Enhancement of storage stability of surimi particles stabilized novel pickering emulsions: Effect of different sequential ultrasonic processes

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\textbf{Abstract}

Preparation of highly stable Pickering emulsions stabilized by food grade particles especially with low concentrations is of concern. In this study, the effects of two-step emulsification procedure with different sequential ultrasonic processes on the storage stability, droplets size, zeta potential, microstructures as well as the rheological behaviors of surimi particles-stabilized Pickering emulsions with 0.6 oil-water ratio were investigated. The results showed that the surimi particles based-emulsions prepared by the homogenization-ultrasonic (H-U) or ultrasonic-homogenization (U-H) method both possessed excellent physical stability during 14 days storage. Particularly, the stability index of emulsions all exceeded 99.5% in the U-H groups. The confocal laser scanning microscopy (CLSM) and cryo-scanning electron microscope (cryo-SEM) images provide evidence that more particles attached on the oil–water interfaces and the network structures formed via particle–particle interactions obviously arrested phase separation in H-U and U-H emulsions. Moreover, the Pickering emulsions obtained by two-step method all exhibited higher viscosity and storage modulus values, which were also conducive to the special storage stability of samples. In short, the storage stability of protein based-Pickering emulsions can be enhanced using homogenization-ultrasonic (H-U) or ultrasonic-homogenization (U-H) procedure.

\textbf{Keywords:}

Pickering emulsions
Storage stability
Rheological characteristics

1. Introduction

Pickering emulsions stabilized by solid particles, by virtue of the special resistance against coalescence, Ostwald ripening, etc., and the potential applications in multiple fields, again spark a renewed interest recently [1–3]. It is well established that the system composed of a liquid dispersed in another incompatible liquid in the form of droplets is thermodynamically unstable, resulting in the occurrence of instability during its process and storage. Hence, solid particles, acting as emulsiﬁying agents, fulfilled a crucial role in maintaining the emulsion stabilization. Herein, given the consumer concerns about the safe and health of inorganic particles (regarded as solid particles), it is extremely critical for the food industry to search and develop food-grade particles. Using proteins derived from animal, plant, or vegetable sources to replace traditional solid particles have received much attention owing to their natural hydrophobic and hydrophilic characteristics [4–6]. Myofibrillar protein, salt-soluble protein, is one of the most important structural compositions in fish muscle. As reported by Liu et al. [7], surimi particles (myofibrillar proteins) could act as solid particles to stabilize Pickering emulsions. However, some special features of myofibrillar protein including large molecular weight, poor dispersibility and solubility in water, as well as weak electrostatic repulsions impeded the adsorption of these particles at the two-phase interface and the particle–particle interactions during emulsification [7,8]. This further poses a huge challenge to fabricate high-quality myofibrillar protein based-Pickering emulsions, especially with low concentrations of proteins. To improve the quality of these emulsions, rational combination of higher energy-efficient emulsification devices may be a very promising approach.

At present, high energy processes in the preparation of emulsion are classified into mechanical rotor–stator shearing, high-pressure homogenization, ultrasound devices, microfluidics, etc. Wherein, the mechanical shearing, mixing the incompatible liquids through a narrow gap between a rotor and a stator, is still the most widely used conventional emulsification technique [9]. Compared to other devices, high intensity ultrasound (HUS) with 20–100 kHz frequency has attracted extensive attentions recently as an alternative emulsification tool due to possessing advantages such as having a simpler system, being easier to

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operate and clean, using less surfactant to produce more stable emulsions particularly for protein-stabilized products [10,11]. In this process, the formation of finer emulsions is related not only to the improvement effect of ultrasound on the functional properties of protein, but also to droplet rupture and re-formation. The potential mechanisms can be explained by the fact that ultrasound can cause acoustic cavitation in a liquid, mainly involving the formation, growth, and implosion of bubbles [12]. Moreover, the occurrence of micro-turbulence and liquid jets follows the collapse of cavitation bubbles, which can generate strong forces and further result in the structural modifications of solid protein particles in a liquid or droplets breakdown [13]. The coarse emulsions firstly were obtained by mechanical rotor–stator shearing and then final emulsions with high quality were produced using ultrasonic irradiation to treat coarse emulsions directly, which has been demonstrated by many researchers [14–17]. Apart from the homogenization-ultrasonic emulsification method, Liu et al. [18] once reported that the emulsion prepared with ultrasonic-treated myofibrillar protein solutions had higher storage stability than the control group. Ma et al. [19] also found when the 10% (w/w) soybean oil and 90% (w/v) sonicated cod protein suspension were homogenized together, the emulsion had lower creaming index. Based on the information above-mentioned, it can be concluded that the two-step emulsification approach involving mechanical shearing and ultrasound is conducive to fabricate high-stability emulsions. It was noteworthy that there existed remarkable differences between the surimi particles and myofibrillar proteins directly extracted from various raw materials in many aspects such as physical state, hydration capacity, conformation, emulsification, etc. [20,21]. However, the effects of different sequential ultrasonic processes on the physical properties and stabilization mechanisms of Pickering emulsions stabilized by surimi particles especially with low concentrations are unclear. Furthermore, there is little information about the applicability of both approaches to the production of surimi particles stabilized Pickering emulsions.

Therefore, in present study, the two-step (homogenization-ultrasonic and ultrasonic-homogenization) emulsification method was applied to prepare the Pickering emulsions. (i) Coarse emulsions including soybean oil, aqueous phase and surimi particles were firstly prepared by mechanical homogenization and then exposed to different ultrasonic conditions (100, 300, 500, 700 W) to produce fine emulsions (H-U groups); (ii) The surimi particle dispersions subjected to ultrasonic processing with different powers (100, 300, 500, 700 W) were obtained and further mixed with soybean oil to fabricate Pickering emulsions under high-speed mechanical homogenization (U-H groups). The storage stability, droplets size, zeta potential, microstructures as well as the rheological behaviors, were characterized and compared. This paper could help extend the applications of surimi particles under low ionic strength environment and meanwhile provide a new option of solid protein extend the applications of surimi particles under low ionic strength behaviors, were characterized and compared. This paper could help extend the applications of surimi particles to construct the Pickering emulsions using different processing methods.

2. Materials and methods

2.1. Materials

Silver carp surimi graded AAA was purchased from a freshwater fish surimi manufacturing enterprise from Honghu, China (Honghu New Hongye Food Co., Ltd.) and the corresponding surimi particles (84.70 wt % protein content on a dry basis) was prepared via lyophilization and crushing methods. The edible soybean oil (Grade A) was purchased from a local Auchan market (Wuxi, China). Nile blue and Nile red were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used in this paper were of reagent grade.

2.2. Preparation of Pickering emulsions

2.2.1. Pickering emulsions prepared by homogenizing method

The oil in water (O/W) Pickering emulsion was prepared using mechanical homogenizer. The soybean oil was added into the aqueous phase containing 2 wt% surimi particles in a ratio of 0.6 (v/v) and then mixed together at 19,000 rpm using a basic homogenizer (model IKA T-10, Wilmington, NC, USA) for 2 min. The prepared emulsion was regarded as control group (0.6–2.0%/C). Additionally, the aqueous phase and dispersed phase (soybean oil) also were emulsified directly at same volume ratio by ultrasonic method. However, large amount of separated oil phase still existed in the sample (data not shown). Hence, the O/W emulsion in this paper was only fabricated by one-step homogenizing method.

2.2.2. Pickering emulsions prepared by two-step homogenization-ultrasonic method

To ascertain the influence of ultrasonic treatment on the features of mini-emulsion formed, the emulsion obtained in part 2.2.1 was placed in the sample chamber of an IJUPIN-1200E ultrasonic processor (1200 W, Wuxi Juiping Instrument Co., Ltd. China) equipped with a frequency of 20 kHz and a 10 mm diameter titanium probe. The probe was immersed in the sample to a 2 cm depth. Ultrasonic power was set as 100, 300, 500 and 700 W, respectively. Sonication time was 3 min (pulse duration: on-time, 3.0 s; off-time, 3.0 s). During the process of ultrasound, the centrifuge tube containing the sample was placed in a beaker filled with ice-cold water to maintain the temperature below 25 °C. Herein, the appropriate treatment group was named as H-U-100, H-U-300, H-U-500, H-U-700, respectively. The preparation process of surimi particles stabilized O/W Pickering emulsion via two-step homogenization-ultrasonic method was exhibited in Fig. 1.

2.2.3. Pickering emulsions prepared by two-step ultrasonic-homogenization method

Surimi particles, firstly, were suspended in ultra-pure water at a concentration of 2.0 wt%, and then the suspension was subjected to different intensity ultrasound (100, 300, 500 and 700 W) under the same conditions as described in part 2.2.2. The edible soybean oil was added to the suspensions above-mentioned, and then the mixture was homogenized for 2 min at 19,000 rpm. The experimental group was labeled as U-H-100, U-H-300, U-H-500, U-H-700, respectively. The preparation process of O/W Pickering emulsion stabilized by surimi particles via two-step ultrasonic-homogenization method was exhibited in Fig. 1.

All prepared O/W Pickering emulsions from part 2.2.1, 2.2.2, and 2.2.3 were stored at 4 °C for 14 days to study the changes in the physical properties, microstructures and rheological behaviors of samples after different processing procedures.

2.3. Characterisation of Pickering emulsions prepared by two-step method

2.3.1. Emulsified phase volume fraction and stability index of Pickering emulsions

The emulsified phase volume fraction (EPVF) and stability index (SI) of Pickering emulsions with or without ultrasonic treatment were recorded to characterize the storage stability of samples. The calculation of EPVF and SI were performed in line with the method described by Wei et al. and Liu et al. [4,7].

\[
\text{EPVF} = \frac{H_e}{H_t} \times 100 \quad (1)
\]

\[
\text{SI} = \frac{\text{EPVF-14 days}}{\text{EPVF-3 hours}} \times 100 \quad (2)
\]

Here, He stands for the heights of white emulsified phase, Ht refers to the total heights of the emulsions. EPVF-3 h and EPVF-14 days
represents the emulsified phase volume fraction of samples at 3 h and 14 days, respectively.

2.3.2. Droplet size and zeta potential analysis of Pickering emulsions

Droplet sizes of surimi particles-stabilized Pickering emulsions with or without ultrasonic treatment were determined by a model BT-9300ST analyzer (Baite Instruments Ltd., Dandong, China). The emulsions (0.5 mL) were added into sample chamber containing a quantity of ultrapure water and the pump speed was set to 1600 rpm. The refractive values of 1.47 (oil phase) and 1.33 (water phase) were employed and the volume-weighted mean diameter ($D_{4,3}$) of samples was collected to exhibit the change of average droplet size.

The zeta potential of samples was analyzed using a Zetasizer nano ZS instrument (Malvern Instruments Ltd., Worcestershire, UK). The samples were diluted properly prior to analysis. All measurements were conducted at 25°C.

2.3.3. Light microscopy and confocal laser scanning microscopy (CLSM)

The optical microstructures of all emulsion samples were obtained at brightfield pattern via a model Axio Vert A1 microscope equipped with an Axiocam 503 camera (Carl Zeiss AG, Germany) under 10× magnification of objective lens. Before observation, the sample was diluted two times using ultrapure water.

To determine whether ultrasonic treatment affects the distribution of surimi particles at emulsion interface or not, the confocal laser scanning microscopy (model TCS SP8, Leica Microsystems, Wetzlar, Germany) was used to observe the microstructures of samples within the micrometer scale. The emulsions obtained from part 2.2.1, 2.2.2, and 2.2.3 were stained using Nile red (1 mg/mL) for oil phase dyeing and Nile blue (1 mg/mL) for surimi particles coloring. The wavelength of 488 and 633 nm was applied to the excitation of Nile red and Nile blue, respectively [22]. Finally, the dual channel pictures were used to indicate the distribution behaviors of solid particles on the oil-water interface.

To further clearly ascertain the microstructure of the O/W emulsions, a cryo-scanning electron microscope (cryo-SEM) was applied (SU8010, Hitachi High-technologies Co., Japan) according to Xiao et al. [23]. Firstly, the emulsion samples were cryo-fixed fixed in the copper specimen holder using slush nitrogen. The holder was then quickly transferred into the cryo-preparation chamber under vacuum. Subsequently, the frozen emulsions were fractured using a pre-cooled razor blade. After sublimated at −80°C for 4 min, the samples were transferred into a vacuum SEM chamber and examined at −175°C under 5 kV.

2.3.4. Rheological properties

The rheological properties of emulsion samples with or without ultrasonic treatment were analyzed by using a HAAKE III rotational rheometer (Thermo Fisher Scientific Inc., USA) at 25°C. The steady shear scanning was performed at shear rates of 0.1–100 s⁻¹ using a C35/1°Ti L cone to obtain the viscosity of emulsions. Here, the Power Law model (3) was utilized to analyze the consistency coefficient ($k'$, Pa•sⁿ) and flow behavior index ($n'$) via fitting the variation trend of viscosity ($η$, Pa•s) with shear rate ($γ$, s⁻¹) [24].

$$\eta = k' \gamma^{n'}$$  \hspace{1cm} (3)

The frequency sweep (scanning frequency ranging from 0.01 to 10 Hz) was conducted at a strain of 0.5% (within the linear viscoelastic region) using a P35 Ti L plate to analyze the storage modulus ($G'$) and loss modulus ($G''$).

2.4. Statistical analysis

The experiments were conducted in triplicate and all data were presented as mean ± standard deviation. The one-way ANOVA was used to calculate the significant differences of results via a version 13.0 SPSS software (Chicago, USA) and a value of $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Changes in storage stability of Pickering emulsions prepared by two-step method

To ascertain the effects of different sequential ultrasonic processes on the formation of surimi particles-stabilized Pickering emulsion, a series of emulsion samples were prepared and meanwhile stored for 14 days.
For the 0.6–2.0% emulsions at 3 h, the EPVF values of all groups ranged from 97.2% to 99.7% (Fig. 2a and 2c). After 14 days storage, the appreciable phase separation phenomenon and a 7% decline in EPVF value were found in the control group (Fig. 2a, 2b and 2c), meaning it was hard to maintain the storage stability of Pickering emulsion prepared only by mechanical homogenizing. This could be ascribed to the poor dispersive property of surimi particles in the aqueous phase with low ionic strength (Figure S1), which might affect the anchoring of these particles onto the water–oil interface and hinder the particle–particle interactions during homogeneous emulsification process to some extent [7]. However, the H-U and U-H groups whatever the ultrasound power still had higher EPVF values (95.5%-99.5%) at 14 days (Fig. 2b and 2c). Meanwhile, the SI values in H-U and U-H groups were significantly greater than that of the control (p < 0.05), particularly the U-H groups (Fig. 2d), which was probably related to the zeta-potential (Figure S2) and structural changes (Figure S3) of surimi proteins or adsorption ratio of particles on the oil/water interface under varied two-step emulsification approaches [25]. This was proved by Bi et al. [26], who obtained stable Pickering high internal phase emulsions stabilized by casein particles exposed to ultrasound conditions. Lee et al. [27] also pointed out sonication technology could simultaneously emulsify and push solid particles adsorption onto droplet interfaces. In brief, the two-step emulsification approaches hold potential as green strategies for the preparation of high-stable surimi particles-based Pickering emulsions.

3.2. Changes in distribution and droplet sizes of Pickering emulsions prepared by two-step method

The optical images and droplet sizes of the control, H-U, and U-H emulsions (0.6–2.0%) were displayed in Fig. 3 and Fig. 4a. The distribution or internal packing of samples revealed via optical microscopy among the control, H-U, and U-H groups (0.6–2.0%) was apparently varied at 3 h (Fig. 3). The control and U-H groups had similar homogeneous spherical shape with different droplet sizes, which was relevant to the surimi particle suspensions after ultrasonic treatment. Instead, the compact emulsions composed of droplets with lower size were observed in the H-U groups. As for this, the coarse oil droplets obtained by mechanical homogenization in the first stage could be ruptured into very fine emulsions with small size in the second step due to the cavitation bubbles generated from sonication [28]. Subsequence, the surimi particles were again adsorbed on the surfaces of these fine emulsions and where the bridging flocculation phenomenon among oil droplets via these particles tended to happen owing to the instantaneous thermal effect, as shown in Fig. 3 and Fig. 6. Similar observation also was reported by Costa et al. [29], who produced chitosan particles stabilized O/W emulsions. After the storage of 14 days, the samples in all groups still had similar distribution or internal packing of droplets but with differential sizes when compared to the emulsions at 3 h. Therefore, the droplet sizes (D<sub>4,3</sub>) of samples were further evaluated.

Compared with the 0.6–2.0%/C group, the H-U and U-H groups had larger emulsion droplet sizes (D<sub>4,3</sub>) (see Fig. 4a). This variance appeared to be due on the one hand to the aggregation and crosslinking behaviors of adjacent emulsion droplets via the surimi particles after ultrasonic processing (H-U groups), and on the other hand to the formation of dense three-dimensional networks in the continuous phase when using the well dispersed surimi particles suspension to produce emulsions (U-H groups) [30]. The Fig. 3 and Fig. 5 also provided visual evidences for this. The D<sub>4,3</sub> value of the 0.6–2.0%/C group in 14 days was greater than that at 3 h, implying the droplet coalescence and creaming phenomenon occurred (Fig. 4a). Meanwhile, there existed a slight increase of D<sub>4,3</sub> values in H-U groups after storage. The reason of this might be that the degree of aggregation and cross-linking of the previous emulsions (3 h) after homogenization-ultrasonic treatment weakened during storage period, leading to the desorption of surimi particles from two-phase interfaces and formation of emulsions with large droplets (Fig. 2 and Fig. 3). The initial close contact among the droplets in the H-U groups could also cause oil transfer from smaller ones to the larger drops to a certain extent during storage [31]. Although such a slight D<sub>4,3</sub> increase in H-U groups, the surimi particles adsorption onto the oil–water interface, as well as, the formation of a weak network structure around oil droplets might provide an enhancement for the stability of samples (Figs. 2, 5, 6, and 7). The droplet size of U-H groups (0.6–2.0%) remained almost constant during storage (Fig. 3 and Fig. 4a), which was congruent with the stability of the U-H treatment (Fig. 2). This implied

Fig. 2. Characterization of storage stability of surimi particles stabilized Pickering emulsions via two-step procedure. (a) Visual observation of Pickering emulsions at 3 h; (b) Visual observation of 0.6–2.0% emulsions at 14 days; (c) The EPVF values of 0.6–2.0% emulsions at 3 h or 14 days; (d) The SI values of 0.6–2.0% emulsions. Different lowercase or uppercase characters in the same set of indicators mean significant differences (p < 0.05).
the two-step ultrasonic-homogenization approach could provide enough energy barrier for this thermodynamically unstable system (0.6–2.0%) to prevent the system from reaching the state with the lowest free energy during 14 days [25]. We deduced the strong adsorption of particles on the O/W interfaces, as well as the formation of network structure of surimi particles after ultrasound were mainly responsible for the notable stability of U-H groups (as evidenced by Fig. 5 and Fig. 6). Zhang et al. [30] and Ai et al. [32] also found that the sono-assembled soy protein isolates or egg white protein peptides treated with ultrasound could easily adsorbed on the oil–water interface, or could form multilayers to encapsulate the oil droplets.

3.3. Changes in zeta potential of Pickering emulsions stabilized by prepared by two-step method

The zeta potential is one of the most decisive factors that determine droplet movement and stability in emulsions [33]. As highlighted in Fig. 4b, the initial zeta potential of all samples (0.6–2.0% emulsions) with or without ultrasound treatment ranged from −30.3 to −47.6 mV at 3 h, and these differences were dependent on the power of ultrasound, the dispersion degree of surimi particles in aqueous phase (Figure S1), and the distribution behavior of surimi particles on the water/oil interface (Fig. 5). There existed no significant differences of the strength of the electrostatic repulsion in H-U groups. However, the absolute zeta potential of all samples (H-U groups) was lower than that of the 0.6–2.0%/C and U-H groups. The weak electrostatic repulsion between

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Fig. 3. Optical microscopic images of Pickering emulsions stabilized by surimi particles via two-step procedure. Images were obtained at 3 h and 14 days after emulsification.

Fig. 4. Changes in $D_{4,3}$ and zeta potential of Pickering emulsions stabilized by surimi particles via two-step procedure. (a) The $D_{4,3}$ values of 0.6–2.0% emulsions at 3 h or 14 days; (b) The zeta potential of 0.6–2.0% emulsions at 3 h or 14 days. Different lowercase or uppercase characters in the same set of indicators mean significant differences ($p < 0.05$).
Fig. 5. CLSM images of Pickering emulsions stabilized by surimi particles via two-step procedure. Images were obtained at 3 h and 14 days after emulsification. Soybean oil was stained red with Nile Red and the surimi particles were stained blue with Nile Blue.

Fig. 6. Cryo-SEM images of Pickering emulsions stabilized by surimi particles via two-step procedure. Images were obtained at 3 h after emulsification. (a) The overall appearance of exposed emulsion droplets; (b) The microstructure of single emulsion droplet; (c) The interface structure of single emulsion droplet.
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droplets might closely associate with the size of droplets and the bridging flocculation between the droplets after homogenization-ultrasonic treatment (Figs. 3 and 4 a). After storage of 14 days, the absolute value of zeta potential all decreased at different degree, especially for the control group (0.6–2.0%/C). This could be explained by the desorption of solid particles from water/oil interface, and consequently reducing the electrostatic interactions between droplets [34]. The visual appearance (Fig. 2 a and 2b), SI (Fig. 2 d) and droplet sizes (Fig. 4 a) results all demonstrated the changes of zeta potential.

For U-H groups, we found the absolute zeta potential of all samples was great higher than 32 mV at 3 h, partly facilitating the stability of emulsions during storage. The visual images of these samples were consistent with this result (Fig. 2). After 14 days storage, the absolute zeta potential of U-H groups (0.6–2.0%/ 100–700 W) decreased by 4.4, 4.7, 2.9 and 4.3 mV, respectively. The reason for this slight decrease in electrostatic interaction might be that during storage the droplets in this system moved and collided leading to these droplets partly associated with each other or fuse together to form larger droplets (Figs. 3 and 4 a). However, apart from the 0.6–2.0% emulsions without ultrasound treatment, the special storage stability of other emulsions subjected to ultrasound treatment (SI values ranged from 97.8% to 99.8%) was of concern. Based on these results, generally, it appeared that whether the ultrasound was used before or after the formation of emulsions or not, the ultrasound (producing cavitation effect and strong microstreaming) would be a green and effective physical processing approach to promote the storage stability of surimi particles-stabilized Pickering emulsions with low concentration.

Fig. 7. Rheological properties of Pickering emulsions stabilized by surimi particles via two-step procedure. (a and b) The viscosity of Pickering emulsions at 3 h or 14 days; (c and d) The storage modulus ($G'$) of 0.6–2.0% emulsions at 3 h or 14 days; (e and f) The loss modulus ($G''$) of 0.6–2.0% emulsions at 3 h or 14 days.
3.4. Changes in microstructures of Pickering emulsions prepared by two-step method

Indeed, it is widely recognized that the microstructure of Pickering emulsions is critical to determine the stability of food emulsions and the aggregation behaviors of droplets during long-term storage [35]. In Fig. 5, the red spheres and blue particles referred to the oil droplets and surimi particles, respectively. Obviously, the distribution and assembly behaviors of surimi proteins in 0.6–2.0%/C, H-U, and U-H systems were diverse (Fig. 5). In 0.6–2.0%/emulsion (Control), an amount of protein particles surrounding oil droplets and some large protein aggregates existed in the continuous phase were found at 3 h. For H-U and U-H groups, the protein particles were anchored effectively onto the water–oil interface, as well as the large protein aggregates observed in control group also virtually abolished, which was preferable for the high stability of this system (Fig. 2). In the case of the H-U system, it was concluded that ultrasound could break down the formed oil droplets into fine emulsions by virtue of the formation, growth, and collapse of cavitation bubbles. Moreover, the surimi particles would be restructured at the newly formed interfaces and cross-linked to form a gel-like structure surrounding the oil droplets in this process [36]. Additionally, this different interface features in U-H groups could be explained by that the ultrasound treatment facilitated the uniform dispersion of salt-soluble surimi particles in aqueous phase (Figure S1), further improving the contact area and adsorption of solid particles in two-phase interface during emulsification process [26]. Further, the cross-linked protein particles after ultrasound in continuous phase is of concern, as this structure could hinder the migration of oil droplets and inhibit the polymerization of adjacent oil droplets [37]. Here, it was noteworthy that the solubility of surimi particles increased after ultrasonic dispersion (100, 300, 500, and 700 W) (see Figure S4). However, when the ultrasound output power was 700 W, the solubility of surimi particles in water just reached to 17.91%, meaning more than 82% of particles still were not dissolved in water. Thus, we could conclude that ultrasound improved the solubility of proteins in water, more importantly, it greatly promoted the dispersion of protein particles in water (Figure S1). This well dispersion of salt-soluble surimi particles in aqueous phase fulfilled a critical role in the formation of Pickering emulsions (U-H groups). As shown in Fig. 5, the emulsion droplets with diverse shape and sizes, and the increased protein aggregates in control group were found after 14 days storage, which was related to the desorption of solid particles from water-oil interface and the destabilization of this system. By comparison with the control, the emulsions in H-U and U-H groups (0.6–2.0%) could keep relatively intact structure and homogeneous sizes well after storage. This further demonstrated why the H-U and U-H systems (0.6–2.0%) had the higher stability (Fig. 2).

Here, to further reveal the role played by protein particles in the formation of Pickering emulsions prepared with two-step method, the cryo-SEM was used to observe the microstructure of the control, H-U-300 and U-H-300 emulsions (regarded as representative samples). The detailed three-dimensional microstructure was disclosed in Fig. 6. For the control (0.6–2.0%), the solid particles anchored onto the two-phase interface provided substantial evidence that surimi particles have the ability to act as Pickering particles to stabilize the emulsions. Actually, apart from the available adsorption, the formation of volume-filling networks of surimi particles fulfilled a more critical role for this system. In the 0.6–2.0%/C group, the continuous oil and ordered protein skeletons surrounding the oil droplets in the H-U and U-H emulsions (0.6–2.0%) were found, especially the U-H-300 sample, which might be the structural basis for the special storage stability of these samples [38,39]. However, it is noted that there also existed distinct difference in the stability of H-U and U-H samples. Therefore, it was speculated that varied rheological behaviors under different processing steps also affected the emulsified phase volume and storage stability of samples.

3.5. Changes in rheological properties of Pickering emulsions prepared by two-step method

3.5.1. Changes in viscosity

The viscosity properties of Pickering emulsions have always been a vital basis in analyzing the droplet–droplet coalescence and creaming. The viscosity as a function of shear rate is shown in Fig. 7. The prepared 0.6–2.0% samples all exhibited marked shear-thinning behaviors at 3 h, holding a high viscosity at initial low shear rates that gradually decreased with increased shear rates [40]. The fitting results of flow behavior index (n) also proved this shear-thinning property of emulsions (Table S1). From Fig. 7a, the pronounced diversity in viscosity values at same shear rates among the control, H-U and U-H groups was found. Wherein, the control and H-U groups had similar viscosity values in magnitude. In contrast, a noticeable increased resistance to flow was observed in the U-H groups (0.6–2.0%) at 3 h when the shear rate ranged from 0.1 to 100 s⁻¹. This phenomenon was attributed mostly to the stable structure of these emulsions such as the increased adsorption of protein particles on the oil–water interface, and the formation of three-dimensional networks of protein in aqueous phase after U-H step, as evidenced by the Fig. 6. Keerati-u-rai et al. [41] also pointed out that the soy protein aggregates distributed on the droplets surface as well as the formation of cross-linking or bridges between droplets both would increase the viscosity of emulsions. After the storage of 14 days, all samples still held shear-thinning behavior (Table S2). Nonetheless, there was an evident decrease in the viscosity feature for the 0.6–2.0%/C sample, which was due to the lower storage stability (Fig. 2) and the limited adsorption of solid particles on the oil–water interfaces (Figs. 5 and 6). Interestingly, the decreased viscosity was not observed in the H-U and U-H groups (0.6–2.0%), which further proved the microstructures of emulsions prepared by homogenization-ultrasonic or ultrasonic-homogenization approach had the potentials to ameliorate the storage stability of samples [42,43]. Briefly, these viscosity behaviors of the H-U and U-H groups (0.6–2.0%) further provided supporting evidence for the varied storage stability (Fig. 2).

3.5.2. Changes in storage and loss modulus

Apart from the viscosity characteristic, the storage and loss modulus are equally crucial to assess the emulsion stability and quality. As showcased in Fig. 7c and 7e, the storage modulus (G′) of the 0.6–2.0% samples treated with or without ultrasound at 3 h was all larger than the loss modulus (G″), suggesting these emulsions were presented in the semi-solid and elastic state [44]. Simultaneously, the H-U and U-H groups (0.6–2.0%) had higher G′ values than the 0.6–2.0%/C emulsion before and after storage (Fig. 7c and 7e). As shown in Fig. 6, the crosslinked protein networks around droplets or in continuous phase after homogenization-ultrasound and ultrasound-homogenization treatments might be thought to play a prominent role for this [39]. Additionally, it is also found that a correlation between both moduli and the internal solid network of O/W emulsion formed was clearly apparent, as reported by Liu and Tang [44]. It was worth noting that the G′ values in magnitude of U-H groups were higher than that of H-U groups, which was consistent with the viscosity behaviors (Fig. 7a and 7b). These results indicated ultrasound-homogenization step facilitated the formation of fully crosslinked and dense networks of surimi particles, which were extremely pivotal to provide steric hindrance or electrostatic repulsion to prevent coalescence or phase separation of Pickering emulsions within the usable period [38,45]. However, a slight increase in G′ and G″ values was detected under the frequency measurement range for all 0.6–2.0% emulsions after storage (Fig. 7c, 7d, 7e and 7f), which implied the surimi particles stabilized emulsions gradually transformed into emulsion gels with poor liquidity during storage (data not shown).
3.6. Proposed mechanisms

In previous work, our team not only prepared successfully the Pickering emulsions stabilized by surimi particles, but confirmed that the Pickering emulsions could maintain good physical stability under various pH and NaCl conditions [7]. However, in view of the salt-soluble property of surimi particles, it is a huge challenge to obtain fully dispersive protein solutions in the aqueous phase without the aid of other external means, which limits the preparation of O/W Pickering emulsions with high stability under lower surimi particle content (0.5–2.5 wt%) to a certain extent. In the present work, the two-step procedure involving homogenization-ultrasonic (H-U) and ultrasonic-homogenization (U-H) was utilized to fabricate the surimi particles based-Pickering emulsions. For H-U systems, the surimi particles stabilized coarse emulsions produced via mechanical homogenization in the first stage and then which were subjected to ultrasonic treatment (100–700 W) in the second step to prepare fine emulsions. With respect to U-H groups, the effective surimi particle dispersions were firstly obtained after the ultrasonic treatment (100–700 W). Subsequently, the final Pickering emulsions stabilized by surimi particles were obtained by mechanical homogenization. Compared to the 0.6–2.0%/C emulsion without ultrasound treatment, the excellent storage stability of Pickering emulsions stabilized by surimi particles (H-U and U-H groups) was observed (Fig. 2). A proposed schematic illustration about why the Pickering emulsions stabilized by surimi particles after homogenization-ultrasonic and ultrasonic-homogenization procedure could remain stable during 14 days storage was shown in Fig. 8. For H-U groups, the intense effects of cavitation phenomenon in the second stage produced smaller oil droplets and simultaneously increased the numbers of surimi particles anchored onto the two-phase interfaces, favoring the stability of emulsions during storage. According to the Figure S1 and S4, the ultrasound treatment promoted the dispersion of protein particles in water, further increasing the contact area between surimi particles and the newly formed interfaces in the homogenization process (U-H groups). These above-mentioned situations were beneficial to overcome the surface energy of dispersing immiscible liquids, which might be the potential internal reason for the difference of storage stability between H-U, U-H groups and the control emulsion (0.6–2.0%/C) [46]. Apart from this, the 0.6–2.0%/C emulsion had higher absolute zeta potential than each H-U and U-H groups whether stored or not (Fig. 4b). It could be deduced that the fully crosslinked and more strong three-dimensional networks of surimi particles was undoubtedly a major factor preventing the move and fuse together of droplets during storage for 0.6–2.0% emulsions (H-U and U-H groups) (Figs. 5 and 6) [38,47]. Dickinson et al. [48] once underlined the inhibiting effect of steric hindrance provided by crosslinked protein particles on the occurrence of Ostwald ripening in protein-stabilized O/W emulsions. After storage process, apparent phase separation phenomenon and a given mass of surimi particles precipitated in the aqueous phase at the bottom of the sample bottle were found in the 0.6–2.0%/C emulsion (Fig. 2). Moreover, there existed a sharp decrease in viscosity for the control group (Fig. 7a and 7b). These results all demonstrated during storage the surimi particles anchored onto the two-phase interfaces previously desorbed and the internal protein particle–particle interactions weakened when samples treated without ultrasound (Fig. 5). Additionally, the 0.6–2.0% emulsions (U-H treatment) had higher EPVF and SI values than the emulsions from H-U groups after storage (Fig. 2). The stronger electrostatic repulsions and initial rheological behaviors (higher viscosity, G' and G'' values) enabled the ultrasound treated-surimi particles stabilized Pickering emulsions with 0.6 oil–water ratio (U-H treatment) to remain excellent storage stability, as compared to the H-U treatment (Figs. 2, 4 and 7) [49]. Therefore, the three-dimensional network structure become more compact and stronger when using the effective-dispersed surimi particle suspensions in the aqueous phase to prepare Pickering emulsions (Figure S1 and S4), which

Fig. 8. Proposed schematic illustration of Pickering emulsions stabilized by surimi particles treated via two-step procedure at 3 h or 14 days.
might the possible reason for the slight difference in the storage stability between H-U and U-H groups (Fig. 2). In short, no matter which two-step method (homogenization-ultrasonic or ultrasonic-homogenization) was employed, the surimi particles-stabilized Pickering emulsions all kept excellently stable during storage period, compared to the control group.

4. Conclusion

In this paper, the surimi particles stabilized Pickering emulsions prepared by homogenization-ultrasonic (H-U) or ultrasonic-homogenization (U-H) method all had higher emulsified phase volume and stability index after 14 days storage. Although these existed slight changes in DL43 and zeta potential of H-U and U-H groups, the emulsions remained stable, especially for the U-H systems. These underlying mechanisms of this special storage stability mainly was ascribed to the effective anchor onto the oil-water interfaces and the compact and strong protein networks formed surrounding the oil droplets or in the continuous phase after ultrasonic. In contrast with H-U groups, using pre-sonicated surimi particles suspensions to produce Pickering emulsions possessed higher viscosity, G’ and G” values. This work provides insights into preparing Pickering emulsions with excellent storage stability using low concentration salt soluble aquatic proteins as solid particles without the aid of salt.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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