Comparison of oil-in-water emulsions prepared by ultrasound, high-pressure homogenization and high-speed homogenization

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ABSTRACT

This study was designed to compare the properties of myofibrillar protein (MP) stabilized soybean oil-in-water emulsions fabricated by ultrasound-assisted emulsification (UAE), high-pressure homogenization (HPH) and high-speed homogenization (HSH). The emulsion properties, droplet characteristics, interfacial proteins, protein exposure extent, micro rheological properties, multiple light scattering results, and 7 d storage stabilities of the three emulsions were specifically investigated. Our results indicate that UAE and HPH were better emulsification methods than HSH to obtain high-quality emulsions with higher emulsifying activity index (UAE 20.73 m²·g⁻¹, HPH 11.76 m²·g⁻¹ and HSH 6.80 m²·g⁻¹), whiteness (UAE 81.05, HPH 80.67 and HSH 74.09), viscosity coefficient (UAE 0.44 Pa·s⁻¹, HPH 0.49 Pa·s⁻¹ and HSH 0.22 Pa·s⁻¹), macroscopic viscosity index (UAE 2.31 mm²·s⁻¹, HPH 0.38 mm²·s⁻¹ and HSH 0.34 mm²·s⁻¹), and storage stability, especially for the UAE. Furthermore, UAE was a more efficient emulsification method than HPH to prepare the fine MP-soybean oil emulsion. The protein-coated oil droplets were observed in the three emulsions. The emulsion droplet size of the UAE-fabricated emulsion was the lowest (0.15 μm) while the interfacial protein concentration (93.37%) and the protein exposure extent were the highest among the three emulsions. During the 7 d storage, no separation was observed for the UAE-fabricated emulsion, while the emulsions fabricated by HPH and HSH were separated after storage for 5 d and 2 h. Therefore, this work suggests that UAE could be a better method than HPH and HSH to fabricate MP-soybean oil emulsion.

1. Introduction

Emulsion-based foods are widely consumed in our daily life such as milk, ice cream, beverage, butter, and sausage [1]. Applying natural proteins to stabilize emulsions has been attracting the interest of researchers and consumers. Myofibrillar protein (MP) is the most important natural protein in muscle, and its emulsifying properties largely determine the quality of emulsified meat products [2,3]. Unlike low molecular weight emulsifiers that can be spontaneously adsorbed onto the oil–water interface to form an emulsion, MP molecules need to use high-energy methods to form an emulsion [1]. Ultrasound-assisted emulsification (UAE), high-pressure homogenization (HPH), and high-speed homogenization (HSH) are the most used high-energy methods to fabricate MP emulsions.

Ultrasound is an energy-efficient and versatile technology applied in food processing such as extraction, freezing, thawing, modification, salting, stabilization, tenderization and emulsification [4–10]. The emulsification character of sonication is widely applied to prepare protein-stabilized emulsions. The forces generated by the collapse of cavitation bubbles could break the large emulsion droplets into smaller emulsion droplets during UAE [11]. Zhou et al. [12] applied UAE to prepare different MP-pork fat ratio emulsions and observed that sonication could significantly decrease the emulsion droplet size and increase the emulsion activity index. To study the effects of defatted Antarctic krill protein concentration on the gel properties of its emulsion, Hu et al. [13] applied an ultrasonic cell disintegrator to prepare the emulsions. The mixture of casein and whey protein-stabilized double emulsions was also fabricated by sonication [14]. The application of the UAE to prepare different oil emulsions stabilized by plant and animal proteins has also been reported [15,16].

HPH is a widely applied food technology in protein modification, milk homogenization, beverage preparation, and emulsion preparation [17]. When the material quickly passes through a cavity with a special internal structure, the material is subjected to mechanical forces such as...
high-speed shearing, high-frequency oscillation, cavitation effect, and convection impact [18]. The large emulsion droplets could be broken into small ones under these mechanical forces. HPH is the mostly used emulsifying method to prepare protein stabilized fine emulsion. For example, applying HPH to prepare soybean protein isolate-soybean oil emulsions, Fernandez-Avila and Trujillo [19] observed that 100 MPa and 200 MPa treatments could allow more proteins to be loaded onto the oil-water interface, thereby improving emulsion oxidative stability. To explore the influences of rice bran rancidity on the rice bran protein-soybean oil emulsions, Li et al. [20] applied two cycles of HPH with 80 MPa to prepare the emulsions. The fine MP-soybean oil emulsions were also prepared with HPH at 30 MPa for two cycles [21]. HSH is widely used in the food industry to mix foods and form protein-stabilized emulsions. During the process of material homogenization, the material enters narrow gaps between the rotor and the stator and is destroyed under the actions of mechanical forces and hydrodynamic effects [22]. Applying HSH to fabricate MP-olive oil emulsion, Li et al. [23] showed that the emulsion prepared at 8,000 rpm was stable during storage. Studying the properties of low-fat MP emulsions prepared by different HSH speeds, Zhou et al. [22] found that the 14,500 rpm of HSH could modify protein structures and improve the emulsifying properties. HSH also has been applied to prepare other protein-stabilized emulsions such as soybean protein isolate [24] and whey protein [25].

2. Materials and methods

2.1. Materials and chemicals

Fresh porcine dorsal muscles and commercial soybean oil were purchased from the local supermarket (Nanjing, China). The used chemicals were at least analytical pure.

2.2. Extraction of pork MP

Pork MP was extracted as the methods described by Zhou et al. [26]. A bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to determine the protein concentration in this study.

2.3. Emulsions preparation

The MP (10 mg⋅mL−1; dissolved in 0.6 M KCl, 10 mM KH₂PO₄, pH 6.0, 4 °C) was mixed with 20% (v/v) soybean oil by a homogenizer (DS-1, Shanghai Specimen Model Factory, Shanghai, China) with 10,000 rpm for 1 min to obtain a coarse emulsion. The 50 mL of the coarse emulsions were used for different treatments. HSH treatment was applied with a PDS00-TP homogenizer (GreenPrima Instrument (Shanghai) Co., Ltd., Shanghai, China) attached with TP12 operating head at 15,000 rpm for 2 min. The speed and time of HSH are the commonly used parameters to fabricate protein emulsion. As for UAE treatment, the sonication power was set as 300 W for 10 min with 2 s on and 3 s off according to our preliminary experiment. A 20 kHz ultrasound processor (YM-1500Y, Shanghai Yuming Instrument Co., Ltd., Shanghai, China) was used in this test, and the actual sonication power was 17.95 W⋅cm⁻². The actual sonication power was determined as the following equation (Equation (1)):

\[
C_p\text{–}m\left(\frac{A}{\pi \phi^2}\right) = \frac{m}{\pi \phi^2} \frac{\sigma}{C} \frac{T}{\gamma}
\]

Where the \(C_p\) is 4.2 J⋅(g⋅°C)⁻¹, \(m\) is the mass of treating medium (g), \(dT/\ dt\) is the heating rate (°C⋅s⁻¹), and \(\phi\) is the diameter of ultrasonic probe (cm). During sonication, the temperature was controlled below 20 °C and a power monitor (PY-G8, Yuyao Pinyi Electrical Appliance Co., Ltd., Zhejiang, China) was used to determine the power consumption. The HPH treatment was conducted with a high-pressure homogenizer (D-3L, PhD-Technology LLC., MINN, USA) under 100 MPa until the power consumption reached the same with UAE. During HPH treatment, a circulation temperature controller (HX-105, Beijing Changliu Scientific Instrument Co., Ltd., Beijing, China) was applied to control the temperature below 20 °C. The emulsions were diluted with 0.6 M KCl and 10 mM KH₂PO₄ (pH 6.0, 4 °C) to different protein concentrations.

2.4. Emulsifying activity index (EAI) and emulsifying stability index (ESI)

Determinations of EAI and ESI of HSH-, HPH-, and UAE-fabricated emulsions (10 mg⋅mL⁻¹) following the method described by Pearce and Kinsella [27] with minor modifications. Fresh and after 10 min of 1 mL samples were dispersed into 300 mL of 0.1% sodium dodecyl sulfate solution. The 500 nm absorbance of the diluted solution was measured, and the EAI and the ESI were calculated as the followed equation (Eqs. (2) and (3)):

\[
EAI (\text{mg} \cdot \text{g}^{-1}) = \frac{2 \times 2.303 \times 300}{C \times (1 - \varphi)} \times 10^4 \times A_0
\]

\[
ESI (\%) = 100 \times \frac{A_{10}}{A_0}
\]

Where \(C\) represents the protein concentration of emulsions (g⋅mL⁻¹), \(\varphi\) is the oil volume fraction (v/v) of emulsions (\(\varphi = 20\%\)), \(A_0\) and \(A_{10}\) represent the absorbance when the time zero and after 10 min, and 300 is the dilution factor.

2.5. Turbidity and whiteness

Different emulsions (1 mg⋅mL⁻¹) were tested at 600 nm absorbance to obtain turbidity. The whiteness values of the emulsions (10 mg⋅mL⁻¹) were obtained with a handheld colorimeter (CR-400, Konica Minolta Sensing INC., Tokyo, Japan) as the following equation (Equation (4)):

\[
\text{Whiteness} = 100 - \sqrt{(100 - L^*)^2 + a^2 + b^2^2}
\]

Where \(L^*\) represents the lightness, \(a^*\) represents the redness/greenness, and \(b^*\) represents the yellowness/blueness.

2.6. Rheological properties

The rheological characteristics of different emulsions (10 mg⋅mL⁻¹) were determined by a rotational rheometer (MCR300, Anton Paar, Graz, Austria) with a 50 mm parallel plate. The shear rate ranged from 0.1 to 1000 s⁻¹. The change values of shear stress (\(\tau\)) with shear rate (\(\dot{\gamma}\)) were obtained. As the method described by Zhou et al. [11], the yield stress (\(\sigma\)), the viscosity coefficient (\(K\)), and the flow behavior index (\(n\)) were obtained by a Herschel-Bulkley model (Equation (5)):

\[
\tau = \sigma + k \times \dot{\gamma}^n
\]
2.7. Emulsion droplet size

The droplet sizes of different emulsions (10 mg·mL⁻¹) were determined with a Malvern 3000 (Malvern Instrument Ltd., Malvern, Nottinghamshire, UK) with the shading range of 10% to 20%, the refractive index of 1.436, and the adsorption index of 0.001.

2.8. Confocal laser scanning microscopy (CLSM)

The images of different emulsion droplets size distribution (10 mg·mL⁻¹) were obtained by a CLSM (TCS SP8X, Leica, Wetzlar, Germany) attached with 40 × objective. The emulsions were stained and observed as the method described by Zhou et al. [12].

2.9. Cryo-scanning electron microscope (Cryo-SEM)

The morphological structures of different emulsion droplets (10 mg·mL⁻¹) were observed with a Cryo-SEM (SU8010, Hitachi Corporation, Tokyo, Japan). After freezing the emulsions with liquid nitrogen and sublimating the water of emulsions for 20 min, the morphological images of emulsion droplets were obtained with Cryo-SEM under the accelerating voltage of 5 kV.

2.10. Determination and characteristics of interface protein

The distribution coefficient and characteristics of interface protein were determined with a centrifugal method [28]. After centrifuging the different emulsions (1 mg·mL⁻¹) at 15,000 g for 30 min, disposable plastic syringes were used to suck the aqueous solution and then through a 0.22 μm filter membrane before determining the protein concentration. The distribution coefficient of interface protein was calculated from the equation (Equation 6):

\[ \text{Distribution coefficient} (\%) = \left(1 - \frac{C_a}{C_i}\right) \times 100\% \]

Where \( C_a \) is the protein concentration in an aqueous solution, and \( C_i \) is the total protein concentration.

After mixing (1:1, v/v) the upper emulsion with 0.6 M KCl (10 mM KH₂PO₄, pH 6.0, 4 °C), the mixture was centrifuged under 15,000 g for 30 min, and then the lower clear liquid was aspirated and discarded. The above steps were repeated three times. The obtained upper emulsion was taken out and then was dissolved with 0.6 M KCl (10 mM KH₂PO₄, pH 6.0, 4 °C; 1:10, m/v). Then the samples were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) with 4% concentrated gel and 12% separated gel.

2.11. Surface hydrophobicity curve

The surface hydrophobicity curve of MP in different emulsions (1 mg·mL⁻¹) was determined by an 8-anilino-1-naphthalenesulphonic acid (ANS) [29]. Briefly, the 10 μL of fluorescence probe solutions (15 mM ANS, KH₂PO₄, pH 7.0) were mixed with 2 mL emulsions. After keeping the mixtures in the dark for 25 min, the fluorescence intensity curves were determined with a multifunctional microplate reader (M2e, Molecular Devices, San Jose, CA, USA) at the excitation wavelength of 374 nm and the emission wavelength from 420 nm to 600 nm.

2.12. Free sulphhydryl (SH) groups

The free SH groups were determined with 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) solution as the method described by Zhou et al. [22].

2.13. Zeta potential and mobility

After diluting the emulsion 500 times with 10 mM KH₂PO₄ (pH 6.0, 25 °C), the zeta potential and the mobility of different emulsions were determined with a Zetasizer (Nano-ZS90, Malvern Instruments Ltd., Malvern, Nottinghamshire, UK).

2.14. Microrheological behavior

A Lab 6 Master instrument (Formulaction, Toulouse, France) was used to characterize the microrheological properties of different emulsions (10 mg·mL⁻¹) within 24 h at 25 °C. The mean square displacement (MSD), the solid–liquid balance (SLB), the macroscopic viscosity index (MVI), and the elasticity index (EI) were obtained with time.

2.15. Multiple light scattering

A Turbiscan Tower instrument (Formulaction, Toulouse, France) was applied for multiple light scattering analyses. The emulsions (20 mL, 10 mg·mL⁻¹) were analyzed for 24 h. The backscattering (ΔBS) and the Turbiscan stability index (TSI) were obtained.

2.16. Storage stability

The pictures of different emulsions (10 mg·mL⁻¹) during the 7 d at 4 °C were obtained using the OnePlus 6 (Shenzhen OnePlus Technology Co., Ltd, Guangzhou, China). The distribution curves of droplet size and the zeta potential of different emulsions during storage were also tested.

2.17. Statistical analysis

All tests repeat three times and the results were expressed as means ± standard error. Data were subjected to a one-way analysis of variance using SPSS software (Ver. 24, IBM Corporation, Armonk, NY, USA). The significant differences were obtained at the \( P < 0.05 \) level under Dunncan’s test.

3. Results and discussions

3.1. Changes in emulsion properties

3.1.1. EAI and ESI

Fig. 1A illustrates the EAI and ESI values of different methods fabricated MP-soybean oil emulsions. Compared with the HSH-fabricated emulsions, HPH- and UAE-fabricated emulsions showed higher EAI and ESI values. The EAI values of UAE-fabricated emulsions (20.73 m<sup>2</sup>·g<sup>−1</sup>) were significantly higher than HPH-fabricated emulsions (11.76 m<sup>2</sup>·g<sup>−1</sup>) \( (P < 0.05) \). The ESI values between HPH- and UAE-fabricated emulsions showed no significant change \( (P > 0.05) \). The incremental EAI value indicates that the proteins showed higher emulsifying activity after HPH and UAE treatment, especially for the UAE-fabricated emulsion. The higher ESI values of HPH and UAE samples indicate that these emulsions had higher stability during storage. HSH is widely used to fabricate coarse emulsions whilst HPH and UAE are widely applied to fabricate fine emulsions [11]. The diminished droplet size (D₄₃), more uniform distribution of droplet size (Fig. 2), and more protein involved in the formation of the emulsion (Fig. 3) might be the reasons for the higher EAI and ESI values in the HPH- and UAE-fabricated emulsions [30]. Under the same power consumption condition, UAE-fabricated emulsions showed a higher EAI value, meaning that UAE is a better method than HPH to ameliorate the emulsifying activity of MP.

3.1.2. Turbidity and whiteness

Turbidity and whiteness are two indicators to reflect the color characters of emulsions affecting consumer acceptance [31,32]. Higher
turbidity and whiteness values mean that the emulsion has a more emulsion-white appearance. Compared with HSH-fabricated emulsions, HPH- and UAE-fabricated emulsions showed higher turbidity and whiteness values, as shown in Fig. 1B. The whiteness of the HPH- and UAE-fabricated emulsions was 80.67 and 81.05, which was significantly higher than the HSH-fabricated emulsion of 74.09. The turbidity of UAE-fabricated emulsion showed the highest value among the three groups (P < 0.05). Particle size is the most important factor affecting the turbidity and whiteness of emulsions [32,33]. The higher turbidity and whiteness values might be due to the lower droplet size of HPH- and UAE-fabricated emulsions.

3.1.3. Rheological properties

The apparent viscosity and the shear stress of different emulsions as a function of shear rate are shown in Fig. 1C and D. Among the three groups, the HSH group showed the highest apparent viscosity and shear stress values, followed by the HPH group, and the UAE group, except for the HPH group which showed the highest value when the shear rate was 10 to 100 s$^{-1}$. The obtained $\sigma$, K, and $n$ values from the Herschel-Bulkley model are shown in the table in Fig. 1D. The determination coefficients ($R^2$) for the three groups were 0.99 indicating this model has an excellent fit. As for the $\sigma$ value, the HSH-fabricated emulsion showed the highest value, followed by the HPH-fabricated emulsion (P > 0.05), while the UAE-fabricated emulsion presented the lowest value (P < 0.05). Among the three emulsions, HSH-fabricated emulsions showed the significantly lowest K value and highest $n$ value (P < 0.05), but no significant differences were observed between HPH- and UAE-fabricated emulsions (P > 0.05).

The apparent viscosity at a specific shear rate value is acceptable to compare the slurries if they are Newtonian fluid, but being inaccurate for non-Newtonian fluid [34]. All three emulsions are non-Newtonian pseudoplastic fluid evidenced by $n < 1$. Therefore, the apparent viscosity and K values showed a different change among the three emulsions. The different trends might be due to the different characters of MP, which properties influence the rheological properties of emulsion [12,35]. The characteristics obtained from the Herschel-Bulkley model could more effectively characterize the true rheological properties of emulsions. The lower $\sigma$ values in HPH- and UAE-fabricated emulsions indicate that more emulsion particles are taken part in the flow and create less resistance to the parallel plate [36], especially for the UAE-fabricated emulsion with yield stress reaching 0.13 Pa.s. In general, the smaller particle size of the emulsion leads to a higher viscosity and a lower flow index, whose emulsion shows a strong shear-thinning character [37,38]. This might be the reason for the higher K values and lower n values of HPH- and UAE-fabricated emulsions.

3.2. Changes in emulsion droplets

3.2.1. Droplet size

The droplet size of emulsion is one of the most important characters to affect the emulsion ability. The D3,2 and D4,3 values of different emulsions are shown in Fig. 2A. HSH-fabricated emulsion showed the
highest D3,2 and D4,3 values, followed by HPH-fabricated emulsion, while the D3,2 and D4,3 values of UAE treatment were the lowest. Compared with the HSH-fabricated emulsion, the smaller size of the HPH- and UAE-fabricated emulsions might be due to the different principles of emulsification. The instantaneous high-pressure generated by a HPH instrument and the cavitation effect of ultrasound are responsible for the decreased particle size of emulsion [11,18]. Both of the two methods are high-energy and high-efficiency emulsification methods. However, under the same power-consuming condition, the D4,3 value of UAE-fabricated emulsion was significantly lower than HPH-fabricated emulsion (P < 0.05), which means that UAE is a more energy-efficient emulsification method than HPH to decrease the emulsion droplet size.

3.2.2. CLSM images and Cryo-SEM images

CLSM images are applied to intuitively illustrate the emulsion profile of the emulsions and cryo-SEM images are used to observe the characteristics of one or several emulsion droplets. Fig. 2B to G show the CLSM images and Cryo-SEM images of HSH-, HPH-, and UAE-fabricated emulsions. The CLSM pictures show similar changes in emulsion droplet size as Fig. 2A. Although both HSH and UAE could reduce the particle size of emulsion droplets, the emulsion droplets prepared by UAE were separated more thoroughly and more uniformly. Protein-coated oil droplets were observed in the three method-fabricated emulsions as shown in Fig. 2E to G. Unlike the HSH- and UAE-fabricated emulsions, HPH-fabricated emulsion droplets were tightly connected by MP. This might be due to the different emulsification
principles between HPH and the other two methods. Ma et al. [39] also observed the tightly connected emulsion droplet for the HPH-fabricated cod protein-soybean oil emulsions.

### 3.3. Interface protein distribution coefficient and SDS-PAGE

During the emulsification process, MP molecules are quickly adsorbed to the soybean oil and form a protein membrane to wrap the oil particles. The protein-membrane could reduce interfacial tension between oil and water, and the emulsion stability is influenced by the amount of the interfacial protein. Fig. 3A is the protein distribution coefficient of adsorbed protein at the cream layer. Compared with the 33.37% adsorbed protein of the HSH-fabricated emulsion, HPH- and UAE-fabricated emulsions had significantly higher contents of adsorbed protein for 85.50% and 93.57%. These changes indicate that HPH and UAE allowed more MP to participate in the emulsification of soybean oil than HSH treatment, which contributed to the higher EAI values of HPH and UAE samples. Although there was no significant difference between the HPH and UAE samples (P > 0.05), the results showed that the adsorbed protein content of emulsion prepared by UAE was more stable (lower standard deviation). To understand which proteins of MP were involved in the formation of protein-coated oil droplets, the SDS-PAGE analysis was performed on the cream layer (adsorbed) and aqueous solution (non-adsorbed).

Fig. 3B shows the main bands of adsorbed and non-adsorbed proteins of HSH-, HPH-, and UAE-fabricated emulsions. The main bands of adsorbed proteins contain myosin heavy chain (MHC) and actin, and the non-adsorbed proteins contain MHC and actin. The changes in main band intensity are consistent with the changes in protein distribution coefficients (Fig. 3A). Among the three emulsification methods, UAE allowed more proteins to be adsorbed, followed by HPH, while HSH treatment adsorbed the lowest amount of proteins. Among the adsorbed proteins, MHC and actin were the main proteins participating in the formation of protein-coated oil droplets. The changes of non-adsorbed proteins indicate that the HMC could possess a stronger ability to be adsorbed on the oil droplet surface compared with actin [40]. The study of Li et al. [41] also found that ultrasound promoted the adsorption of myosin and actin on oil droplets, and the adsorption of MHC was greater than that of actin. The above changes indicate that UAE is a more effective method to form the protein-coated emulsion.

### 3.4. Surface hydrophobicity curve, free SH, zeta potential, and mobility

The surface hydrophobicity curves of MP in different emulsions are shown in Fig. 4A. Compared with HSH-fabricated emulsions, HPH- and UAE-fabricated emulsions showed a higher surface hydrophobicity value (P < 0.05). This change indicates that HPH and UAE effectively unfolded MP molecules than HSH. No significant change was observed between HPH and UAE-fabricated emulsion (P > 0.05), which means that the two methods showed similar effects to unfold protein molecules. The emulsifying capacity of the protein shows a positive correlation with their surface hydrophobicity values [42]. The higher surface hydrophobicity values might be also the reason for the higher EAI values of HPH- and UAE-fabricated emulsion.

Free SH refers to the SH groups exposed to an aqueous solution. The results of free SH groups of different methods fabricated emulsions are shown in Fig. 4B. Among the three treatments, UAE treatment led to the highest free SH group content of 181.20 μmol·g⁻¹, followed by HPH of 155.72 μmol·g⁻¹, and HSH of the 72.38 μmol·g⁻¹. The highest free SH content of UAE-fabricated emulsion indicates that more protein molecules were exposed after sonication compared with other treatments. The other studies also pointed out that the incremental free SH groups indicated the unfolding of proteins after high-speed homogenization [22], high-pressure homogenization [43], and ultrasound [35].

The stability of emulsion shows a positive correlation with its absolute potential value, and a higher absolute zeta potential value indicates the emulsion is less prone to coalescence [1]. The zeta potential and the mobility of different emulsions are shown in Fig. 4C. Generally, the absolute value of the mobility is proportional to the zeta potential. A similar change was observed in the three emulsions between zeta potential and mobility, and HSH-fabricated emulsion showed the lowest absolute zeta potential and mobility, with no significant changes being observed between the HPH and UAE groups. All the zeta potential values showed negative values at the pH above the MP isoelectric point. The higher absolute zeta potential and mobility of HPH and UAE groups indicate that more negatively charged groups were exposed compared to the HSH treatment. The changes of surface hydrophobicity, free SH, zeta potential, and mobility indicate that HPH and UAE treatment are more effective methods to unfold MP than HSH treatment, especially for UAE treatment.
3.5. Storage stability of different emulsions

3.5.1. Microrheological properties

Compared with the common rotational rheometer technique, the Lab 6 Master instrument could test the rheological properties during sample storage without destroying the sample. The viscoelastic properties of the samples were obtained by testing the Brownian motion with time. The MSD values of HSH-, HPH- and UAE-fabricated emulsions with the change of decorrelation time during 24 h were obtained as shown in Fig. 5 A to C. Generally, the MSD curves of the viscoelastic sample were divided into three stages with respect to the decorrelation time [44]. Firstly, the particles are free to move during the initial decorrelation time, so that the curve is linear, and the linear slope is related to the viscosity of the dispersant medium. Then the particles are blocked by a “cage” formed by the surroundings, the slope of the MSD curve decreases, and the MSD curve enters the plateau stage. This stage is related to the elasticity of the sample. At a longer decorrelation time, the particles are escaped from the “cage” and the MSD curves rise linearly again. The macroscopic viscosity of the sample is related to this stage, which corresponds to the moving speed of the particles. The MSD curves of the three emulsions all showed the characteristics of the viscoelastic samples. The plateau stages of all emulsions were increased as the incremental storage time, especially for the UAE-fabricated emulsion, in which the third stage almost disappeared. This change indicates that, compared with HSH- and HPH-fabricated emulsion, UAE-fabricated emulsions were more easily to form a “cage” to prevent the escape of particles.

The SLB values are obtained from the slope of MSD curve at the plateau stage. The lower slope means that the product is more solid than liquid, and the higher value means that the sample shows a stronger liquid-like behavior. The calculated SLB values of different emulsions are shown in Fig. 5D. The elastic/solid-viscous/liquid condition of the sample can be determined according to the SLB value. Generally, SLB = 0 indicates the sample is purely elastic/solid-like, 0 < SLB < 0.5 indicates the sample is mainly elastic/solid-like, SLB = 0.5 indicates the sample is half elastic/solid-like and half viscosity/liquid-like, 0.5 < SLB < 1 indicates the sample is mainly viscous/liquid-like, and SLB = 1 indicates the sample is purely viscous/liquid-like [45]. As the storage time increased, the SLB of all emulsions gradually decreased and then remained stable. The SLB value of HSH-fabricated emulsion decreased from 0.92 to 0.50 after 2.74 h, the HPH-fabricated emulsion decreased to 0.50 after 56 s, whilst the UAE-fabricated emulsion was lower than 0.5 throughout the storage period. The HSH-fabricated emulsion had the highest SLB value, followed by HPH and UAE after the SLB curves became horizontal. The above changes indicate that UAE-fabricated emulsion showed a more solid-like structure, followed by HPH and HSH. The obvious decrease of the SLB value of HSH-fabricated emulsion might be due to the separation of the emulsion within about 2 h (Fig. S1).

The EI values are obtained from the height of the plateau stage. The lower height means that the size of the “cage” is smaller, indicating that the sample has higher elasticity. As shown in Fig. 5E, all the EI curves showed a gradual increase and then horizontal with the incremental storage time. Compared with the EI curve of HSH-fabricated emulsion,
the HPH- and UAE-fabricated emulsions firstly tended to be horizontal with the extension of storage time. This phenomenon might be due to the separation of HSH-fabricated emulsion within the short storage time (Fig. S1). Among the three emulsions, the EI values (after horizontal) of HSH were the highest, followed by HPH, while UAE was the lowest. This change indicates that HSH-fabricated emulsion had the highest elasticity values, followed by HPH and UAE within the long-term storage. The Lab 6 Master instrument detects the cream layer once the emulsion separates. The higher EI values of HSH-fabricated emulsion might be due to the incremental volume fraction of the emulsion in the creaming layer caused by separation.

The MVI curve is obtained from the slope of the MSD curve after the plateau stage. Indeed, the lower slope value means that the particle moves slower for long decorrelation times. The MVI values could be used to express the macroscopic viscosity, and the MVI curves of HSH-, HPH-, and UAE-fabricated emulsion with the change of time are shown in Fig. 5F. The values of different MVI were increased with the increase of storage time. Compared with the MVI curve of HSH-fabricated emulsion, the HPH- and UAE-fabricated emulsions firstly tended to be horizontal with the extension of storage time. This change might be due to the instability of HSH samples, and separation occurs in a short storage time (Fig. S1). The MVI values of UAE-fabricated emulsion showed the highest MVI values, followed by HPH and HSH, and the MVI values of the HSH reached the same as HPH after storage of around 18 h. The FI

**Fig. 5.** Changes of microrheological properties of different emulsions.
The situation such as clarification, creaming, and flocculation/coalescence of the emulsion during storage could be reflected by the ΔBS values. Fig. 6A to C are the ΔBS results of HSH-, HPH- and UAE-fabricated emulsions during 24 h. The ΔBS values of HSH emulsions gradually decreased during 0 to 10,000 μm with the incremental storage time, while increased during 10,000 to 35,000 μm, indicating that the
clarification was observed at the bottom and the creaming was observed at the middle. The changed ΔBS values of HPH- and UAE-fabricated emulsions indicate that the emulsions were flocculated/coalesced during 24 h storage. The TSI values of three emulsions with time are shown in Fig. 6D. A higher TSI value indicates the lower stability of the emulsion. The TSI values of the HSH-fabricated sample showed the highest TSI values, followed by HPH and UAE samples. This indicates that the MP-soybean oil emulsion prepared by UAE was the most stable, followed by HPH, while the HSH showed the lowest stability. The bottom and middle of the emulsions had the most noticeable change among the three emulsions. To explore the changes in TSI values at the bottom and middle, and the mean ΔBS values during storage, a radar chart was drawn as shown in Fig. 6E. The TSI values (at the bottom and middle area) and mean ΔBS values of HSH-fabricated emulsions were higher than the HPH- and UAE-fabricated emulsions during the 24 h storage indicating that both the bottom and middle stability of HPH- and UAE-fabricated emulsions were higher than HSH. Except that the TSI values and the mean ΔBS values of HPH-fabricated emulsion after 24 h

Fig. 7. The emulsion pictures, particle size distribution (A to E), and zeta potential (F) during 7 d storage. Pictures A to E represent the 0, 1, 3, 5, and 7 d of storage, respectively. Different letters (a and b) in picture F indicate the significant differences between different groups.
storage were lower than the UAE emulsions, there was no obvious difference in the other storage period. This change might be elucidated by the connected emulsion droplet of HPH-fabricated emulsion (Fig. 2F).

3.5.3. Long-term stability

Fig. 7A to E respectively contain the emulsion pictures and droplet size distribution of three emulsions stored for 0, 1, 3, 5, and 7 d. With the prolongation of storage time, the three emulsions gradually separated. The firstly separated emulsion was the HSH-fabricated, and then the HPH-fabricated emulsion started separation at 5 d storage, and the UAE-fabricated emulsion showed a slight separation at the 7 d storage. The results indicate that UAE-fabricated emulsion showed the highest storage stability, followed by HPH and HSH. For the freshly prepared emulsions, the droplet size distribution of UAE-fabricated emulsion is the most to the left, the HPH-fabricated emulsion moves to the right, and the HSH-fabricated emulsion is the most to the right. This is consistent with the emulsion droplet observed in Fig. 2. The distribution curves gradually moved to the right with the incremental storage time, which indicates that the emulsion droplets coalesced and the size gradually increased, especially for the HPH- and UAE-fabricated emulsion. The zeta potential values of different emulsions during storage are shown in Fig. 7E. The absolute zeta potential value of HSH-fabricated emulsion during the 7 d storage was lower than that of HPH and UAE. The absolute zeta potential value of HSH-fabricated emulsion showed no significant change with incremental storage time (P > 0.05), whilst the absolute zeta potential value of HPH- and UAE-fabricated emulsions increased firstly and then decreased. The increased droplet size and the attenuated absolute zeta potential values might be the reasons for the separation of the HPH- and UAE-fabricated emulsions.

4. Conclusions

The properties of the myofibrillar protein (MP)-soybean oil emulsion fabricated by ultrasound-assisted emulsification (UAE), high-pressure homogenization (HPH), and high-speed homogenization (HSH) were compared. HPH and UAE are better emulsification methods than HSH to emulsify MP-soybean oil emulsion. HSH and UAE formed the separated protein-coated emulsion droplets, while the HPH formed the MP-connected emulsion droplets. Under the same power consumption condition, the emulsion prepared by UAE showed a smaller particle size and better storage stability than HPH. The low size and uniform distribution of emulsion droplets, and the high values of viscosity (viscosity coefficient and macroscopic viscosity index), unfolded protein molecules content, absolute zeta potential, and interfacial protein concentration might contribute to the better stability of the emulsions. Therefore, UAE is a better method to fabricate MP stabilized soybean oil emulsions compared to HSH and HPH treatment.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.julsonch.2021.105885.

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CRediT authorship contribution statement

Lei Zhou: Conceptualization, Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. Wangang Zhang: Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. Jingyu Wang: Methodology, Writing – review & editing. Ruyu Zhang: Writing – review & editing. Jian Zhang: Methodology, Writing – review & editing.

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.
