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Comparative analysis of the effect of ultrasonic treatment with ice and without ice cooling on the stability of oil-in-water emulsions stabilized by fava bean (*Vicia fava* L.) protein

Susma Bhattarai,*[a] Sanjaya Karki,[b] Yottha Srithep,[c] Kunwadee Kaewka[a] and Arissara Phosanam *[a]

Ultrasonic (US) treatment has attracted significant attention for improving emulsion stability; however, its effects on fava bean protein (FBP)-based emulsions remain underexplored. This study investigates the effect of US-treatment on the stability of oil-in-water emulsions formulated with FBP concentrate. Emulsions were sonicated for 5–30 minutes under two conditions: ice ultrasonic treatment (IUT) and without ice ultrasonic treatment (WIUT). Stability was assessed through physicochemical analysis, including color, ζ-potential, microscopic imaging, FTIR spectroscopy, centrifugal stability, and storage stability. The results showed that US-treatment significantly increased lightness (L^*) by 26.57% (IUT) and 26.35% (WIUT) and improved centrifugal stability from 35.60% to 77.52% (IUT) and 82.03% (WIUT) after 30 minutes. Moreover, US-treatment reduced the droplet size and turbidity while enhancing emulsion stability by promoting protein adsorption at the oil–water interface. US-treated samples exhibited significantly higher absolute ζ-potential values than the control. With increasing treatment duration, absolute ζ-potential reached 24.96 mV (IUT) and 28.68 mV (WIUT) after 30 minutes, indicating enhanced emulsion stability. FTIR analysis revealed structural modifications in proteins and stronger protein–oil interactions. Over a 15-day storage period, the US-treated emulsion exhibited improved stability, with WIUT samples performing better than those treated under IUT. Overall, these findings highlight the potential of ultrasonic processing for developing stable emulsions with extended shelf life, applicable to plant protein-based food formulations.

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Sustainability spotlight

With the rising global demand for plant-based products, this study highlights an eco-friendly approach, *i.e.*, ultrasound for improving the emulsion stability of fava bean protein. Although fava beans are a source of high-quality proteins, their native emulsifying activity is limited and requires modification. In this study, ultrasound treatment was applied under two conditions: with ice (IUT) and without ice (WIUT), to evaluate its effectiveness in enhancing emulsion stability. The results showed that ultrasound treatment effectively improved the stability of the protein, with significant differences observed between IUT and WIUT conditions, indicating the influence of temperature on protein functionality. Moreover, this approach supports Sustainable Development Goals (SDGs) 3, 9, and 13 by promoting healthier protein diets, encouraging innovation in food processing, and supporting climate action through energy-efficient technologies. Overall, the findings demonstrate that ultrasound is an effective and sustainable strategy for stabilizing fava bean protein emulsions, highlighting its potential for sustainable processing and application in various food formulations.

1. Introduction

Emulsions are widely used in food products such as ice cream, soybean milk, and beverages, as well as in cosmetics and pharmaceuticals, primarily due to their ability to enhance texture, stability, and the effective delivery of active ingredients.[1] Emulsions offer various benefits, including encapsulation of functional compounds to improve the sensorial and physicochemical properties of final products.[2] They play a key role in the texture, taste, and stability, contributing to the creaminess of salad dressings and mayonnaise, the smoothness of ice

[a]*Department of Food Technology and Nutrition, Faculty of Natural Resources and Agro-Industry, Kasetsart University Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon 47000, Thailand. E-mail: arissara.p@ku.th; susmabhatta23@gmail.com; kunwadee.ka@ku.th*

[b]*Kasetsart University International College (KUIC), Kasetsart University, 50 Ngam Wong Wan Road, Chatuchak 10900, Bangkok, Thailand. E-mail: karkisanjaya75@gmail.com*

[c]*Manufacturing and Materials Research Unit, Faculty of Engineering, Mahasarakham University, Kantarawichai, Mahasarakhan, 44150, Thailand. E-mail: yottha.s@msu.ac.th*

cream, and the rich consistency of chocolate, enhancing sensory appeal.[3]

Due to the rising demand for healthier and more sustainable foods, recent research has focused increasingly on plant-based proteins as natural emulsifiers.[4–6] These proteins are valued for their nutritional benefits and amphiphilic properties, which enable them to self-aggregate and form continuous, uniform layers around oil droplets, thereby enhancing emulsion stability.[7] In addition, they help stabilize oil droplets and air bubbles, form gel networks, and increase system viscosity.[8] Despite extensive research on plant proteins as emulsifiers, studies specifically on fava bean protein are limited, although it holds great potential as an emulsifying agent.

Fava bean (*Vicia fava* L.) is one of the world's oldest cultivated crops and ranks sixth among legume crops in global production. Its dietary significance derives from its high protein content and bioactive compounds, which contribute to health maintenance and disease prevention.[9] It is widely cultivated across Africa, Asia, Europe, and Latin America, with global production reaching approximately 6 million tons in 2021.[10] Fava beans contain high protein levels (20–41% of dry weight) with potential emulsifying capabilities; however, their native emulsifying properties are limited.[7,11] Emulsion stability can be compromised by factors such as storage, heating, and oxidation. Therefore, innovative strategies are needed to prevent oxidation, enhance stability, and prolong shelf life.[12] High-energy processing techniques, such as homogenization, micro-fluidization, and ultrasonic treatment, have been shown to enhance emulsion stability. Among these, ultrasonic treatment is recognized as a safe, eco-friendly, and efficient technology.[13–15]

Ultrasound (US) treatment or sonication employs high-frequency sound waves to introduce structural changes in food systems.[12,15–20] High-intensity ultrasound promotes the breakdown of oil droplets and alters protein structures. These structural changes affect physicochemical properties such as solubility, surface hydrophobicity, cross-linking potential, and antioxidant activity.[18] For instance, Tian *et al.*[21] showed that ultrasound significantly alters protein conformation and physicochemical properties by disrupting non-covalent interactions and breaking down peptide aggregates. Likewise, Ma *et al.*[22] reported that high-intensity ultrasound enhanced the Maillard reaction and improved the conjugation between soy protein isolate (SPI) and pectin. The increased SPI–pectin grafting was attributed to ultrasound-induced unfolding of SPI, which enhanced hydrophobicity and emulsifying properties, resulting in more effective conjugates.

While US-treatment results in structural modification of proteins, enhancing functional properties, the effectiveness of the process also depends on the surrounding conditions, particularly temperature. Higher temperatures reduce viscosity and tensile strength, promoting greater bubble formation and increasing the cavitation effect.[23] During sonication, bubbles grow until collapsing violently, creating extreme conditions that generate physical, chemical, and thermal effects, thereby altering emulsion properties depending on the modification intensity.[24] Therefore, it is necessary to maintain the surrounding conditions of US-treatment, essentially temperature, to avoid compromising protein integrity.

Food emulsions typically consist of immiscible liquids, most commonly oil and water.[25] Vegetable oils, such as sunflower, soybean, rapeseed, corn, and canola, are commonly used in the formulation of plant-based food emulsions.[26] In this context, Lima *et al.*[27] examined the synergistic effect of various plant proteins as emulsifiers and stabilizers in oil-in-water (O/W) emulsions containing 3% sunflower oil. Their findings revealed that fava bean protein formed the most stable emulsion and exhibited strong resistance to centrifugal force, highlighting its potential as a functional ingredient in emulsion-based formulations.

Although previous studies have examined the effects of ultrasound treatment on plant protein functionality, its specific impact on the stability of oil-in-water (O/W) emulsions stabilized by fava bean protein remains largely unexplored. In addition, the influence of ultrasound duration on emulsion stability under ice and without ice conditions is yet to be thoroughly investigated. Evaluating key emulsion properties such as appearance, particle size, stability, and rheology is also essential for understanding the system's behavior.[28] Therefore, this study aimed to evaluate the effect of ultrasonic treatment duration, with and without ice cooling, on the stability of fava bean protein–sunflower oil emulsions. In our study, the fava bean protein sample predominantly comprised protein (90%); therefore, the interference of minor components, such as carbohydrate, fat, and other trace elements, on emulsion formation was not considered. The findings support the broader use of alternative proteins in food by examining the turbidity, ζ-potential, particle size, microscopic structure, and storage stability of the protein–sunflower oil emulsions.

2. Materials and methods

2.1 Materials

The commercial fava bean protein (FBP) used in this study, obtained from Macrobiotic World (Thailand), contained an average nutritional composition of 90 g of protein, 3 g of carbohydrates, and 1.5 g of fat per 100 g of sample, along with trace amounts of fibers and minerals such as iron, magnesium, phosphorus, and zinc. Sunflower oil was purchased from a local market in Thailand. All other chemicals, including Tween 80, were of analytical grade.

2.2 Preparation of an emulsion

The protein solution was prepared following the method described by Zhang *et al.*[12] with minor modifications. Briefly, 5% (w/v) fava bean protein (FBP) was dispersed in 10 mM sodium phosphate buffer (pH 7.0) and then left overnight at 4 °C to hydrate. The solution was then stirred for 2 hours at room temperature the next day.

Subsequently, 0.5% Tween 80 was added to the protein solution, followed by 10% (w/v) sunflower oil (SFO). A coarse emulsion was prepared using a high-speed homogenizer (HG-15D, Korea) at 13 000 rpm for 3 minutes. The mixture then

underwent an ultrasonic treatment using an ultrasonic processor (VC505, 500 W, Sonic and Materials Co. Ltd, USA) at 60% amplitude for variable durations (5, 10, 15, 20, 25, and 30 minutes). Ultrasonication was applied in a pulsed mode of 4 seconds on and 2 seconds off. The treatment was performed under two conditions: with ice (IUT), where the sampling beaker was in contact with an ice cube to control the temperature, and without ice (WIUT). The IUT samples showed temperatures ranging from 16.0 °C to 28.2 °C for treatment durations of 5 min to 30 min, while the WIUT samples showed a temperature range of 46.3 °C to 73.9 °C. The control emulsions had a temperature of approximately 20 °C. After sonication, 0.02% of sodium azide (KA122, N.S.W. 2126, Australia) was added to prevent microbial growth in the emulsions. An emulsion without ultrasonic treatment served as a control for comparison. All samples were stored at 4 °C until further analysis. Each sample was prepared and analyzed in triplicate.

2.3 Color measurement

The color of the emulsion samples was measured using a Color Flex EZ colorimeter (Hunter Lab, USA). Color properties were recorded as CIE Lab values: L^* (lightness), a^* (red-green), and b^* (yellow-blue).

2.4 Emulsion turbidity determination

The turbidity of the emulsion was assessed using the method of Wang *et al.*[15] with minor modifications. Briefly, 1 mL of each emulsion sample was diluted 300-fold with deionized (DI) water, and the absorbance was measured at 600 nm by using a spectrophotometer. Turbidity was calculated using the following equation:

$$T = \frac{2.302 \times A \times D}{I} \quad (1)$$

where A is the absorbance of the emulsion, D is the dilution factor, and I is the optical path length (0.01 m).

2.5 Centrifugal stability of the emulsion

The centrifugal stability of the fine emulsion was determined by measuring the separation between the cream and water layers after centrifugation. Prior to centrifugation, each emulsion sample was diluted 300-fold with distilled water and subjected to centrifugation at 4000 rpm for 30 minutes. The bottom layer of the emulsion was carefully removed using a syringe needle, and its absorbance was measured at 600 nm using a UV-vis spectrophotometer.[29] Centrifugation stability (CS) was calculated using the following equation:

$$\text{CS } (\%) = \frac{A - A_1}{A} \times 100\% \quad (2)$$

where A is the absorbance before centrifugation, and A_1 is the absorbance after centrifugation.

2.6 Particle size distribution and zeta potential (ζ-potential) analysis

The particle size distribution and ζ-potential of the emulsion were measured using a Zetasizer-ZS system (Laboratory Service Unit, Suranaree University of Technology, Thailand). Each emulsion sample was diluted to 40% (w/v) with deionized water, and measurements were conducted at 25 °C.

2.7 Microscopic visualization

The microstructure of the freshly prepared emulsions was examined using a light microscope (CKX3-HOUN, Tokyo, Japan) equipped with a charge-coupled device (CCD) camera. Aliquots of 100 μL were diluted 10-fold with distilled water. Then, 20 μL of the diluted emulsion was placed on glass microscope slides and covered with a coverslip. The samples were observed at 40× magnification at ambient temperature.[30]

2.8 Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared (FTIR) spectroscopy (MTR-OPTKBR Spectrum 100, CPU32) was used to obtain the infrared spectra of the emulsion. The spectra were recorded from 4000 to 500 cm^{-1} with 16 scans at a resolution of 4 cm^{-1}.[31,32] Spectral data in the range of 500–4000 cm^{-1} were plotted using the OriginPro software (version 2022b).

2.9 Creaming index (CI) determination

The creaming index was determined as described in previous work.[33] Briefly, the fresh emulsion was placed in a clear 10 mL glass bottle, sealed, and stored at room temperature, in the dark, for 15 days. After storage, the upper layer consisted of the emulsion, while the lower layer was clear. CI was calculated according to the following formula:

$$\text{CI } (\%) = \frac{H_e}{H_t} \times 100\% \quad (3)$$

where H_e represents the height of the clear liquid layer at the bottom, and H_t is the height of the total emulsion.

2.10 Statistical analysis

All experiments were conducted in triplicate, and results are expressed as mean ± standard error (SE). Statistical significance was assessed using a one-way analysis of variance (ANOVA), followed by Duncan's multiple range test.

3. Results and discussion

3.1 Color of the emulsion

Color parameters are key factors influencing consumer acceptance of food products. Table 1 presents the effect of ultrasonic (US) treatment on the color parameters (L^*, a^*, and b^*) of FBP powder and emulsions (FBP: sunflower oil emulsion). The FBP powder exhibited a high L^* value of 80.10 ± 0.09. Ultrasonic treatment has a substantial impact on L^* values in nanoemulsions ($p > 0.05$). As shown in Table 1, the L^* values of both IUT and WIUT increased with increasing treatment time.

Table 1 Color of the emulsion from IUT and WIUT[a]

Sample	Color parameter		
	L^*	a^*	b^*
FBP	80.10 ± 0.09	1.04 ± 0.04	17.16 ± 0.07
IUT			
Control	64.13 ± 1.76^{C}	-2.17 ± 0.26^{B}	3.90 ± 1.41^{D}
5 min	78.30 ± 0.60^{B}	-0.74 ± 0.43^{A}	11.03 ± 1.05^{A}
10 min	79.90 ± 0.92^{A}	-0.65 ± 0.04^{B}	8.03 ± 0.69^{B}
15 min	79.82 ± 0.26^{A}	-0.69 ± 0.03^{A}	8.50 ± 0.50^{B}
20 min	80.50 ± 0.40^{A}	-0.51 ± 0.06^{A}	7.40 ± 0.85^{B}
25 min	79.80 ± 0.18^{A}	-0.70 ± 0.09^{A}	8.75 ± 0.73^{B}
30 min	81.17 ± 0.32^{A}	-0.38 ± 0.17^{A}	5.44 ± 0.50^{C}
WIUT			
Control	64.13 ± 1.76^{c}	-2.17 ± 0.26^{c}	3.90 ± 1.41^{c}
5 min	79.38 ± 0.94^{b}	-0.54 ± 0.41^{b}	9.40 ± 3.06^{a}
10 min	80.24 ± 0.37^{ab}	-0.37 ± 0.22^{b}	8.42 ± 0.41^{ab}
15 min	81.00 ± 0.52^{a}	-0.17 ± 0.19^{ba}	6.73 ± 0.60^{bc}
20 min	81.42 ± 0.13^{a}	0.07 ± 0.12^{a}	5.42 ± 0.21^{cd}
25 min	81.18 ± 0.17^{a}	0.18 ± 0.21^{a}	5.37 ± 0.26^{cd}
30 min	81.03 ± 0.08^{a}	0.26 ± 0.07^{a}	5.20 ± 0.24^{cd}

[a] The data refer to the mean ± standard deviation of triplicate measurements. Different uppercase and lowercase superscripts along the column indicate significant differences ($p < 0.05$) between the IUT and WIUT samples, respectively.

However, no significant increase in lightness was observed after 15 minutes of treatment in either IUT or WIUT. Thus, longer ultrasound treatment produces a lighter colored emulsion than the control. A similar trend was observed for a^* values for both WIUT and IUT. The b^* value of the control sample was significantly lower than that of the US-treated samples. However, as the treatment time increased, b^* values significantly decreased in US-treated samples. These color changes are likely due to pigment degradation and protein degeneration caused by ultrasonic treatment. Additionally, the reduction in particle size may have contributed to color changes. Ultrasonic treatment reduces protein particle size, increasing particle number and enhancing light scattering, which results in a whiter appearance.[16,34]

3.2 Turbidity of the emulsion

Turbidity, as a macroscopic indicator, reflects emulsion turbidity and is strongly associated with droplet size, shape, and distribution.[15] Emulsion turbidity is negatively correlated with emulsion stability. Fig. 1 shows the turbidity of the US-treated emulsion under IUT and WIUT conditions at treatment times ranging from 5 to 30 minutes. The higher turbidity in the control sample compared to US-treated emulsions suggests greater protein aggregates. Ultrasonic treatment significantly altered the turbidity of the emulsions. Turbidity decreased with increasing ultrasonic time under both IUT and WIUT conditions. Ultrasonic cavitation disrupted emulsion droplets, reducing particle size and enhancing droplet uniformity. As a result, a significant decrease in turbidity was observed. These findings align with the results reported by Du *et al.*[35] who

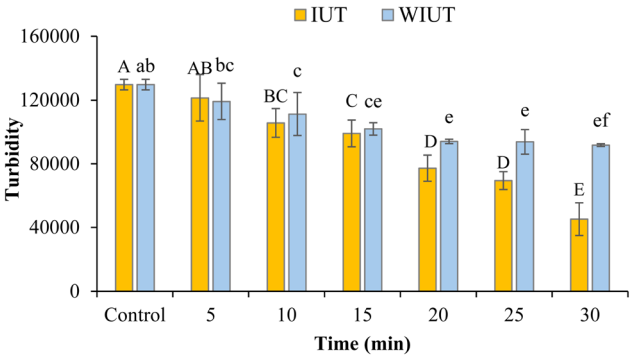

Fig. 1 Turbidity of FBP–oil emulsions at different US-treatment times and treatment conditions (IUT and WIUT). Different uppercase and lowercase superscripts indicate significant differences ($p < 0.05$) for the IUT and WIUT samples, respectively.

attributed turbidity reduction to decreased particle size following ultrasonic treatment. At 30 minutes of US-treatment time, the turbidity was 45 165.24 in IUT and 91 757.72 in WIUT. The higher turbidity in WIUT may be due to heat generated during sonication, which causes protein unfolding and denaturation, altering the surface charge and promoting aggregate formation.[36]

3.3 Centrifugal stability of the emulsion

Centrifugation is an effective method for evaluating emulsion stability, as it induces phase separation between the oil and water phases.[37] Fig. 2 illustrates the centrifugal stability of the FBP emulsion subjected to US treatment under two different conditions (IUT and WIUT). The control emulsion exhibited the lowest centrifugal stability in both IUT and WIUT, likely due to the presence of larger droplet sizes, indicating poor stability. Centrifugal stability increased with longer US treatment time, suggesting that US enhances the stability of FBP-stabilized emulsions. This improvement is likely attributed to reduced particle size, which enhances protein–oil interactions. Rajasekaran *et al.*[38] reported that ultrasonicated shrimp oil emulsion exhibited significantly higher centrifugal stability, attributed to

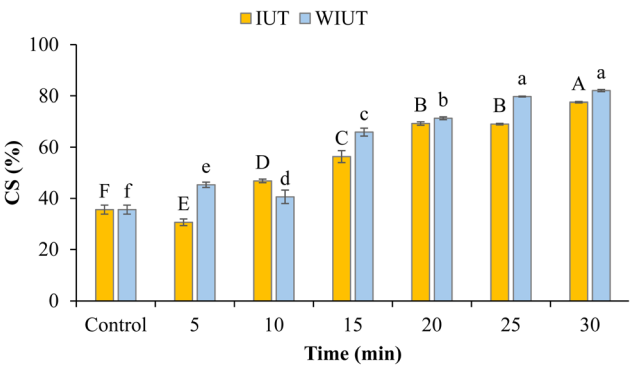

Fig. 2 Centrifugation stability of emulsions (IUT and WIUT). Different uppercase and lowercase superscripts indicate significant differences ($p < 0.05$) for IUT and WIUT samples, respectively.

the formation of a thick interfacial layer and reduced interfacial tension between the oil and water phases. Our findings are consistent with this observation, as US-treated samples had higher stability than untreated ones. Furthermore, WIUT generally resulted in greater centrifugal stability than IUT at all treatment times, suggesting that thermal energy during US enhanced protein adsorption at the oil–water interface. This improvement may be due to heat-induced protein unfolding, which exposes hydrophobic groups, enabling stronger interaction with oil droplets. Statistical analysis showed no significant difference in centrifugal stability between 25- and 30-minute US-treatments in WIUT, indicating that extending the treatment time beyond 25 minutes does not improve stability, possibly due to partial protein denaturation due to the combined heat and cavitation effects. Overall, the results suggest that US treatment significantly enhances emulsion centrifugal stability. However, extending the US treatment time beyond 25 minutes, particularly WIUT, may limit further stability enhancement. Therefore, optimizing the treatment time is important to achieve maximum stability.

3.4 Particle size distribution and ζ-potential analysis

The size and aggregation state of oil droplets play a crucial role in the physical stability of oil-in-water emulsions. Therefore, the average particle diameter of the emulsions was measured after US treatment under both IUT and WIUT conditions to assess their physical stability. As shown in Fig. 3a, the control sample exhibited a broad particle size distribution peak around 700–800 nm, indicating the presence of larger protein aggregates, as further confirmed by Fig. 4a. However, following US-treatment (both IUT and WIUT), the particle size distribution changed significantly. A 5-minute US-treatment in both cases, IUT and WIUT conditions, resulted in a shift toward smaller particle sizes compared to the control. Moreover, longer treatment led to further particle size reduction, likely due to cavitation induced by ultrasonics. The shear forces, micro-jetting, and turbulence generated by ultrasonic cavitations can disrupt non-covalent bonds in large protein aggregates, thereby reducing particle size.[12,39] Notably, the 15-minute WIUT treatment led to a marked reduction in particle size, with a sharp peak at approximately 300–400 nm. In contrast, IUT exhibited a broader particle size distribution, with the peak centered around 800 nm. A consistent reduction in particle size was observed following US-treatment, as ultrasound generates disruptive energy that breaks oil droplets through cavitation, shear, and turbulence. This process also partially unfolds proteins, enhancing their surface hydrophobicity and adsorption at droplet interfaces – factors that collectively reduce interfacial tension and promote the formation of smaller droplets.[39–41]

WIUT treatment beyond 15 minutes caused the particle size peak to shift toward larger sizes and with a broader distribution, suggesting partial re-aggregation. This may be attributed to excessive cavitation, which may have disrupted the protein network and promoted aggregate formation.[42] Conversely, under IUT, a 25-minute treatment resulted in a significant size reduction in particle size, with a peak shifting toward the peak

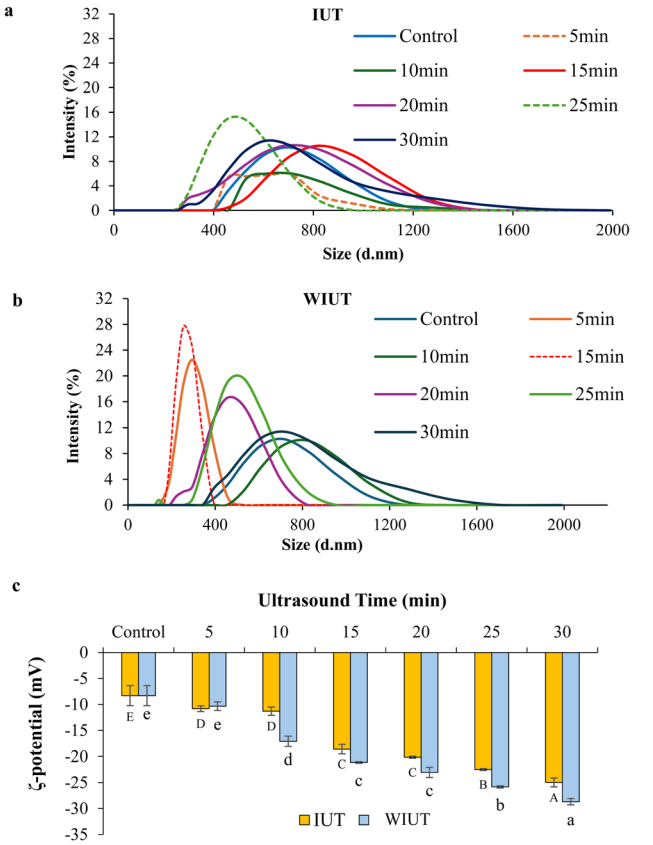

Fig. 3 Particle size distribution of fava bean protein–sunflower oil emulsions under different US-treatments: (a) IUT (b) WIUT and (c) corresponding ζ-potential.

of smaller sizes and centering around 450 nm. At 30 minutes, the particle size distribution broadened and shifted towards larger sizes, indicating that it was beyond 25 minutes, leading to partial re-aggregation. These findings highlight that while increasing the treatment time reduces particle size, excessive durations, beyond 25 minutes for IUT and 15 minutes for WIUT, may cause partial re-aggregation, potentially compromising emulsion stability. Similarly, prolonged US treatment time has been reported to induce protein aggregation in β-lactoglobulin.[43]

In general, ζ-potential is a key indicator of electrostatic interactions and plays a critical role in determining emulsion stability. A higher absolute ζ-potential corresponds to stronger electrostatic repulsion between droplets, which reduces the likelihood of aggregation and enhances stability.[44] An absolute ζ-potential in the range of 30–60 mV typically indicates good nanoemulsion stability, while values below 15 mV are associated with a higher risk.[45] Fig. 3c presents the ζ-potential of FBP-SFO emulsions under various US treatment conditions, revealing that all samples exhibited negative potential. Notably, control emulsions exhibited an absolute ζ-potential of 8.30 mV, which led to flocculation (Fig. 8), whereas US-treated emulsions showed significantly higher ζ-potential values. The absolute ζ-potential progressively increased with treatment time, reaching 24.96 mV in IUT and 28.68 mV in WIUT after 30 minutes of US

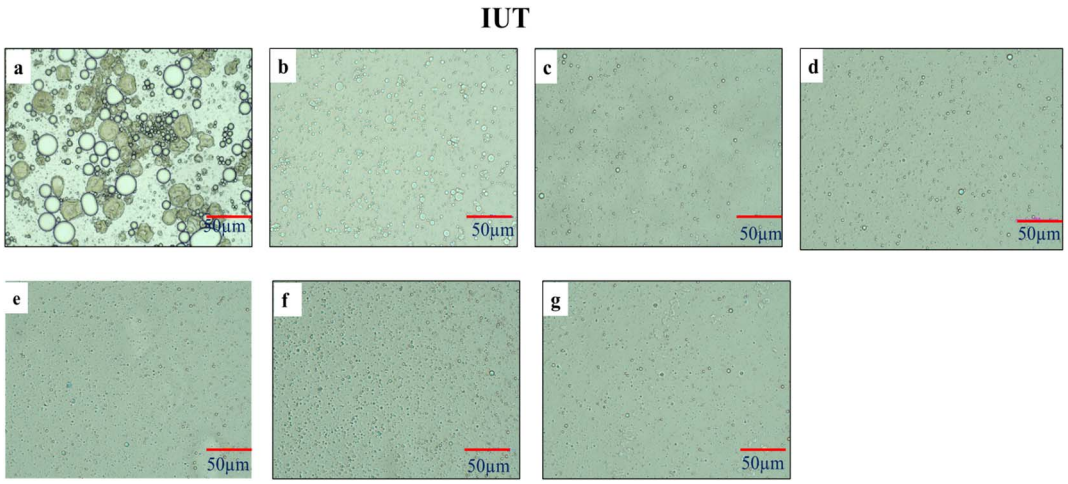

Fig. 4 Microscopic structure of the emulsion (IUT with different US-treatment times): (a) control, (b) 5 min, (c) 10 min, (d) 15 min, (e) 20 min, (f) 25 min and (g) 30 min.

treatment. This effect is attributed to sonication-induced protein unfolding, which exposes additional negatively charged ($-COO^-$) groups on the droplet surface, thereby increasing the ζ-potential. Several studies suggest that higher absolute ζ-potential values are associated with more stable delivery systems.[46,47] Thus, our findings indicate that the 30-minute WIUT treatment yields the most stable emulsion, as further supported by storage stability data (Fig. 8).

3.5 Emulsion morphology

Optical microscopy was used to examine the microstructure of various FBP–SFO emulsions. Fig. 4(IUT) and 5 (WIUT) illustrate the microscopic structure of the emulsion, both displaying spherical droplet morphology, though with notable differences in size and distribution. The emulsion without US treatment (Fig. 4a – control) exhibited a dispersed microstructure with a large average droplet size, primarily due to high interfacial tension at the oil–water interface, which promoted droplet aggregation and the formation of larger oil droplets.[33] In contrast, US-treated FBP emulsions induced conformational changes in FBP, thereby reducing interfacial tension at the water–oil interface. Similar findings were reported by Chen *et al.*[48] who noted that ultrasound affected the structural behavior of myofibrillar protein-stabilized emulsions. Additionally, the emulsion exhibited significantly improved stability, with spherical droplets remaining well dispersed, thereby preventing flocculation in US-treated samples. As the ultrasonic treatment time increased, droplets decreased, and distribution became more uniform. This effect is attributed to US-induced

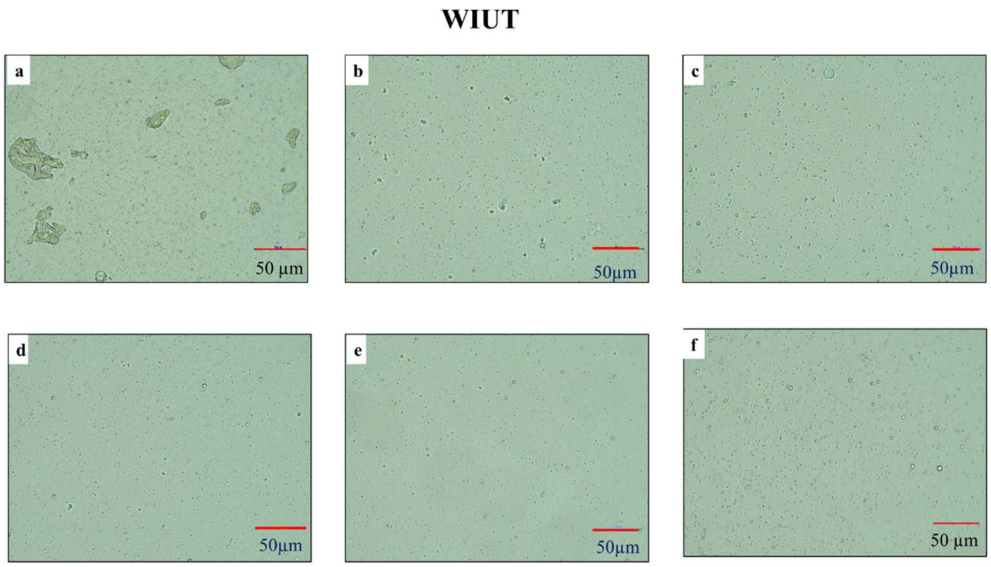

Fig. 5 Microscopic structure of the emulsion (WIUT at different US-treatment times): (a) 5 min, (b) 10 min, (c) 15 min, (d) 20 min, (e) 25 min, and (f) 30 min.

cavitation, which reduces particle size and facilitates rapid and uniform distribution of the FBP–SFO emulsifier at the oil–water (O/W) interface. Both US treatment conditions, IUT and WIUT, resulted in reduced particle size with increasing treatment duration. Microscopic analysis revealed that WIUT produced smaller droplets, which facilitated protein adsorption onto the oil droplet surfaces. Enhanced interfacial protein adsorption increased the repulsive force between oil droplets, thereby stabilizing the emulsion by preventing aggregation and phase separation. These findings are consistent with previous studies on ultrasonicated cod proteins, which also demonstrated uniform oil droplet morphology.[20]

3.6 FTIR spectra of emulsions

FTIR spectroscopy is a powerful technique for analyzing the molecular composition, structure, and interactions.[11] The FTIR spectra of SFO, FBP, and emulsions subjected to various US treatments are shown in Fig. 6a and b. In the spectra of pure SFO, a characteristic absorption peak was observed at 2923.48 cm^{-1} indicating the presence of O–H and C=O groups. A strong absorption band at 1744.01 cm^{-1} was attributed to ester functional groups in the oil spectrum. Similarly, the FBP spectrum (Fig. 6a) displayed a peak at 3437.30 cm^{-1} associated with amino acids. The absorption band at 1635.83 cm^{-1} (amide

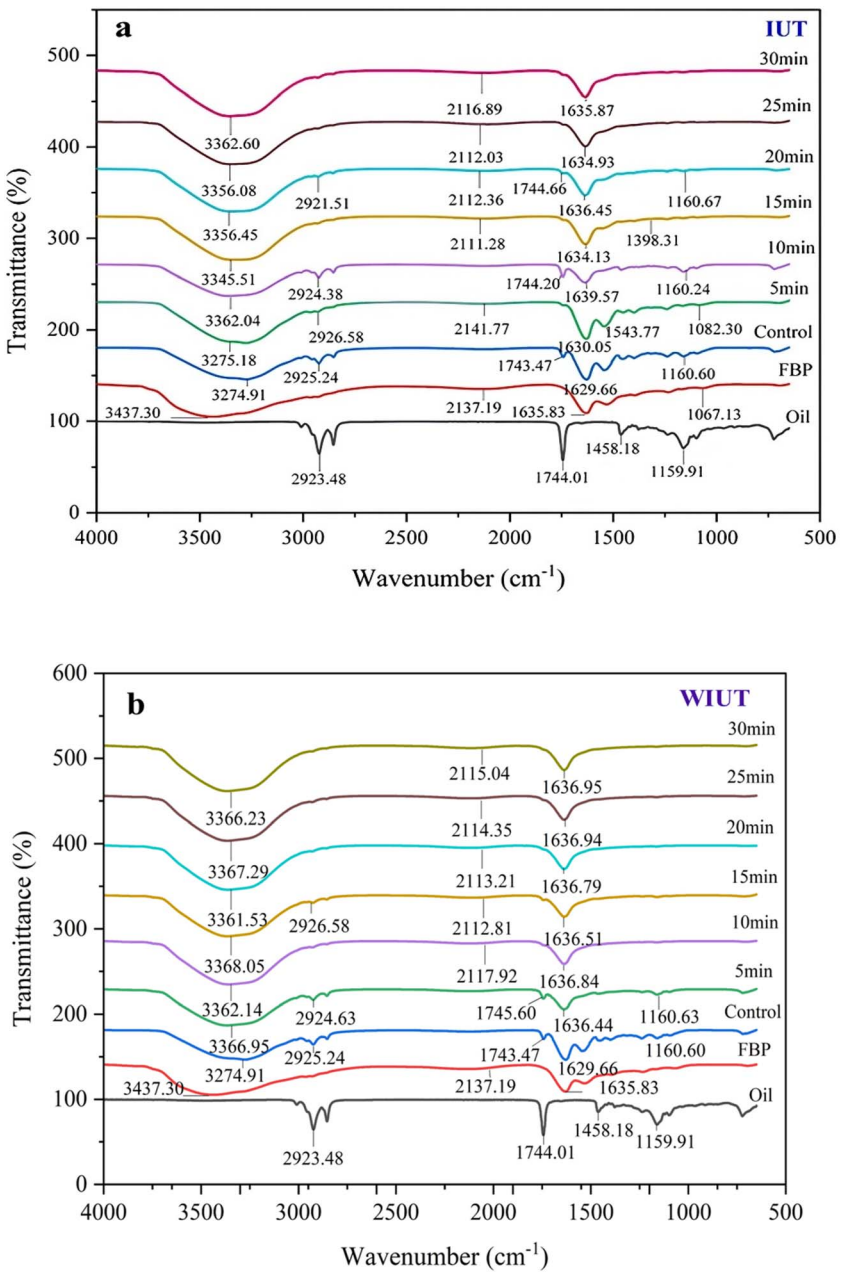

Fig. 6 FTIR spectra of the emulsion subjected to different US-treatments: (a) IUT (with ice cooling) and (b) WIUT (without ice cooling).

I) corresponds to the stretching vibrations of C=O and N–H groups.

Sonication partially unfolds globular proteins, enhancing exposure of amino groups on their surface and facilitating interactions with oil molecules. The FTIR analysis of FBP–SFO emulsions (Fig. 6a and b) revealed notable spectral changes after US treatment, particularly in the amide I (~1650 cm^{-1}) and C=O (~1740 cm^{-1}) bands. The untreated emulsions (control sample) showed distinct amide and lipid peaks with minimal overlap, indicating limited interaction between protein and lipid components. After US treatment, shifts in the amide I band indicated protein unfolding, enhancing the FBP's ability to stabilize oil droplets *via* a cohesive interfacial layer. In our study, US-treated emulsions exhibited higher peak intensities than the control, shifting from 1630.05 cm^{-1} to 1635.87 cm^{-1} (IUT) and from 1636.44 cm^{-1} to 1636.95 cm^{-1} (WIUT). These shifts may reflect protein structure changes that facilitate hydrophilic and hydrophobic interactions, enhancing the binding of C–O groups to the fava bean proteins.[49] The peak in the 3200–3400 cm^{-1} region corresponds to O–H stretching, associated with inter- and intramolecular hydrogen bonding.[50] A decrease in these peaks was observed at the oil–water interface in both treatments. With prolonged treatment, sonicated emulsion exhibited lower peak intensities than the control, reaching ~3362.60 cm^{-1} (IUT) and ~3366.23 cm^{-1} (WIUT) after 30 minutes, possibly due to cavitation disrupting hydrogen bonds. This reduction suggests partial protein unfolding, which may enhance emulsifying capacity by favoring protein-oil over protein–water interactions. The characteristic band at 1160 cm^{-1} in both treatments corresponds to the C–O stretching vibrations of ester groups and CH_2 bending, typical of vegetable oils.[51] Similarly, band shifts observed with increasing US-treatment time strongly indicate enhanced protein and oil interactions during sonication. Overall, the FTIR results confirm that US treatment alters protein conformation, thereby enhancing protein–oil interactions.

3.7 Creaming index (CI) and storage stability

The CI reflects the degree of phase separation between oil and water during storage and serves as a key indicator of emulsion stability. A higher CI corresponds to lower emulsion stability.[20,33] Changes in CI and visual observation of FBP emulsions stored at room temperature for 15 days are presented in Fig. 7a, b, and 8. All samples exhibited noticeable stratification over time; however, no visible oil separation was observed. Fig. 7 displays the CI of emulsions over a 15-day storage period. The control sample exhibited the highest CI, reaching 93.33% after 15 days of storage. In comparison, CI decreased progressively with increasing US-treatment time, improving emulsion stability in both IUT and WIUT samples by minimizing phase separation. The CI of WIUT-treated emulsions was consistently lower than that of IUT-treated emulsions under identical storage conditions. After 30 minutes of US treatment, the CI decreased to 19.05% for IUT and further dropped to 7.33% for WIUT. These results suggest that WIUT more effectively inhibits

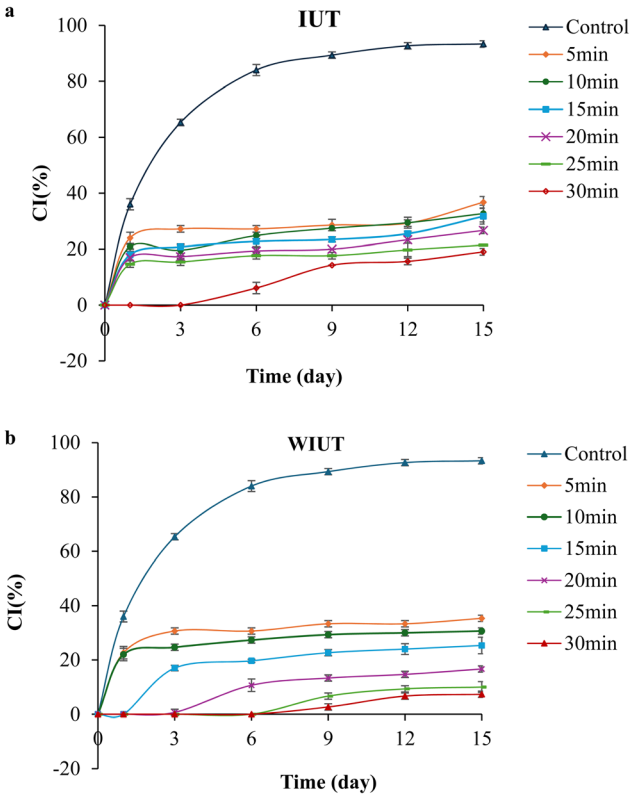

Fig. 7 CI of the FBP-SFO emulsion under different US-treatments: (a) IUT and (b) WIUT.

phase separation, possibly due to the moderate thermal effect associated with ultrasound treatment without ice cooling. The heat-pretreated soy proteins were reported to significantly reduce the CI compared to unheated proteins.[52] Likewise, Peng *et al.*[30] found that emulsions stabilized with heated pea protein exhibited a lower CI. This supports the idea that partial heat treatment enhances protein emulsifying capacity by reducing oil droplet migration during storage.

Fig. 8 presents the visual appearance of the emulsions at different time points during storage. On the first day, protein sedimentation was observed in all IUT samples except the 30-minute US-treated sample, which showed sedimentation only after six days. In contrast, sedimentation occurred from day one in the control, 5-minute, and 10-minute WIUT samples, whereas the 30-minute WIUT-treated sample exhibited sedimentation only after nine days. Emulsions stabilized by WIUT for 25–30 minutes demonstrated greater physical stability than those treated with IUT. These findings suggest that US treatment without ice effectively inhibited droplet aggregation and flocculation. US treatment increased protein surface hydrophobicity and reduced particle size, thereby enhancing FBP adsorption at the oil–water interface and strengthening repulsive interactions between oil droplets.[33] Similar effects have been reported for ultrasonicated soy protein,[14] cod proteins,[20] and pea proteins.[30]

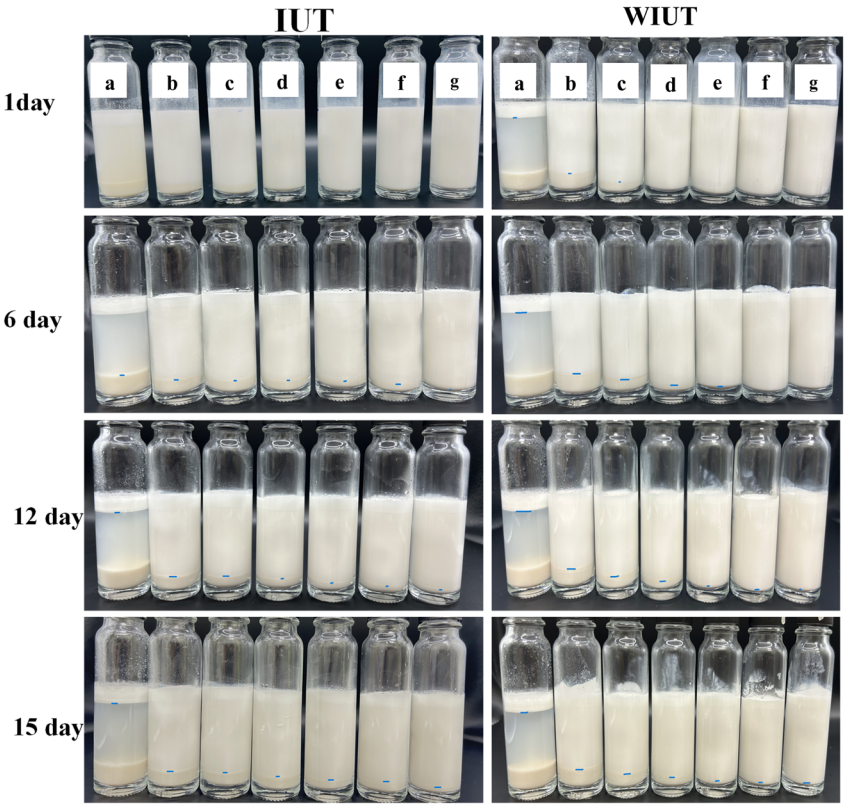

Fig. 8 Storage stability of emulsions under different US-treatments, (a) control, (b) 5 min, (c) 10 min, (d) 15 min, (e) 20 min, (f) 25 min, and (g) 30 min.

4. Conclusion

This study demonstrated that US-treated FBP–SFO emulsions exhibited greater stability compared to untreated samples. The significant reduction in particle size and turbidity suggests that the US-treatment altered interface characteristics, thereby enhancing emulsification efficiency. Between the two different conditions, IUT and WIUT, WIUT exhibited higher centrifugal stability, smaller particle size, and greater absolute ζ-potential value across all durations. These results suggest that US treatment without ice cooling is more effective in forming stable emulsions, likely due to enhanced molecular mobility and improved protein interactions at the oil–water interface. However, treatment duration remains a critical factor, as prolonged sonication may negatively impact emulsion stability by inducing protein denaturation and aggregation. Microstructural analysis revealed that the emulsion formed after 30 minutes of WIUT exhibited the most uniform distribution of droplets, facilitating improved protein adsorption onto oil droplet surfaces. These findings indicate that US treatment can significantly enhance the emulsifying properties of proteins and highlight the potential of FBP as a natural, plant-based functional ingredient for food applications such as ice cream. Further research should investigate the effect of varying ultrasonic intensities to optimize processing conditions and assess the potential for industrial scalability.

Author contributions

Susma Bhattarai: conceptualization, investigation, formal analysis, writing – original draft, data curation. Sanjaya Karki: writing – original draft, writing – review & editing, data curation, methodology. Yothha Srithep: writing – review and editing. Kunwadee Kaewka: conceptualization, supervision, writing – review & editing. Arrisara Phosanam: writing – review and editing, supervision, methodology, conceptualization.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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