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Research Article

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Comparative Study on Stability of Emulsion and Foam Stabilized by Rambutan Seed Protein, Rambutan Seed Protein with High Intensity Ultrasonication Modification, and Soy Protein

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Abstract

Rambutan (*Nephelium lappaceum*) seed proteins (RSP) with/without high intensity ultrasonication modification were used as natural emulsifiers. Emulsions were prepared by using RSP powder of 1% (w/w) and corn oil of 5% (w/w). Emulsions prepared by rambutan seed protein with high intensity ultrasonication modification (US protein) showed high heat stability at 60 - 90 °C and the heat stability of emulsions was comparable to emulsions prepared by soy protein (SP). Heated emulsions prepared by rambutan seed protein without high intensity ultrasonication modification (No-US protein) contained more bridging flocculation than US protein emulsions. US protein emulsions and No-US protein emulsions exhibited high stability against coalescence after freeze thawing while SP emulsions transformed into emulsion gel. US protein emulsions and No-US protein emulsions displayed relatively similar surface charge characteristics and microstructure in varied pH (2 - 12) and salt concentrations (100 - 300 mM). Foam prepared by US protein and No-US protein powder of 1% (w/w) had comparable capacity and higher capacity than SP foam. No-US protein foam had comparable stability as SP foam and higher stability than US protein foam.

Keywords

Rambutan, Seed protein, High intensity ultrasonication, Emulsion stability, Foam stability, Heat stability, Freeze thaw stability, Soy protein isolate

Introduction

Currently, plant-based proteins have been widely interested according to the aspects of availability, economical cost, environmental sustainability, and healthy dietary protein choice [1]. One of the potential uses of plant-based proteins is as functional food ingredients such as emulsifiers and stabilizers. Plant-based proteins have natural emulsifying ability according to the amphiphilic nature of protein inducing protein binding at oil-water and air-water interface. Polymeric protein layering on oil droplet or air droplet surface created steric and electrostatic stabilization effects. Plant protein emulsifiers could be applied in food emulsion and foam products such as mayonnaise, dressing, milk, coconut milk, cream, sauce, soup, cake batter, and whip cream. Proteins extracted from new plants could be novel emulsifiers for future plant-based foods.

Emulsifying performance of plant-based proteins strongly depended on pH, salt, and temperature. Stability of emulsion was able to tune and control by adjusting these environmental parameters. Both pH and salt affected protein surface charges. By changing pH of environment, net charges of interface proteins were altered which altering electrostatic interaction among dispersed oil droplets. Salting screened protein charges. Increasing of salt concentrations reduced electrostatic repulsive force between oil droplets and induced droplet flocculation. Sarkar et al. [2] found that emulsions stabilized by tomato seed protein were

stable at all pH conditions, except at pH 2 to 4 that near the protein isoelectric points. At the pH near the protein isoelectric points, droplet flocculation occurred since the net charge of proteins approached to zero. Addition of calcium chloride salt at concentrations of higher than 50 mM caused droplet aggregation. Emulsions stabilized by tomato seed protein became unstable at temperatures higher than 80 °C according to protein coagulation by heat. Similarly, other emulsion studies demonstrated that emulsions prepared by tree peony seed proteins [3], chenopodium seed protein [4], jackfruit seed protein [5], Chinese quince seed protein [6], and date palm seeds protein [7] also had these pH, salt, and temperature dependence behaviors. Besides emulsions, foams prepared by chenopodium seed protein [4], tree peony seed protein [3], and Chinese quince seed protein [6] also displayed the pH dependence characteristics.

High intensity ultrasonication is considered as a green technology and ultrasonic processor is one of unit operation with clean operation [8, 9]. Ultrasound wave in a frequency range of 20 to 100 kHz passed through a small area sonotrode generated high intensity energy of 10 to 1,000 W/cm^2. High intensity ultrasonication had been used to reduce sizes of proteins and modify structures of proteins. The degree effects of high intensity ultrasonication depended on types of proteins and original structural conformation of proteins. Since the emulsifying activities of proteins related to protein molecular size, molecular conformation, and interfacial arrangement, the emulsifying ability of sonicated plant-based proteins became enhanced. Previous research studies showed that small sonicated protein particles transferred rapidly to adsorb at interface, and this possibly lowered interfacial tension and better reduced oil droplet sizes. Sonicated proteins with high surface hydrophobicity were more effectively absorbed at oil-water interface forming stable interfacial membranes. Emulsions stabilized by modified plant-based proteins exhibited better emulsion stability. Creaming stability of emulsions was improved with lesser droplet flocculation and lesser coalescence. Higher emulsion stability index and higher emulsion activity index of emulsions prepared by sonicated SP [10, 11], black bean protein [12], and peanut protein [13], had been reported.

Foaming ability was also enhanced by applying proteins treated with high intensity ultrasonication as stabilizer [8, 14]. The foam formation depended on adsorption rate of proteins at air-water interface, interfacial attachment of hydrophobic domains, and unfolding and reorganizing of proteins at interface. Broken protein particles diffused at faster rates to absorb at air-water interface. Proteins had better interfacial adsorption and partially unfolded interfacial proteins had higher cohesiveness. Produced foam contained high volume with small bubble sizes. The increased capacity of foam had been noticed in foams prepared by sonicated SP [15] and jackfruit seed protein [16].

In agricultural countries, fruit seeds are one of the major industrial wastes produced from industrial food processing plants. Since fruit seeds contain some proteins, extraction, and utilization of proteins from fruit seeds will help in reducing environmental burdens from seed waste and creating new agricultural by-product. The recent work from our research group, RSP was extracted from rambutan seeds received from canned fruit companies in Thailand. High intensity ultrasonication had been applied to modify protein structures [17]. It had been found that some polypeptides were disrupted into smaller units and emulsions prepared by the modified RSP had lesser flocculation and better creaming stability than emulsions prepared by non-modified RSP. In the previous study [17], emulsions were stored only at room temperature condition. This continuing work, more investigation on the effects of environmental factors on characteristics and stability of emulsions prepared by RSP, with (US) and without (No-US) high intensity ultrasonication modification, had been performed. The effects of heating at different temperatures and freezing thawing on stability of emulsions stabilized by US protein and No-US protein were compared. The effects of pH and salt concentrations on oil droplet characteristics were examined. Properties of foams prepared by US protein and No-US protein such as foam capacity and foam stability were investigated. Commercial SP isolate was used for a compared study.

Materials and Method

Materials

Rambutan seeds were supplied by Malee Group Public Company Limited (Sampran, Nakorn Pathom, Thailand) and Universal Food Public Company Limited (UFC, Muang, Nakorn Pathom, Thailand). Sodium di-hydrogen phosphate and di-sodium hydrogen phosphate 12-hydrate were supplied by Kemaus (Australia). 37% hydrochloric acid was supplied by QReC (New Zealand). Sodium hydroxide was supplied by KemAus (Elago Enterprlses Pty Ltd, NSW, Australia). SP isolate (SINOPRO 910) was supplied by Shandong Sinoglory Health Food Co, Ltd. (Shandong, China). The protein content in SP isolate was 90%. Corn oil was supplied by Mazola Refined Corn Oil, Lam Soon Public Co., Ltd. (Samutprakarn, Thailand). Distilled water was used for emulsion preparation.

Protein extraction and protein powder preparation

RSP was extracted and protein powder were prepared as reported in the previous work [17]. RSP was extracted from ground rambutan seeds by alkaline extraction method using an overhead stirrer (Model RW20 Digital, IKA, Germany) at a controlled stirring speed and a stirring time of 20 min. A weight ratio between ground seeds and phosphate buffer (pH 8) of 1:20 was used. After removal of solid residues by filtration, the protein solutions were treated by an ultrasonic processor (UP400S, Hielscher Ultrasonics GmbH, Germany, with a titanium sonotrode of 2.2 cm diameter) at an amplitude of 80% and a cycle mode of 0.5. The sonication time of 20 min and the sonication power of 320 W were used. This US power was approximated from 80% of the maximum power of 400 W [14, 18, 19]. The sample container was put in an ice water bath during sonication to prevent samples became overheated.

RSP was precipitated at the protein's isoelectric points by pH adjustment to 3.8 - 4.0 using 1 - 3 M HCl solutions. The centrifugation (Universal Model PLC-012, Gemmy Industrial Corporation, Taipei, Taiwan) at a rotation time of 10 min was applied to separate concentrated proteins. The concentrat-

ed proteins were layered on plates and air dried in a conventional tray drier at a controlled temperature of 45 °C for 12 to 36 h. The dried proteins were ground into powder and kept in capped bottles. The protein content in the dried powder was 52.3 ± 7.7% determined by Kjeldahl method using a protein conversion factor of N × 6.25.

Emulsions preparation, storage, and characterization

Emulsion samples of 100 g contained corn oil of 20% (w/w) and protein powder (No-US protein, US protein, and SP isolate) of 1% (w/w) were prepared and homogenized by the same ultrasonic processor. Protein powder was dissolved in phosphate buffer (pH 8) first and mixed with corn oil. The mixtures of corn oil and protein solutions were sonicated at an amplitude of 80% and a 0.5 cycle mode for 4 min. The homogenized sample container was put in an ice water bath to prevent samples from becoming overheated. All the prepared emulsion samples were kept in bottles sealed with caps.

Emulsion samples of 10 g were put into glass test tubes. For the pH study, the pH of each emulsion sample was adjusted to 2, 4, 6, 8, 10, or 12 by using 1 - 3 M hydrochloric acid solutions and 1 - 3 M sodium hydroxide solutions. For the ionic strength study, sodium chloride salts were added and dissolved to each emulsion sample at a concentration of 100, 200 or 300 mM. For the heating study, the emulsions test tubes were put in beakers containing water at a controlled temperature of 60, 70, 80, 90 or 100 °C for 1 h. The beakers were put on a magnetic stir plate connected with a temperature controlled thermocouple. For the freezing study, glass bottles containing emulsion samples were put in a freezer at a controlled temperature of -21 °C for 22 h. The emulsions were thawed at room temperature for at least 2 h.

Droplet size distribution measurement

A laser particle size distribution analyzer (Mastersizer S, Malvern Instruments Ltd., Worcestershire, UK) was used for determining droplet size distributions of emulsions, either with or without applying the sonication mode. The refractive indices of water and corn oil were 1.33 and 1.47, respectively. Both surface-volume mean diameters (d_{32}) and volume-weighted mean diameters, (d_{43}) of emulsions were reported.

Optical microscopy

An optical microscope (Alphaphot-2, YS2-H, Nikon Corporation, Japan) at the total magnification of 1000× was used for examining emulsion microstructure. A microscope eyepiece camera (AM423 Dino-Eye USB, AnMo Electronics Corporation, Taipei, Taiwan) and DinoCapture software installed on a computer were used.

Zeta potential measurement

A particle electrophoresis instrument (Zetasizer nano ZSP, Malvern Instruments, United Kingdom) was used for determining electrical charges of oil droplets. The emulsion samples were diluted to 1/100 by using phosphate buffer (pH 8). The sample was put into an electrophoresis cell that had electrodes at both ends. The direction and velocity of oil droplets moved in the applied electrical field were observed under a microscope and the oil droplet surface charge or zeta-potential was calculated.

Determination of foaming properties

Protein solutions with protein powder of 1% (w/w) were prepared in phosphate buffer (pH 8). The protein solutions were stirred on a magnetic stirred plate until protein powder was fully dissolved. The samples were filtered through filter paper. Protein solutions in an amount of 15 ml were put in a 50 ml centrifugal tube with scales labeled on the tube. The samples were blended by a high-speed homogenizer (HG-15A, DAIHAN Scientific, Co., Ltd., Korea) at a speed of 10,000 rpm for 2 min to incorporate air into the samples. The total foam volume was recorded immediately (0 h) and after 1 and 2 h standing. Foam capacity and foam stability were calculated by equations 1 and 2, respectively.

$$Foam\ capacity\ (\%) = \frac{V_0}{V} \times 100 \quad [1]$$

$$Foam\ stability\ (\%) = \frac{V_{1\text{-}2}}{V_0} \times 100 \quad [2]$$

Where: V is the initial volume of protein solution (V = 15 mL). V_0 is the foam volume at 0 h. $V_{1\text{-}2}$ is the foam volume at 1 or 2 h [3, 6].

Statistical analysis

All the experiments were conducted at least duplicates. The reported data were the mean ± standard deviation. Statistical analysis was performed by using SAS Enterprise guide 7.1 software (SAS Institute Inc, Cary, NC, USA) employing ANOVA procedure and Tukey test ($p < 0.05$).

Results and Discussion

Effects of heating

The first column of figure 1 shows the micrograph of US protein emulsions after heated at different temperatures for 1 h. US protein emulsions heated at 60 °C, 70 °C, 80 °C, and 90 °C had mostly dispersed and non-flocculated oil droplets. US protein emulsions heated at 100 °C contained some flocculated oil droplets. Table 1 shows that the mean droplet sizes d_{32} and d_{43} measured without sonication of US protein emulsions heated at temperatures below 100 °C were 1.2 - 1.4 µm and 2.0 - 3.0 µm, respectively. Only US protein emulsion heated at 100 °C had larger mean droplet sizes d_{32} and d_{43} of 2.4 µm and 9.6 µm, respectively, and its size distribution was broad with double peaks (Figure 2A). The board double peaks occurred due to the presence of droplet flocculation induced by heat. Heat changed native structures of RSP. The hydrophobic sites that originally located within hydrophobic inner domain of protein were exposed to the surface and the surface hydrophobicity of protein increased [20]. The hydrophobic interaction between denatured proteins at interface of different oil droplets possibly connected oil droplets together [2]. By using the sonication mode during the size determination (instead of the non-sonication mode), the droplet size distributions of US protein emulsions at all heating temperatures, including at 100 °C, appeared similarly as one narrow peak (Figure 2B). Flocs of oil droplets that were observed at 100 °C were separated apart by sonic vibration. The mean droplet sizes d_{32} and d_{43} measured with sonication of all heated US protein emulsions (60 to 100 °C) were 1.1 - 1.2 µm and 1.3 - 1.5 µm, respectively (Table 1).

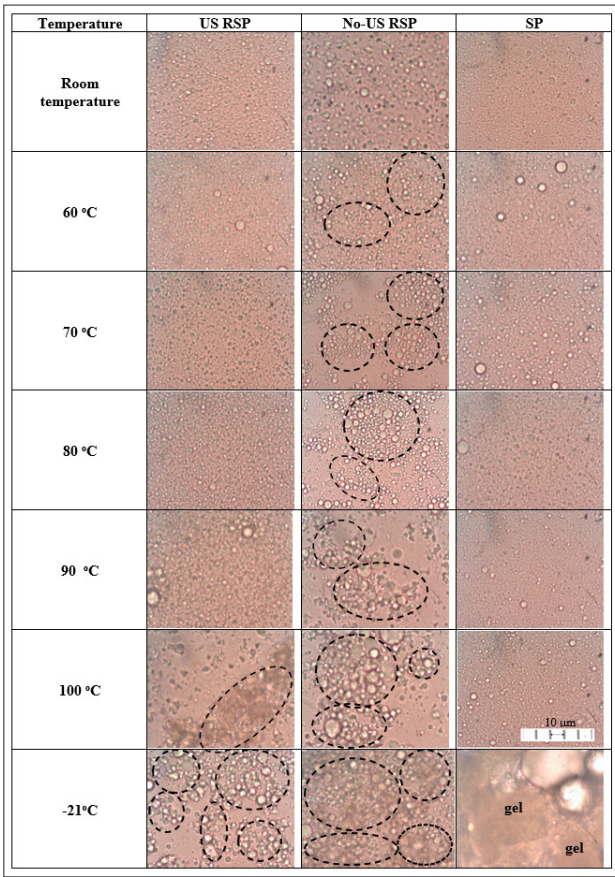

Figure 1: Micrograph of emulsions (5% corn oil, 1% protein powder) stored at different temperatures (magnification of 1000×).

The second column of figure 1 shows that No-US protein emulsions heated at 60 °C, 70 °C, and 80 °C for 1 h contained some flocculated oil droplets and emulsions heated at 90 °C and 100 °C for 1 h became extensively flocculated. The mean droplet sizes d_{32} and d_{43} measured without sonication of No-US protein emulsions heated at 60 to 80 °C were 1.5 - 1.7 µm and 2.6 - 3.5 µm, respectively, and emulsions heated at 90 to 100 °C were 1.9 - 2.2 µm and 4.1 - 4.7 µm, respectively (Table 1). The droplet size distributions measured without sonication of No-US protein emulsions at all heating temperatures were broad (Figure 2C). The size distributions measured with sonication became narrower (Figure 2D) due to separation of oil droplets flocs. Correspondingly, the mean droplet sizes measured with sonication of No-US protein emulsions at all heating temperatures were smaller than the mean droplet sizes measured without sonication. The mean droplet sizes d_{32} and d_{43} measured with sonication of all heated No-US protein emulsions (60 to 100 °C) were 1.1 - 1.5 µm and 1.5 - 2.7 µm, respectively (Table 1).

By comparison between US protein emulsions and No-US protein emulsions, obviously more degrees of flocculation were observed in No-US protein emulsions. Droplet flocculation was observed in No-US protein emulsions even at mild heating temperature of 60 °C. The mean droplet sizes (d_{32} and d_{43}) measured without sonication of No-US protein emulsions were larger from more flocculation, as compared between No-US emulsions and US emulsions at the same heating temperature (Table 1). The previous work showed that high intensity ultrasonication reduced molecular sizes of RSP into smaller units [17]. Thus, No-US protein contained more amounts of large-size proteins than US protein to cause more bridging flocculation. The denatured large-size protein could create more bridging between oil droplets. In both No-US protein emulsions and US protein emulsions, no coalescence and no oilly off or oil phase separation were observed in all emulsions.

The SP emulsions result from the micrograph (the third column of Figure 1), the droplet size distributions (Figure 2E and figure 2F), and the mean droplet sizes (Table 1) did not show any droplet flocculation or coalescence after heated at 60 to 100 °C for 1 h. A single narrow peak emulsion was ob-

Table 1: The mean droplet sizes of emulsions (5% corn oil, 1% protein powder) stored at different temperatures.

Temperature	US RSP				No-US RSP				SP			
	d_{32}		d_{43}		d_{32}		d_{43}		d_{32}		d_{43}	
	w/o sonication	w sonication	w/o sonication	w sonication	w/o sonication	w sonication	w/o sonication	w sonication	w/o sonication	w sonication	w/o sonication	w sonication
Room temperature	1.655 ± 0.771^{abA}	1.162 ± 0.224^{aA}	1.545 ± 0.064^{Bb}	1.254 ± 0.104^{Ba}	1.390 ± 0.438^{bB}	1.141 ± 0.211^{aA}	1.893 ± 0.605^{aB}	1.454 ± 0.232^{aA}	0.704 ± 0.019^{cB}	0.686 ± 0.001^{eB}	0.922 ± 0.001^{dB}	0.942 ± 0.001^{bB}
60 °C	1.405 ± 0.304^{ab}	1.056 ± 0.105^{a}	2.645 ± 0.715^{b}	1.360 ± 0.240^{a}	1.705 ± 0.898^{b}	1.137 ± 0.009^{a}	3.450 ± 2.843^{a}	1.499 ± 0.045^{a}	0.932 ± 0.001^{a}	0.926 ± 0.001^{a}	1.260 ± 0.000^{b}	1.230 ± 0.006^{a}
70 °C	1.205 ± 0.106^{cb}	1.061 ± 0.102^{a}	1.987 ± 0.231^{b}	1.340 ± 0.113^{a}	1.545 ± 0.672^{b}	1.100 ± 0.085^{a}	2.622 ± 1.539^{a}	1.454 ± 0.122^{a}	0.931 ± 0.000^{a}	0.924 ± 0.001^{b}	1.280 ± 0.000^{a}	1.210 ± 0.000^{a}
80 °C	1.265 ± 0.078^{ab}	1.067 ± 0.127^{a}	2.207 ± 0.349^{b}	1.309 ± 0.097^{a}	1.675 ± 0.856^{b}	1.244 ± 0.217^{a}	3.387 ± 2.607^{a}	1.597 ± 0.189^{a}	0.801 ± 0.000^{b}	0.802 ± 0.001^{d}	0.942 ± 0.000^{c}	0.942 ± 0.000^{b}
90 °C	1.415 ± 0.247^{ab}	1.124 ± 0.192^{a}	3.069 ± 0.709^{b}	1.377 ± 0.217^{a}	2.180 ± 1.541^{a}	1.515 ± 0.544^{a}	4.717 ± 4.403^{a}	2.730 ± 1.665^{a}	0.801 ± 0.000^{b}	0.803 ± 0.001^{c}	0.942 ± 0.000^{c}	0.944 ± 0.001^{b}
100 °C	2.420 ± 1.725^{a}	1.165 ± 0.262^{a}	9.596 ± 6.243^{a}	1.490 ± 0.424^{a}	1.935 ± 1.223^{b}	1.169 ± 0.069^{a}	4.090 ± 3.592^{a}	1.582 ± 0.087^{a}	0.799 ± 0.000^{b}	0.802 ± 0.001^{d}	0.942 ± 0.000^{c}	0.943 ± 0.001^{b}
-21 °C	2.905 ± 0.276^{A}	1.038 ± 0.093^{A}	7.420 ± 1.245^{A}	1.579 ± 0.129^{A}	2.900 ± 0.877^{A}	1.101 ± 0.126^{A}	6.760 ± 3.465^{A}	1.620 ± 0.127^{A}	35.100 ± 0.361^{A}	14.750 ± 0.763^{A}	372.500 ± 111.300^{A}	360.655 ± 111.300^{A}

Note: Data are provided as means ± standard deviation of at least duplicate analyses. Different lower case letter symbols above the bar indicate significantly difference of data of different heating temperatures in the same column ($p < 0.05$). Different upper case letter symbols above the bar indicate significantly difference of data between freezing temperature and room temperature in the same column ($p < 0.05$).

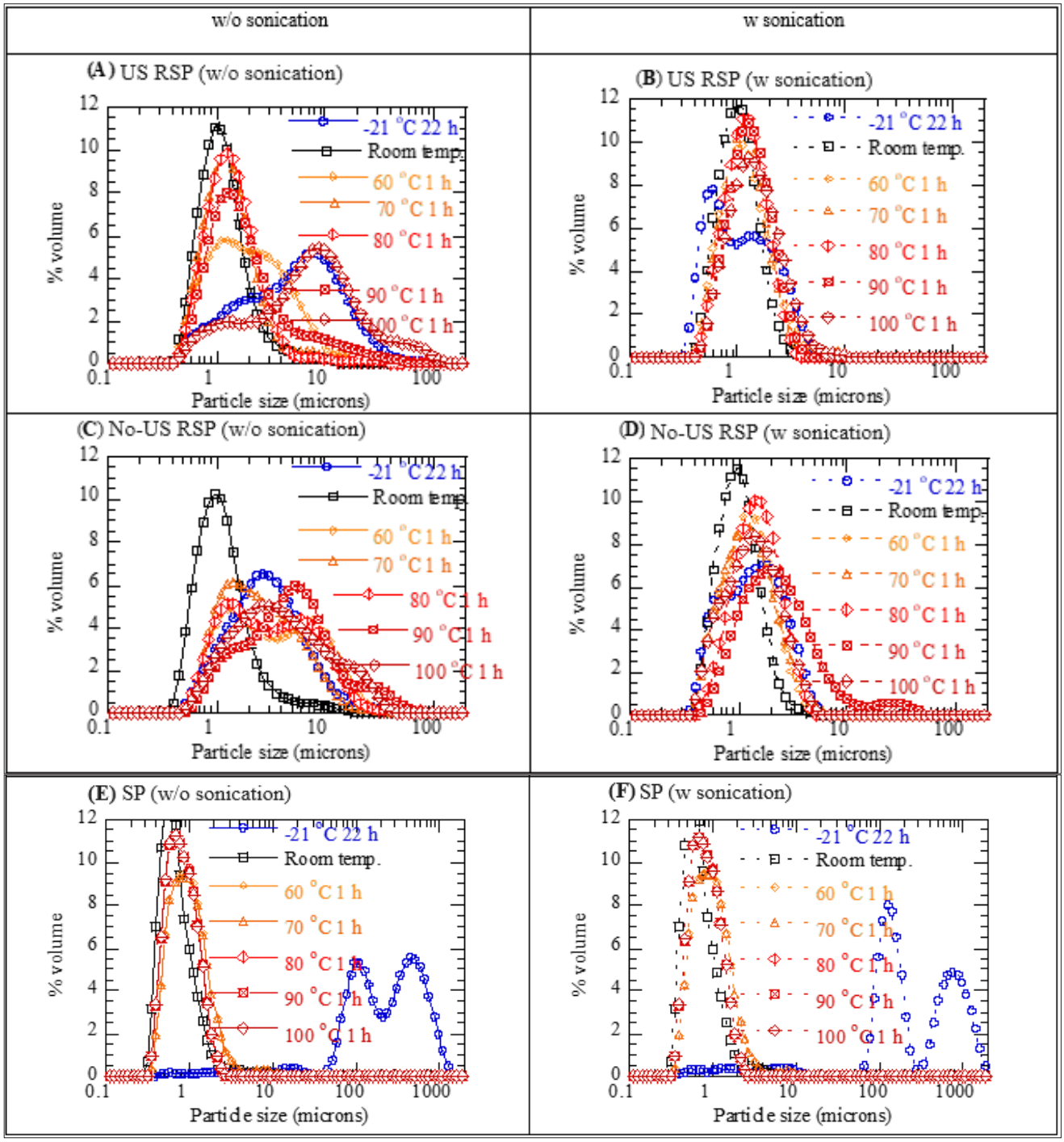

Figure 2: The droplet size distributions of emulsions stored at different temperatures, without (A, C, E) and with sonication (B, D, F).

served during size measurement without and with sonication (Figure 2E and figure 2F). The mean droplet sizes d_{32} and d_{43} measured without and with sonication of SP emulsions heated at all heating temperatures (60 to 100 °C) were 0.7 - 0.9 µm and 0.9 - 1.3 µm, respectively (Table 1).

Effects of freeze thawing

The last row of the micrograph in figure 1 shows that both US protein emulsions and No-US protein emulsions after frozen at -21 °C and thawed became extensively flocculated. The droplet size distributions of both emulsions measured without sonication appeared as a large broad peak from flocculated oil droplets (Figure 2A and figure 2C). The size distributions measured with sonication became a narrower single peak (Figure 2B and figure 2D), indicating the flocs of oil droplets induced by freeze thawing were separated apart by sonic vibration. The mean droplet sizes (d_{32} and d_{43}) measured with sonication of both emulsions were smaller than the mean droplet sizes measured without sonication (Table 1). The mean droplet sizes d_{32} and d_{43} measured with sonication reduced to be 1.1 µm and 1.6 µm, respectively, similarly as the sizes of the initial emulsions. Both US protein emulsions and No-US protein emulsions remained stable against coalescence. The outside appearance of both US protein emulsions and No-US protein emulsions remained as white liquid emulsions without oilly off or oil phase separation (Figure 3).

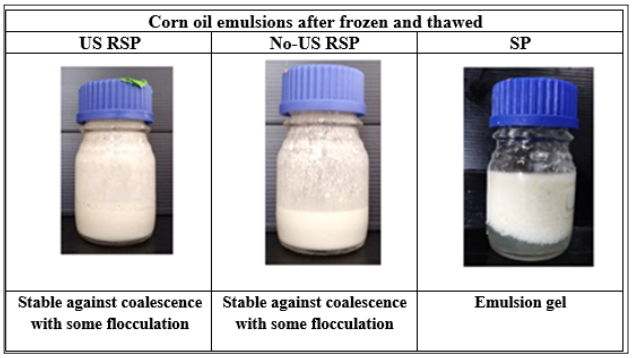

Figure 3: The visual appreance of emulsions (5% corn oil, 1% protein powder) after frozen (-21 °C, 22 h) and thawed.

Differently, SP emulsions completely transform into emulsion gels after freeze thawing (Figure 1). The white semi solid floated on top of emulsion samples (Figure 3). Figure 2E shows that these large particles appeared as a second peak at around 100 to 1,000 µm. The second peak of these particles did not disappear from the size distribution measured with sonication (Figure 2F). Other studies reported that emulsions prepared by SP became flocculated after freezing [21, 22] and the flocculation of oil droplets leaded to emulsion gel formation [23]. The aggregated SP by cold aggregation of unabsorbed storage globulins was also detected in the aqueous phase [21]. Since RSP contained a relatively higher content of hydrophobic amino acids [17], US protein and No-US protein were supposed to have a compact structure with lesser tendency to form gel. Also, the protein contents in RSP powder were lower than in SP isolate that might not facilitate the gel formation. Further investigation on the gelation behaviors of RSP was also suggested.

Effects of pH and salts

Figure 4 and figure 5 show the micrograph and the oil droplet surface charge (zeta potential) of all three types of emulsions at different pH, respectively. US protein emulsions show extensive droplet flocculation only at pH 4. The oil droplet surface charge of US protein emulsions at pH 4 was a small negative value of -10.5 ± 6.3 mV which was the pH that most closing to zero or neutral net charge. This pH was supposed to be nearest to the isoelectric points of RSP that numbers of positively charged amino groups being balanced by numbers of negatively charged carboxyl groups. Since the repulsive force between oil droplets was mostly reduced at this pH, oil droplets could come into closer contact. Differently, at pH 2, 6, 8 10, and 12, US protein emulsions contained mostly dispersed and non-flocculated oil droplets. The surface charges of oil droplets were a positive value at pH 2 (the pH that lower than the protein isoelectric points) and negative values at pH of 6 to 12 (the pH that higher than the protein isoelectric points). As pH increased from 6 to 12, the negative charges gradually increased as the carboxyl groups became more negative, and the amino groups became neutral. More dispersion of non-flocculated droplets in US protein emulsions at higher pH conditions was by more electrostatic repulsion from increasing charges at oil droplet surface.

Similar to US protein emulsions, the surface charge of

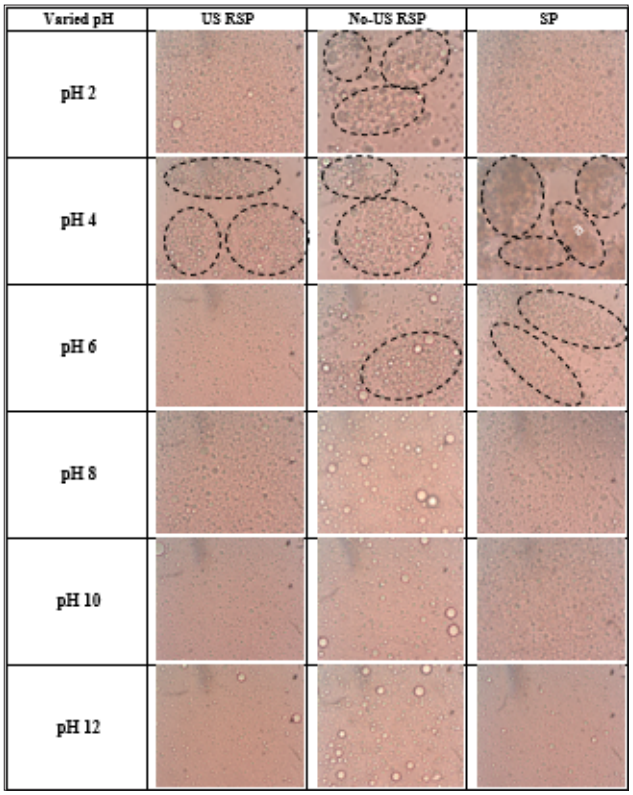

Figure 4: Micrograph of emulsions (5% corn oil, 1% protein powder) at different pH conditions (magnification of 1000×).

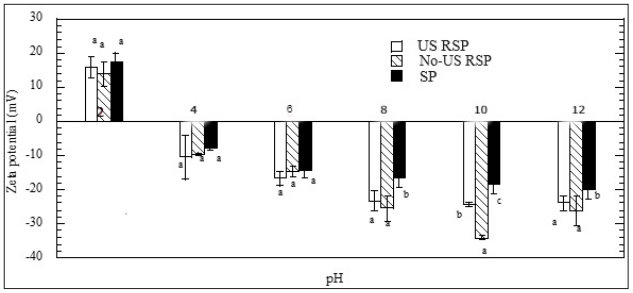

Figure 5: Oil droplet surface charges (zeta potential) of emulsions (5% corn oil, 1% protein powder) at different pH conditions. Different letters at the same pH indicate significantly different data ($p < 0.05$).

oil droplets in No-US protein emulsions was a small negative value of -9.5 ± 0.4 mV at pH 4 (Figure 5) and extensive flocculation occurred at this pH 4 (Figure 4). A slight flocculation observed at pH 2 and pH 6 was due to low surface charges that not sufficient to create full repulsion between oil droplets. The surface charges at pH 2 and pH 6 were +13.9 ± 3.4 mV and -14.6 ± 1.5 mV, respectively. Dispersion of non-flocculated droplets in US protein emulsions at other higher pH of 8, 10, and 12 (Figure 4) was from the negatively charged proteins surrounding oil droplet surface (Figure 5).

The surface charge characteristics of both US protein emulsions and No-US protein emulsions were quite similar. The surface charges of oil droplets in US protein emulsions and No-US protein emulsions at pH varied from 2 to 12 were within a same range of -34 to + 16 mV (Figure 5). Therefore, the magnitudes of electrostatic repulsive force among oil drop-

lets stabilized by these two types of proteins were supposed to be in a similar range. From the previous study [17], the conformation of RSP did not significantly alter by the applied high intensity ultrasonic power and time. The relative similarity of surface charge characteristics of US protein emulsions and No-US protein emulsions might reflect such unaltered structural conformation of RSP after the high intensity ultrasonication treatment.

The pH depedence characteristics of SP emulsions in Figure 4 show extensive oil droplets flocculation at pH 4. The pH of around 4 was also supposed to be the isoelectric points of SP with a low net charge (Figure 5). Slight flocculation was also observed in SP emulsions at pH 6 due to non-sufficient charge repulsion at the zeta potential of -14.4 ± 1.2 mV. By increasing pH further from 8 to 12, the oil droplet surfaces were more negatively charged and non-flocculated droplets were detected. It was noticed that the charges of SP emulsions were less negative at the higher pH conditions of 8, 10, and 12 compared to RSP emulsions.

Besides the surface charge of protein that depending on pH, it also depended on the ionic strength. Figure 6 shows the micrograph of emulsions stabilized by US protein, No-US protein, and SP at varied sodium chloride concentrations from 100 to 300 mM. Oil droplets in all emulsions became extensively aggregated at a relatively low salt concentration of 100 mM. The net repulsive force between oil droplets stabilized by US protein, No-US protein, and SP became relatively weak in the presence of salt due to the charge screen effect.

Foaming properties

Figure 7A shows the capacity of foam prepared by US protein, No-US protein, and SP. The capacity of US protein foam was comparable to No-US protein foam (not significantly difference, $p > 0.05$), but higher than SP foam (significantly difference, $p < 0.05$). SDS-PAGE analysis from the previous work revealed that RSP consisted of two main protein bands at the molecular weight of 55 and 36 kDa and the 36 kDa band consisted of two subunits of 22 and 18 kDa [17]. SP had two major globular proteins: glycinin and β-conglycinin. Glycinin, with molecular weight of 300 to 400 kDa consisted of six subunits of acidic A (35 kDa) and basic B (20 kDa) subunits. β-conglycinin with molecular weight of 150 to 250 kDa consisted of three subunits of 67, 71, and 50 kDa [24, 25]. Since the molecular size of proteins in rambutan protein seemed to be smaller than in SP [17], they probably diffused faster to adsorb at air-water interface and facilitated foam formation. This could be a reason for foam prepared by RSP foam having a higher capacity than foam prepared by SP. The foam capacity of US protein foam and No-US protein foam were comparable and the surface tension between air-water interface of both proteins were also comparable [17].

Figure 7B shows that the stability of US protein foam after 1 h standing was slightly lower than the stability of No-US protein foam. However, the stability of US protein foam after 2 h standing was obviously much lower than No-US protein foam (significantly difference, $p < 0.05$). No-US protein as protein mixtures consisted of both large-size and small-size

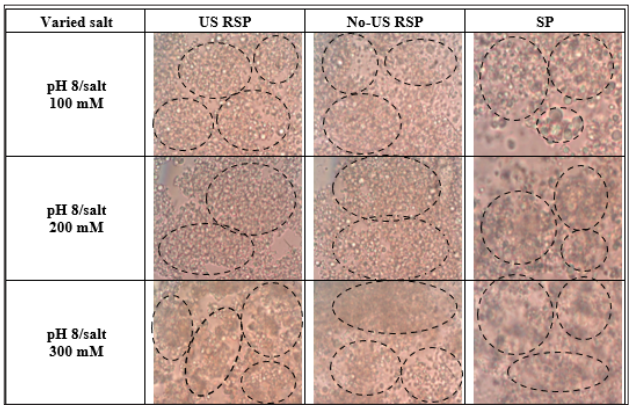

Figure 6: Micrograph of emulsions (5% corn oil, 1% protein power) at different NaCl concentrations (magnification of 1000×).

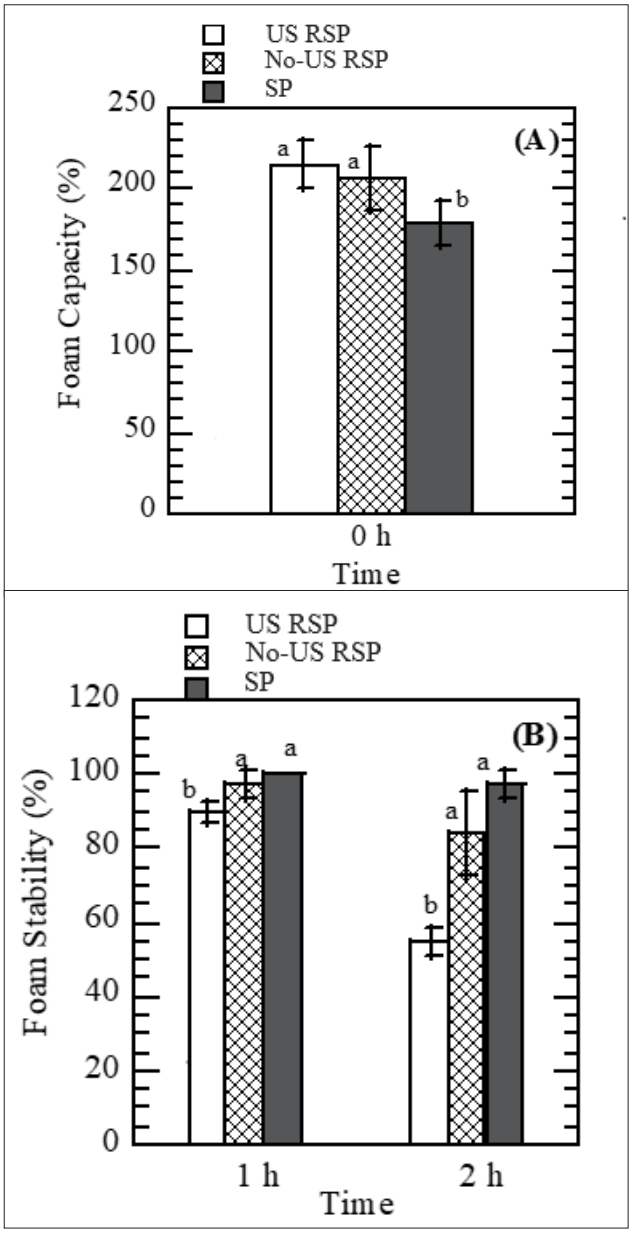

Figure 7: (A) Foam capacity and **(B)** foam stability of foams prepared by 1% protein powder. Different letters at the same time indicate significantly different data ($p < 0.05$).

proteins, while US protein consisted of lesser large-size proteins [17]. No-US protein could better surround air droplets and form different interfacial membranes that better protected air droplets such as reducing the Ostwald ripening. The stability of No-US protein foam after 1 an 2 h standing was comparable to SP foam (not significantly difference ($p > 0.05$). SP was also a mixture of proteins with different molecular sizes that were also supposed to produce high foam stability. The use of mixed emulsifiers or mixed surfactants to reduce Ostwald ripening rate had been reported before [26, 27].

Conclusion

This study displayed that RSP both with and without high intensity ultrasonication modification exhibited emulsifying and foaming abilities. For the heating process at 60 to 90 °C of 1 h, US protein emulsions demonstrated high stability against both coalescence and flocculation. No-US protein emulsions demonstrated high stability only against coalescence. For the freezing-thawing process, US protein emulsions and No-US protein emulsions demonstrated high stability against coalescence with some flocculation. The droplet surface charges of US protein emulsions and No-US protein emulsions in variation of pH conditions from 2 to 12 were within the same range. The isoelectric points of US protein and No-US protein were around 3.8 to 4.0. Droplet flocculation occurred in US protein emulsions and No-US protein emulsions at the pH approached the protein isoelectric points and in the condition of sodium chloride concentrations of 100 mM and more. No-US protein foam had comparable capacity to US protein foam. No-US protein foam had higher stability than US protein foam suggesting interfacial membranes of No-US protein were better for air droplets stabilization. This study revealed that RSP was one of plant seed proteins that having potential future use as an alternative emulsifier to SP. Even though the protein contents in the prepared US protein powder and No-US protein powder were relatively lower than in SP isolate, their emulsifier properties were comparable. The use of higher concentrations of RSP powder could be further investigated for suitable future applications.

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Conflict of Interest

None.

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