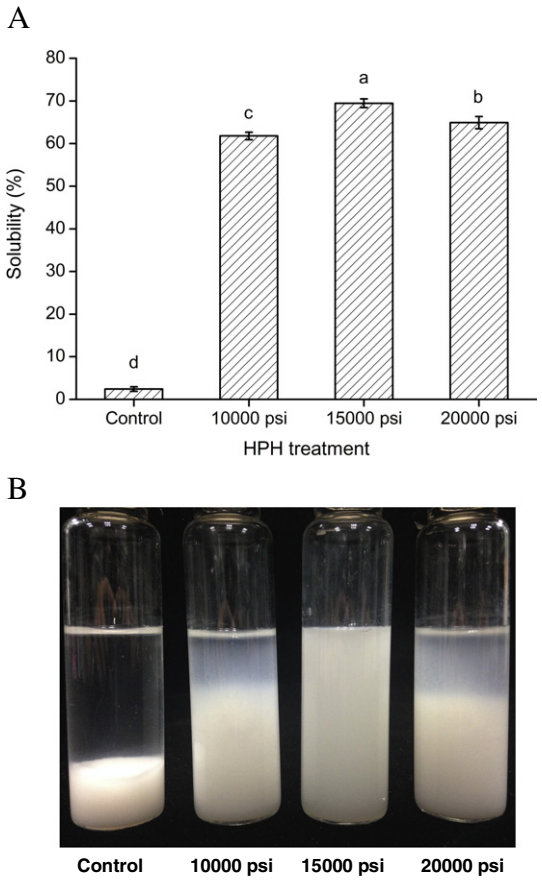


**Fig. 1.** (A) Effects of HPH pressures on the solubility of MPs in water (5 mg/mL). Values are means ± SD (n = 3), a–d indicates that the different letters are significantly different ($P < 0.05$). (B) Photographic representation of MP dispersions in water processed by different HPH pressures treatments after 14 days storage at 4 °C. HPH pressures are 0 psi (0.1 MPa), 10,000 psi (69 MPa), 15,000 psi (103 MPa) and 20,000 psi (138 MPa), respectively.

solubilization effect at 20,000 psi HPH was found in Fig. 1A. Fig. 1B showed the visual aspect of MPs in water treated by various HPH pressures after 14 days storage at 4 °C. MPs in the non HPH-treated samples (control) migrated to the bottom and two phases, a white sediment and a clear aqueous phase, were observed. The protein migration confirmed the phenomenon of sedimentation, it occurred due to density difference between the particles and the medium (Alvarez-Sabatel et al., 2015). On the contrary, the sample under 15,000 psi HPH treatment exhibited a homogeneous white structure along the entire sample (Fig. 1B), suggesting that HPH could significantly improve sample homogeneity. Also at the pressure levels of 10,000 psi and 20,000 psi, the samples presented a small layer of non-retained water. A similar phenomenon was presented for inulin aqueous dispersion after HPH treatment (Alvarez-Sabatel et al., 2015). It was reported that HPH could reduce sedimentation of fruit juices during storage (Leite et al., 2014) and the heated whey proteins aqueous solutions (Iordache & Jelen, 2003). These facts indicated that HPH was capable of increasing the solubility of chicken breast MPs in water and improving its homogeneity and stability during storage. It was noted that 15,000 psi for two passes was the optimal HPH condition in solubilization of chicken breast MPs in water.

The solubility of MPs was relative to various factors such as pH (Omana, Xu, Moayedi, & Betti, 2010), ionic strength (Hultin et al., 1995), protein structure (Hayakawa et al., 2009) and protein–water/protein–protein interaction (Chen et al., 2016). Since pH and ionic strength adjustment were reported as essential to achieve high solubilization of the chicken breast muscle proteins in water (Krishnamurthy et al., 1996), our immediate interest was in testing the effects of HPH pressures on pH and ionic strength of MP aqueous suspensions. Results showed that no significant change was detected (pH 6.92–7.05 and ionic strength 1.3 mM) (data not shown). It seemed that pH and ionic strength were not significant factors that enhance solubility of MPs in water by HPH. HPH could provide intense mechanical forces which can possibly fragment highly-ordered macromolecules (Song et al., 2013; Wang, Li, Wang, Liu, & Adhikari, 2012) and improve the protein functionality (Leite et al., 2014), Thus it was speculated that physical force (intense shear, impact force and cavitation) applied by HPH might modify the protein structure and affect protein–protein/protein–water interaction, enabling solubilization of chicken breast MPs in water.

### 3.2. Effects of HPH pressures on protein profile

Possible protein hydrolysis during HPH poses a concern since it may negatively affect the functional properties of the processed proteins (Khiari et al., 2014). Thus, the polypeptide compositions of chicken breast muscle proteins and water-soluble MPs treated by different HPH pressures were examined by SDS–PAGE in Fig. 2. A typical polypeptide composition of MPs was observed in non-HPH treated sample in Lane 1 (Fig. 2): The bands corresponding to myosin heavy chain (MHC), actin, tropomyosin and myosin light chain (MLC) were consistent with previous report (Hayakawa et al., 2009). The supernatant of non-HPH treated sample obtained by centrifugation presented quite low concentration of protein (Lane 2 in Fig. 2), confirming the insolubility of MPs in water (Fig. 1 A). However, it was found that most of the proteins in HPH treated samples (Lane 3, 5 and 7 in Fig. 2) remained in the supernatant (Lanes 4, 6 and 8 in Fig. 2), and there was no difference in polypeptide composition between the non-treated sample and HPH-treated samples (Fig. 2), indicating that HPH could result in solubilization of MPs in water. The SDS-PAGE patterns of the MPs did not show any sign of hydrolysis during HPH process. This suggested that the solubilization of MPs in water by HPH was not caused by the fracture or shortening of any polypeptides in MPs. With HPH treatment, it was supposed that some highly-ordered structures of myofibrils might be disrupted or dissociated and almost all MPs could be recovered in the supernatant after centrifugation.

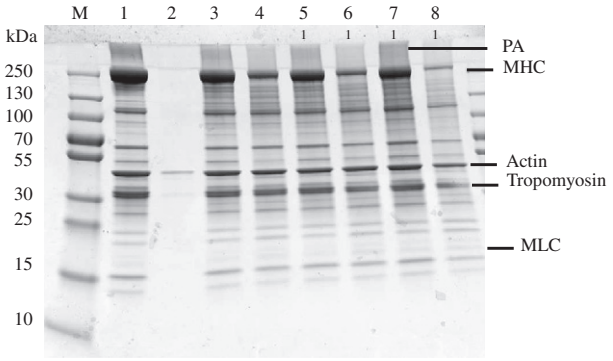


**Fig. 2.** SDS–PAGE pattern of chicken breast muscle proteins in water treated by different HPH pressures. Lane M: molecular weight markers, Lane 1: control sample, Lane 2: supernatant of control after centrifugation (20,000 *g* for 20 min at 4 °C), Lane 3: 10,000 psi (69 MPa) treated suspension, Lane 4: supernatant of 10,000 psi (69 MPa) treated suspension after centrifugation, Lane 5: 15,000 psi (103 MPa) treated suspension, Lane 6: supernatant of 15,000 psi (103 MPa) treated suspension after centrifugation, Lane 7: 20,000 psi (138 MPa) treated suspension, Lane 8: supernatant of 20,000 psi (138 MPa) treated suspension after centrifugation. PA: protein aggregation, MHC: myosin heavy chain, MLC: myosin light chain.

Notably, there appeared to be more protein aggregations (PA) in 20,000 psi (138 MPa) HPH-treated sample compared to that of 10,000–15,000 psi HPH. As reported, HPH shares some of the action mechanisms of high hydrostatic pressure (HHP) (Zamora & Guamis, 2015). Harsh pressures higher than 100 MPa (14,500 psi) can facilitate muscle protein aggregation (Chen et al., 2014). In addition, HPH can induce changes in protein structure and particles reassembling (Dumay et al., 2013). Probably due to protein aggregation, the solubility of MPs in water began to decrease when HPH pressure increase to 20,000 psi (Fig. 1A).

Therefore, it can be concluded HPH treatment could solubilize chicken breast MPs in water with no degradation to the individual proteins. High HPH pressure (20,000 psi) might give rise to protein aggregation and adversely affect the solubility of MPs in water.

### 3.3. Effects of HPH pressures on particle properties of MPs suspensions

#### 3.3.1. Particle size distribution (PSD)

The PSD, *Z*-average hydrodynamic diameter (*Z*-average), hydrodynamic diameter (nm) of the more intense peak ($D_H$) and polydispersity index (PDI) of MPs in water as a function of HPH pressures were investigated by DLS (Fig. 3A and Table 1). In the case of non-HPH sample, a relatively wide distribution with large *Z*-average (2066 nm) and $D_H$ (1849 nm) was observed (Fig. 3A and Table 1), reflecting the highly-ordered myofibril structures with various large particle size in water. After being processed by HPH, the PSD showed a substantial shift toward the left of the distribution with pressure increased to 15,000 psi and then a slightly shift toward the right as the pressure increased from 15,000 psi to 20,000 psi (Fig. 3A). Comparing with the non-HPH sample, the *Z*-average and $D_H$ of HPH-treated MPs decreased sharply to submicron (around 600 nm) or nanometer range (around 80 nm) ($P < 0.05$) (Fig. 3A and Table 1), suggesting that HPH could reduce the particle size of MPs in water and make the distribution more uniform as less polydisperse ($P < 0.05$) of these distributions was monitored (PDI significantly decreased to nearly 0.3) (Table 1). Particles or macromolecules can be ruptured by the mechanical associated forces of HPH inducing a significant reduction of size down to the micron/submicron range (Dumay et al., 2013). By an intense HPH, the high-amylose maize starch macromolecular chain and its cross-liking can be fragmented to a small size (Wang et al., 2012). For inulin water suspensions, HPH is capable of reducing the presence of large particles and the samples with the smaller mean particle sizes were obtained after processing at 103 MPa HPH (Alvarez-Sabatel et al., 2015). Soy protein

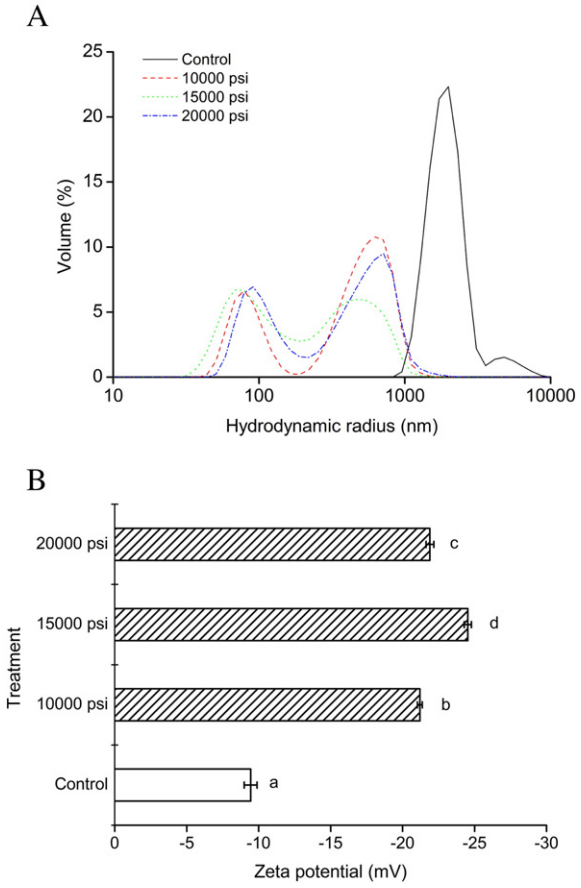


**Fig. 3.** Particle properties of chicken breast muscle protein aqueous suspensions: (A) particle size distribution (PSD) of suspensions treated by various HPH pressures; (B) zeta potential of samples treated by various HPH pressures, monitored by DLS at 25 °C. Values are means ± SD (n = 3), a–d in (B) indicates that the different letters are significantly different ($P < 0.05$). HPH pressures are 0 psi (0.1 MPa), 10,000 psi (69 MPa), 15,000 psi (103 MPa) and 20,000 psi (138 MPa), respectively.

isolate suspensions exhibited a considerable reduction in particle size after HPH treatment due to the disruption of colloidal particles (Song et al., 2013). Therefore, it was believed that HPH was an effective mechanical method of reducing MP particles size in water to submicron range, or even nanometer.

Both of the HPH-treated samples presented bimodal distribution with small particle size: one peak around 80 nm and another peak around 600 nm (Fig. 3A and Table 1). Although it was not possible to assign the protein structure based on DLS experiments, it was assumed that the first small contribution was ascribed to the monomer species or oligomers and the second one was probably due to the contribution of myofilament structures (Chen et al., 2016; Shimada, Takai, Ejima, Arakawa, & Shiraki, 2015). Muscle myofibril structure is mainly comprised of thin and thick myofilaments, the backbone of the thin filaments consists of the protein actin while the largest component of the thick filament is the protein myosin (Pearce, Rosenvold, Andersen, & Hopkins, 2011). It had been verified that no hydrolysis or fragmentation of individual proteins in MPs took place under HPH (Fig. 2), thus it was reasonable that the highly-ordered myofibril structure with large particle size might be destructed and undergo random disruption, depolymerization or dissociation by the applied mechanical forces when samples passed through the homogenizer under high pressure, causing the reduction of particle size. Some monomer species (myosin or actin) and myofilament (thick filament) might be released (as observed in Fig. 5), contributing to bimodal PSD. In the case of 10,000 psi (69 MPa) treated sample, filament or fragment structure might be dominant species in MP aqueous suspensions as it displayed a $D_H$ of 571 nm (Table 1). With HPH pressure increased to 15,000 psi (103 MPa), the $D_H$ significantly ($P < 0.05$) shifted to nanometer size (72 nm) while the *Z*-average decreased to 262 nm ($P < 0.05$) (Table 1), indicating that the contribution of monomer or oligomers population was more pronounced in 15,000 psi HPH treated sample. When HPH pressure increased to 20,000 psi (138 MPa), further reduction of the particle size did not occurred. On the contrary, *Z*-average and $D_H$ significantly increased ($P < 0.05$) to 324 nm and 610 nm, respectively comparing with that at 15,000 psi HPH (Table 1). This could be attributed to aggregation of disrupted protein particles as we discussed in Fig. 2. Upon high pressure (207 MPa and 296 MPa) of HPH treatment, it was highlighted that small inulin particles would reorganize into agglomerates, resulting in large particle size (Alvarez-Sabatel et al., 2015). Large aggregates were also formed when milk casein concentrate solution was subjected to a 300 MPa HPH treatment (Sørensen et al., 2014). Overall, HPH could break the macromolecular structure of myofibril to small particle size species in which the monomer and sub filament structures were obtained. 15,000 psi of HPH sufficiently modified the structure most.

**Table 1**
Hydrodynamic properties of MP suspensions in water under 0 psi (0.1 MPa), 10,000 psi (69 MPa), 15,000 psi (103 MPa) and 20,000 psi (138 MPa) HPH treatment, respectively, obtained from DLS data.

| Treatment | Hydrodynamic properties | | |
|---|---|---|---|
| | *Z*-average (nm)* | $D_H$ (nm)** | PDI*** |
| Control | 2066 ± 33 a | 1849 ± 341 a | 0.98 ± 0.03 a |
| 10,000 psi | 390 ± 1 b | 571 ± 57 b | 0.33 ± 0.03 c |
| 15,000 psi | 262 ± 5 d | 72 ± 6 c | 0.35 ± 0.05 c |
| 20,000 psi | 324 ± 3 c | 610 ± 54 b | 0.43 ± 0.01 b |

Note: values were mean of triplicate values ± S.D.
a–d different letters in the same column indicate significant differences at $P < 0.05$.
* *Z*-average hydrodynamic diameter obtained by DLS.
** Hydrodynamic diameter (nm) of the more intense peak shown in Fig. 3.
*** PDI is the polydispersity index.

The solubility of MPs in water may be especially related to PSD. Due to the decrease of the particle size, the increase of the particle specific area might increase the ability of water-particle interactions (Ronkart et al., 2010). An improved interaction between inulin and water molecules was produced by HPH treatment because of increased surfaces and inulin nuclei exposure (Alvarez-Sabatel et al., 2015). Moreover, when the nano−/submicron size particles were small enough for Brownian motion, it would greatly prevent gravitational separation phenomena during storage as we observed in Fig. 1B and reduce centrifuged aggregation (Dumay et al., 2013; Tadros, 2011). Hence, particle size reduction was probably responsible for the improved solubility of the HPH-processed MPs in water (Fig. 1A).

### 3.3.2. Zeta potential

Zeta potential is related to the charge residing on the surface or near-surface of a suspended particle, determining their dispersion and aggregation (Chen et al., 2016). In general, potential value is zero at the isoelectric point (pI), and it increases in magnitude when pH is moved away from the pI (Jia, You, Hu, Liu, & Xiong, 2015). Because of the pH of the samples (near 7.0) was higher than the pI of MPs (about 5.5), the zeta potential of MP suspensions in the present study were negative (Fig. 3B). The negative charge of proteins was governed by negatively charged amino acid residues, such as aspartic acid and glutamic acid, in MPs at neutral pH (Jia et al., 2015). Compared to the non-HPH sample, MP aqueous suspensions treated by HPH possessed significantly higher ($P < 0.05$) absolute zeta potential (around 25 mV in Fig. 3B). This was consistent with previous report which showed that the absolute zeta potentials of soy protein isolate suspensions were increased by HPH (Song et al., 2013). This behavior could be attributed to the exposing of more charges groups to the protein surface. When HPH was applied, myofibrils broke down to small size particles as aforementioned. It was reasonable that small treated protein particle led to a higher surface

area and more charges sites may be exposed on the surface of the suspended particles. As a result, the absolute zeta potentials of MP suspensions in water would be increased. Since most extensive disruption of myofibril structure thereby greatest particle size reduction was produced at 15,000 psi (Fig. 3A and Table 1), it was found that HPH at 15,000 psi endowed MP aqueous suspension with the highest absolute zeta potential (Fig. 3B).

It is well known that increasing the surface charge (increasing the absolute zeta potential) on colloidal particles could strengthen the inter-particle electrostatic repulsion and disrupt existing protein aggregates and discourage further aggregate formation (Song et al., 2013; Teng, Luo, & Wang, 2012; Yuan et al., 2014). With respect to the non-HPH sample, a low absolute zeta potential (about 10 mV) was presented, meaning that the electrostatic repulsion among protein particles was weak. This might easily promote formation of particle aggregates and preventing their dissociation (Li et al., 2007). Therefore, low solubility of MPs in water was obtained (Fig. 1A). By strong electrostatic repulsion, it was reported a physical stable nanosuspension solely stabilized would have minimum absolute zeta potential of 30 mV (Di Marzio, Marianecci, Petrone, Rinaldi, & Carafa, 2011). The high absolute zeta potential (around 25 mV) in HPH treated samples indicated very stable MP aqueous suspensions, thus it was expected that the solubility of HPH treated samples would be increased. The higher absolute zeta potential produced by HPH, the stronger electrostatic repulsion among protein particles and thereby the more enhancement solubility of MPs in water can be rendered.

The results on particle properties of MP suspensions suggested myofibril would undergo disruption and depolymerization during HPH treatment. HPH could induce MP aqueous suspensions with small particle size species (likely monomer or myofilament structure) and high absolute zeta potential, facilitating solubilization of MPs in water.

### 3.4. Effects of HPH pressures on rheological property of MP suspensions in water

Flow behavior by measuring the evolution of the viscosity as a function of shear rate is useful in determining ingredient functionality in product development and providing information about the strength of protein–protein interaction (Fernández-Ávila, Escriu, & Trujillo, 2015; Lin et al., 2015). Depending on HPH treatment, MP aqueous suspensions behaved either as exhibited shear thinning or Newtonian fluids (Fig. 4).

It was observed that the viscosity of the protein suspensions (5 mg/mL) without HPH treatment presented a constant decrease with increasing shear rate, indicating a shear thinning behavior or pseudoplastic behavior (Fig. 4). The pseudoplastic behavior was already early recognized in the literature for MPs (Chapleau & de Lamballerie-Anton, 2003) and actomyosin (Liu, Zhao, Xiong, Qiu, & Xie, 2008)

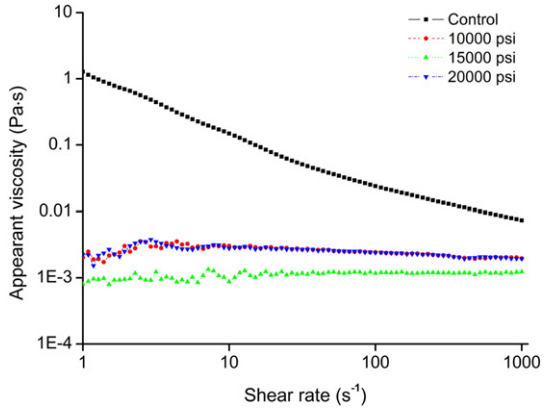


**Fig. 4.** Flow curve (apparent viscosity as a function of shear rate) of MP aqueous suspensions (5 mg/mL) after 0 psi (0.1 MPa), 10,000 psi (69 MPa), 15,000 psi (103 MPa) and 20,000 psi (138 MPa) HPH treatment, respectively.

dissolved in 0.6 M KCl/NaCl solution. In the present study, Non-HPH MPs suspended in water maintained some intact and integrated myofibril structure (as shown in Fig. 5A), tending to participate in entanglements. The steric hindrance effect and internal friction could lead to a high viscosity (Fig. 4). As the shear rate increased, the connection between fibrils was reduced and the asymmetric dispersed molecules tended to align themselves with the shear planes so that frictional resistance was reduced (Song et al., 2013), making suspension flow more easily. Thus, the shear-thinning behavior of non-HPH treated MP aqueous suspensions was demonstrated.

By applying HPH, all the suspensions at a protein concentration of 5 mg/mL displayed a sharp decrease in apparent viscosity, exhibiting Newtonian fluids over the range of shear rates investigated (Fig. 4). These facts could be attributed to the decrease of the particle size of MPs by HPH (Fig. 3A and Table 1), which could lead to an increase in mobility in water. When liquid medium was subjected to HPH, a strong physical force including shear, turbulence, impact and cavitation can be generated, being able to modify the flow properties of suspensions (Ronkart et al., 2010; Song et al., 2013; Sørensen et al., 2014). It had been reported that the decrease of hydrodynamic radius was associated with a decrease of the intrinsic viscosity. By HPH treatments, a decrease of apparent viscosity was attributed to depolymerization of polysaccharides (Villay et al., 2012). In the present case, intact myofibril structure had been disrupted to small size particles in submicron or even nanometer range (Fig. 3A and Table 1), thus less protein–protein interaction and thereby less resistance to flow appeared to be produced by HPH treatment. Therefore, a relatively low viscosity was presented in the HPH treated MP aqueous suspensions (Fig. 4). Probably due to the sufficient reduction of particle size (Fig. 3A and Table 1), effective water binding and high solubility of MPs in water (Fig. 1A) induced by HPH, the suspensions were characterized by similar apparent viscosity and exhibited Newtonian behavior (Fig. 4) (Sørensen et al., 2014; Ulbrich & Flöter, 2014). Because of the smallest particle size and highest water solubility, MPs in water treated at 15,000 psi presented the lowest viscosity. It can be concluded that the flow ability of MP suspensions in water was enhanced by HPH treatment.

### 3.5. Effects of HPH pressures on microstructure of MPs in water

Changes of myofibrillar structure at each HPH pressure were monitored by AFM measurements in Fig. 5. Untreated suspensions showed a bundle of myofibrils with an intact and linear featured structure (Fig. 5A and 5a), which is consistent with the appearance of rabbit skeletal muscle myofibril observed by AFM at the same resolution size (Yoshikawa, Yasuike, Yagi, & Yamada, 1999). This was also highlighted by previous report (Ito et al., 2003), which affirmed that some of the myofibrils with highly-ordered structures remained in MP aqueous suspensions.

With HPH treatment, myofibrils with some highly-ordered structures were completely disrupted, exhibiting dispersive particles with reduced particle size (Fig. 5B, 5C, 5D, 5b, 5c and 5d). This was in accordance with our PSD results as discussed in Section 3.3.1. By section analysis of height images, the height of the particles in suspensions obviously decreased from 110 nm to lower than 40 nm as observed in Fig. 5 (1–4). It had been concluded that HPH can change the surface morphology and decrease the roughness of cellulose suspensions by AFM observation (Lin et al., 2015). The maize starch and soy protein isolate particles were sheared to small fragments when exposing to super-high shear rate induced by HPH treatment(Song et al., 2013; Wang et al., 2012). Upon 10,000 psi HPH treatment, some filaments with knobby structure and monomers or oligomers with bead-like or myosin-like features were identified (Fig. 5B and 5b), evidencing that integrated structure of myofibrils indeed degraded into shorter and thinner filament fragments and released some monomer or oligomer proteins as we discussed above. With HPH pressure increasing to 15,000 psi, the filament might further split and disassociate into sub unit (thick, thin

filament, oligomers or monomers) (Fig. 5C). At this condition, it appeared that the monomers or oligomers became the dominant species uniformly distributed as we afore discussed in Section 3.3.1. Since the highly-ordered structure of myofibril was completely destroyed at 15,000 psi HPH, higher pressure at 20,000 psi might accelerate the formation of protein aggregation as some large irregular aggregates appeared in the suspensions treated by 20,000 psi (Fig. 5D and 5d), confirming our founding in the proteins profiles (Fig. 2). Due to the involvement of inter- and/or intra-molecular aggregation or accumulation, samples treated by 20,000 psi presented higher height than those treated at lower pressures (10,000 psi and 15,000 psi) as shown in Fig. 5 (2–4).

According to our results and previous studies, a proposed mechanism of solubilization of chicken breast MPs in water by HPH treatment was depicted in Fig. 6: Non-HPH treated samples generally maintained the intact highly-ordered myofibril structure (comprised of thin and thick myofilaments) (Fig. 5) with large particle size (Fig. 3A and Table 1) and relative low absolute zeta potential value (Fig. 3B). The protein–water interaction and protein–protein electrostatic repulsion were analyzed to be weak, resulting in low water solubility and stability

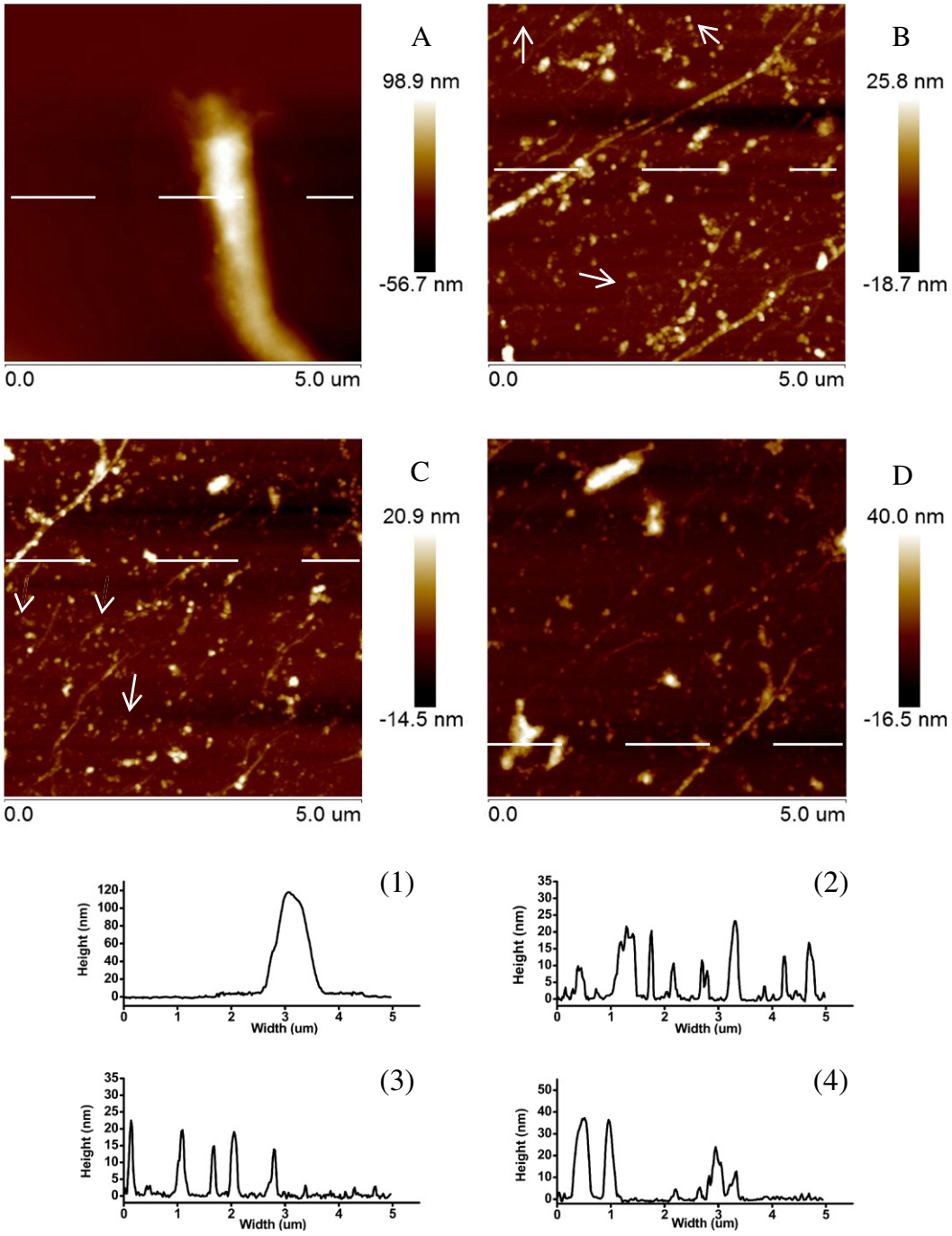


**Fig. 5.** The representative AFM images of MP aqueous suspensions treated at various HPH pressures: AFM height images of MP aqueous suspensions treated with (A) 0 psi (0.1 MPa), (B) 10,000 psi (69 MPa), (C) 15,000 psi (103 MPa) and (D) 20,000 psi (138 MPa) HPH; White arrows in (B-D) indicate myosin-like structure; (1–4) are section analyses (height) along the corresponding white dashed lines in (A–D); (a–d) are three-dimensional structures of MP aqueous suspensions treated with (A) 0 psi (0.1 MPa), (B) 10,000 psi (69 MPa), (C) 15,000 psi (103 MPa) and (D) 20,000 psi (138 MPa).

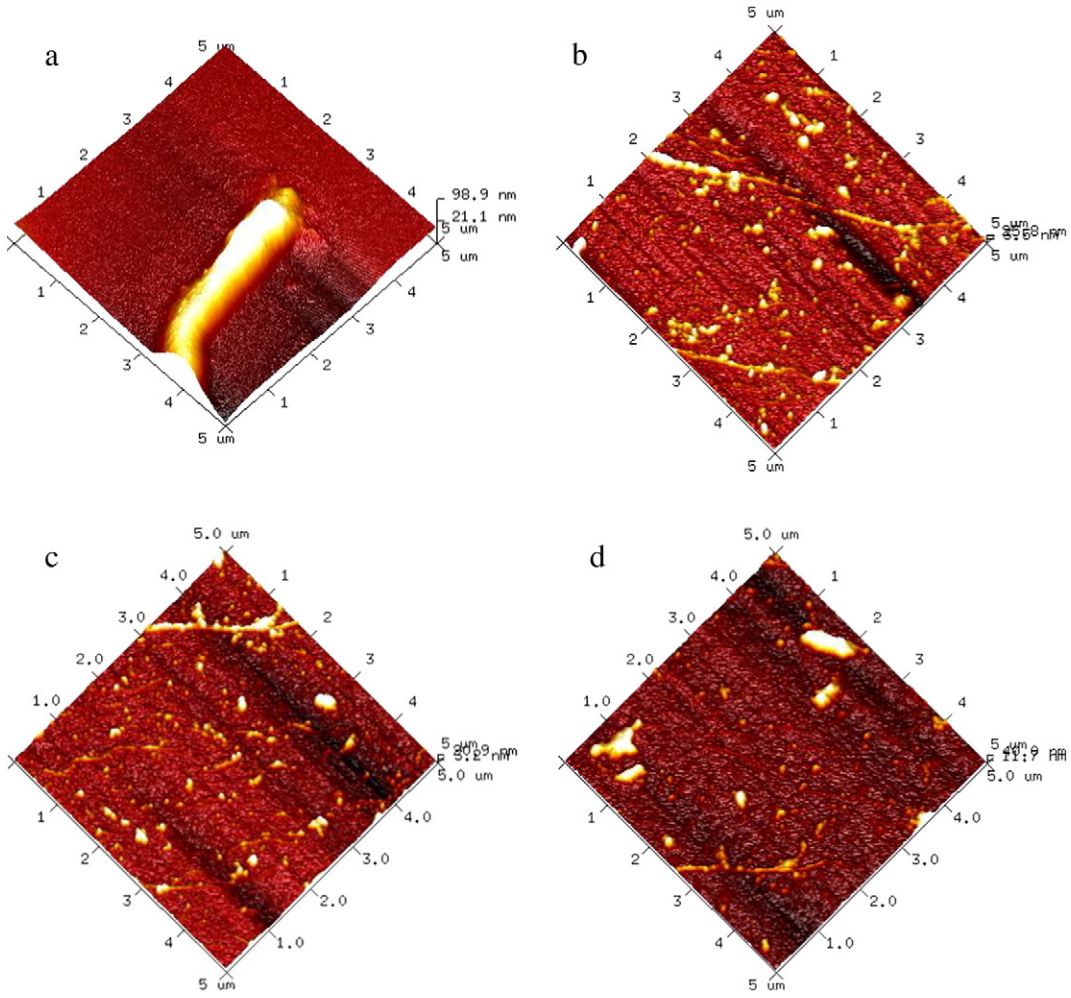


**Fig. 5** (*continued*).

(Fig. 1). When subjecting to HPH (15,000 psi), the intense physical force including intense shear, impact force and cavitation was effective in altering the protein structure and modifying particle properties. By applying HPH, the myofibril can be entirely disrupted and underwent random depolymerization or dissociation, producing some filaments, oligomers and myosin monomers suspensions (Fig. 5) with submicron/nm range particle size (Fig. 3A and Table 1) and high absolute zeta potential (Fig. 3B). At this condition, it was suggested that strong water binding and intermolecular electrostatic repulsion can effectively avoid subsequent aggregation and flocculation in MP aqueous suspensions. Thus, solubilization MPs in water with high stability and flow ability was achieved by HPH (Fig. 1 and Fig. 4). No

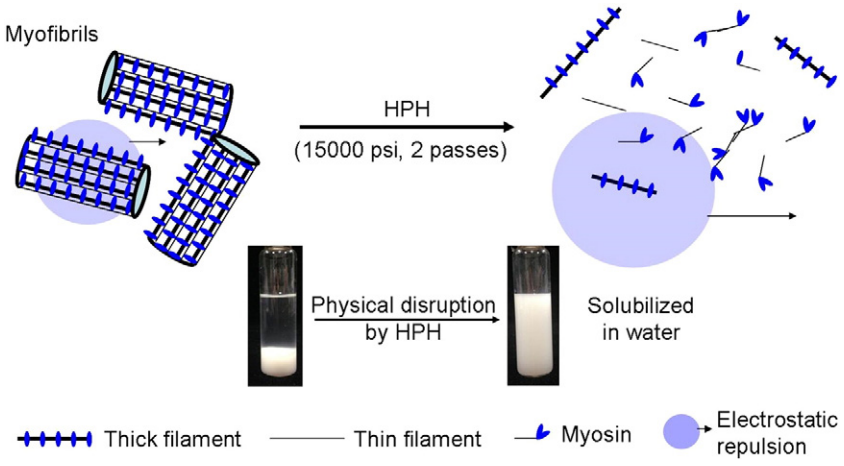


**Fig. 6.** Proposed schematic illustration of solubilizing chicken breast MPs in water by 15,000 psi (103 MPa) HPH treatment for two passes. See texts for details.

individual protein degradation or hydrolysis was observed in the HPH treated samples (Fig. 2). Further study is in progress to investigate the physicochemical characteristics and functionality of water-soluble MPs induced by HPH.

We established a new method by applying HPH treatment to facilitate solubilization of chicken breast MPs in water. It would be of great interest to test the potential of HPH to solubilize MPs in water for developing new food items such as protein-rich beverages, nutritional emulsifiers, as excellent source of proteins for dysphagia patients and for those with difficulty to masticate foods.

## 4. Conclusions

By applying 15,000 psi (103 MPa) for two passes HPH treatment, the solubility and stability of MPs in water could be significantly enhanced without any degradation of individual protein polypeptides. HPH treatment can produce MP aqueous suspensions with small particle size species (most likely sub-filament or monomer structure) and high absolute zeta potential. The flow ability was also increased by HPH treatment. Great reduction of particle size and enhancing of intermolecular electrostatic repulsion induced by HPH might result in increased solubility of chicken breast MPs in water. HPH seemed a promising technique for promoting solubilization of MPs in water.

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