Effect of slit dual-frequency ultrasonic emulsification technology on the stability of walnut emulsions

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ABSTRACT

A highly hygienic walnut emulsion beverage was prepared by using a slit dual-frequency emulsification technique. The optimal ultrasonic parameters were studied as a model system: the ultrasonic time of 50 min, the ultrasonic power density of 260 W/L, and a dual-frequency ultrasonic combination of 28/68 kHz. Walnut emulsion with an average mean volume diameter of 2.05 µm, a Zeta potential absolute value of 40 mV was obtained after ultrasonic treatment, and the emulsion stability could be maintained for more than 14 days without phase separation. At the lowest ultrasonic energy input, the vibrating emulsion could promote droplet aggregation. However, excessive energy input could result in sample overtreatment and reduced emulsion activity. The laser scanning confocal microscope (LSCM) and transmission electron microscope (TEM) confirmed that walnut emulsion processed by slit dual-frequency ultrasonic had pretty high stability. Therefore, the slit dual-frequency ultrasonic emulsification technique was found to be well suited for the preparation of complex and fine oil-in-water food emulsions.

1. Introduction

Walnut emulsions are made by decortication, refining, filtration, blending, emulsification, and sterilization of walnut kernels [1], which are considered as a kind of vegetable protein beverage with high nutritional value and easy absorption. The walnut emulsion is composed of a major constituent of about 60–65% fat, 15–18% protein, and a trace number of active ingredients [2,3]. In recent years, the full-fat walnut emulsion has become increasingly popular among consumers due to its distinct flavor and exceptional taste. However, owing to its high oil content and complex emulsion system, walnut emulsions are prone to coalescence, flocculation, and Ostwald ripening [4].

To improve the stability of the emulsion, it is imperative to add appropriate emulsifiers, including polysaccharides and surfactants [5,6]. Furthermore, exploring more efficient emulsification methods has become urgent. Commonly used methods are high-energy emulsification, including high-pressure homogenization (HPH), high-speed homogenization (HS), microfluidization (MF), and ultrasonic emulsification (UE). HPH is a technique for breaking large droplets into microscopic droplets by passing them through thin slits in high-pressure valves [7], resulting in nanoemulsions and emulsifying agents [8] that can stabilize food emulsions. HSH uses high-speed rotating blades to break up droplets, which are commonly used in the preparation of coarse emulsions [9]. The microfluidizer of MF has two micro-channels, and the fluid in the channels is strongly sheared in the impact region under high pressure to produce a fine emulsion [10]. UE, on the other hand, breaks large droplets into small droplets with the help of the ultrasonic cavitation effect and disperses the emulsion system simultaneously [11]. The advantages of UE include the ability to generate stable emulsions with the same energy input, resulting in low energy consumption and low cost [12], making it appropriate for large-scale food industry production. As a result, UE under the study of stabilizing emulsions has attracted a lot of attention [13,14]. Currently, invasive ultrasonic with horn [15–18] is widely employed in the dairy industry for emulsification, crystallization, fermentation, functional modification, and fat separation [19]. Jena and Das published a study in which they used an ultrasonic approach to prepare coconut emulsion with gum Acacia and maltodextrin and found that the fat particle size was reduced after treatment [20]. Shanmugam et al. reported that ultrasonic preparation of flaxseed oil and skimmed milk emulsion resulted in a stable cream with a particle size of 0.4 µm and a stable creaming index for 9 days [21]. According to Homayoonfai et al., the particle sizes of walnut oil emulsions generated by ultrasonic range from 190 to 280 µm [4]. Various researchers [9] have reported that ultrasonic treatment could

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significantly enhance the physical stability and antioxidant capacity of walnut emulsions. The frequency of the ultrasonic equipment employed above, on the other hand, is single and fixed, making it only appropriate for intermittent small-batch production and unsuitable for continuous large-batch industrial production. Furthermore, severe cavitation corrosion may result in product pollution or degradation. Despite the use of continuous contact-free flow designs for UE [22,23], issues like limited throughput and uneven treatment persist. Current studies typically focus on a single oil–water or oil–water-protein system [4,21,24], There are, however, few reports on the application of UE in complex systems such as vegetable protein beverages.

A slit dual-frequency ultrasonic device was proposed to be employed in the preparation of walnut emulsions to solve the above problems. The effect of this technology on the stability of walnut emulsions was studied by particle size distribution determination, zeta-potential determination, creaming index analysis, storage stability analysis, laser scanning confocal microscope (LSCM), and transmission electron microscope (TEM). The optimum ultrasonic emulsification parameters were investigated in this study. Also, the technique of ultrasonic emulsification stability-enhancing ability of emulsins was established.

2. Materials and methods:

2.1. Reagents

Walnut kernels were donated by Yunnan Morre Garden Biotechnology Development Co., Ltd., Yun Nan, China. Monoglyceride, sorbitan monostearate, nile red, fast green, and guar gum were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd., China. Sucrose fatty acid esters, xanthan gum, and konjac glucomannan were purchased from Shanghai McLin Biochemical Technology Co., Ltd., China. Potassium sorbate was purchased from Sinopharm Chemical Reagent Co., Ltd., China.

2.2. Design of slit sonochemical reactor and experimental set-up

The slit sonochemical reactor is composed of the main cavity and two vibration plates. A certain number of ultrasonic piezoelectric transducers with different frequencies are arranged on the vibration plate to provide ultrasonic vibration. The ultrasonic waves are transmitted to the liquid in the cavity through the two vibration plates to achieve dual-frequency ultrasonic processing. The emulsion was first dispersed in a magnetic stirrer at 40 °C before being fed through a peristaltic pump at a rate of 50 mL/min to create a circulation system (Fig. 1).

2.3. Preparation of walnut emulsions

The peeled walnut kernels were mixed with deionized water at a weight ratio of 1:10, rough ground by a refiner, and fine ground by a colloidal mill to obtain the crude. It was boiled in 95–100 °C water and filtered with three layers of 200 mesh nylon screen to remove the slag slurry and obtain a coarse slurry. To obtain a stable emulsion, the surfactants and polysaccharides are compounded. The specific compounding ratio and dosage are as follows: Monoglyceride:SP-60: sucrose ester = 1:1:1 (surfactant ratio), 0.3% additive; xanthan gum: konjac gum: guar gum = 3:1:8 (polysaccharide ratio), 0.2% additive. Afterward, it is dispersed evenly in the emulsion by colloid milling. Finally, potassium sorbate is added to inhibit the growth of microorganisms. The walnut emulsion beverage formula design is as follows: 8.5% walnut kernel, 8% sucrose, 0.1% monoglyceride, 0.1% SP-60, 0.1% sucrose ester, 0.025% xanthan gum, 0.083% konjac gum, 0.067% guar gum, 0.005% potassium sorbate, 85% deionized water. To reduce the average particle size of emulsions to obtain fine emulsions, slit ultrasonic was used for ultrasonic emulsification. It is necessary to screen the ultrasonic

Fig. 1. Design of slit sonochemical reactor and experimental equipment of ultrasonic emulsification for preparing walnut emulsions.
parameters and select the best process conditions for ultrasonic emulsification. Table 1 explains the experimental design of this study.

2.4. Creaming and storage stability

Creaming stability in terms of creaming index, which checked for phase separation or creaming. Also, the amount of creaming was measured by storing them in a 40 mL transparent screw bottle at 4 °C for 14 days. The emulsion stability against creaming was monitored by measuring the height of the lower clear liquid (H50%) and the height of the total emulsion (H100%) in the bottle. Creaming stability was obtained using equation (1).

\[ CI(\%) = \left( \frac{1 - H50}{H100} \right) \times 100\% \]  

(1)

On the 7th and 14th days of storage stability, the emulsions were filmed and recorded to observe whether the phase separation occurred. If the CI is 100%, there is no phase separation in the emulsions.

2.5. Particle size and zeta potential measurements

The particle size distribution, volume mean diameter D [4,3], Dv50, and Dv90 was measured on fresh and stored samples using a laser diffraction method by Mastersizer 3000 (Mastersizer 3000, Malvern, Worcestershire, UK). Dv90 and Dv50 respectively represent diameters with 90% and 50% volume distribution lower than this value. The Zeta potential of emulsions was measured using a Malvern Zetasizer Nano ZS (Zetasizer Nano ZS, Malvern, Worcestershire, UK). The emulsions were diluted 10 fold using MilliQ water before measurements.

2.6. Microstructure

The microstructure of the sample was evaluated with laser scanning confocal microscope (Leica TCS SP5; Leica, Heerbrugg, Germany). Nile red solution (0.01% (w/v)) in methanol (40 μL) and 0.01% (w/v) Nile blue solution in methanol (40 μL) was mixed into the freshly prepared emulsion (1 mL) [25]. The samples were stained for fats and proteins, whereby 10 μL of each sample was placed in cover glass. Oil droplets and protein were observed at 488 nm and 633 nm excitation wavelengths in the two-channel mode, respectively [26]. Areas rich in dyed oil droplets (green) and protein (red) appeared as bright patches, while distilled water appeared as a black background in microscopic photographs.

The microstructure of the sample was also evaluated with a transmission electron microscope (Hitachi HT7800; Tokyo, Japan). The sample was dropped onto the copper web for 3 min. The excess droplets have sucked the edge of the copper net with filter paper. After that, it was dyed with tungsten phosphate negative dye for 2 min.

2.7. Statistical analysis

When necessary, one-way ANOVA with a 95% confidence level was used. The ANOVA data with a p less than 0.05 significance level were considered statistically significant. All the experiments were repeated three times. The walnut emulsions were prepared in a fresh sample of the same batch. All measurements were performed on the same day of the sonication and upon storage, wherever necessary.

3. Results and discussion

3.1. Effect of ultrasonic time on the stability of walnut emulsions

Under the conditions of the ultrasonic power density of 300 W/L, ultrasonic frequency combination of 20/40 kHz, the ultrasonic temperature of 40 °C, creaming and storage stability, particle size distribution, and zeta potential were used to screen the effects of ultrasonic time of 10, 20, 30, 40, 50 and 60 min on the emulsification effect of walnut emulsions.

Fig. 2. A shows the particle size distribution of walnut emulsion under different processing times. It could be found that the peak with the larger volume density moves first to the right and then to the left, indicating the large particle size increased first and then decreased. The particle size tended to increase at the treatment time of 10 min to 30 min, and gradually decreased at the treatment time of 40 min to 60 min. Table 2 shows the volume mean diameter of walnut emulsions processed by ultrasonic at 10–60 min. The data from Fig. 2.A and Table 2 also elucidate that the proportion of large particle size in emulsion has increased at short time ultrasonic procession. The zeta potential in Fig. 2.B also illustrates this problem. After 30 min of treatment, the zeta potential shows a decreasing trend, and then the zeta potential increased significantly. Fig. 3 shows the stability of walnut emulsion treated with different ultrasonic times when stored at 4 °C. The creaming index was an important indicator to measure the stability of the emulsion. If the creaming index is 100%, it means that the emulsion is stable at this time. The emulsion treated for 50 min showed 100% emulsification stability before storage for 9 days, but the emulsion treated for 60 min was relatively stable for only 7 days. The phase separation recorded in Fig. 3 also supports this phenomenon, which occurred on the 7th day after treatment for 60 min and remained for 9 days after treatment for 50 min.

The mean diameter of emulsion particles decreased as ultrasonic time increased, while the degree of dispersion increased [20]. Perisamy et al. reported the effect of ultrasonic time on the nano-emulsion of nigella essential oil and found that when the ultrasound time was 10–60 min, the particle size of the emulsion gradually decreased, and finally remained at 0.05–0.1 μm [27]. Ramisetti et al. also indicated that the droplet size of coconut oil–water emulsion could not be further reduced after 30 min, and it was always maintained at 1.5–2 μm [28]. However, the particle size of walnut emulsion increased in the first 30 min following slit ultrasonic emulsification in this study, which could be since walnut emulsion is not just an O/W emulsion but also contains some solid particles (protein, crude fiber). The vibration of ultrasonic waves in the transmission process causes a slight aggregation of particles. Prolonged ultrasonication tends to increase emulsion particle size while decreasing emulsion dispersion degree [12], That is, the creaming

| Ultrasonic Time(min) | D[4,3] (μm) | Dv50 (μm) | Dv90 (μm) |
|----------------------|-------------|-----------|-----------|
| 0                    | 14.1        | 5.81      | 16.1      |
| 10                   | 8.69        | 7.63      | 18.2      |
| 20                   | 7.90        | 7.11      | 16.1      |
| 30                   | 6.04        | 5.36      | 12.2      |
| 40                   | 5.29        | 5.03      | 12.3      |
| 50                   | 4.92        | 4.41      | 9.55      |
| 60                   | 4.10        | 3.72      | 7.75      |
stability of the emulsion treated for 60 min was found to be relatively poor as the particle size and absolute potential of the emulsion increased. According to the results, 50-minute ultrasonic treatment is the optimum time for slit ultrasonic emulsification of walnut emulsion with a size distribution of 5–10 µm.

3.2. Effect of ultrasonic power density on the stability of walnut emulsions

Under the conditions of the ultrasonic time of 50 min, ultrasonic frequency combination of 20/40 kHz, the ultrasonic temperature of 40 °C, creaming and storage stability, particle size distribution, and zeta potential were used to screen the influence of ultrasonic power density of 100, 140, 180, 220, 260 and 300 W/L on the emulsification effect of walnut emulsion.

Fig. 4. A displays the influence of different processing power densities on the particle size distribution of walnut emulsion. The volume density of emulsion with a large particle size (10 µm) gradually decreased as ultrasonic power density increased, while that of emulsion with a small particle size (1 µm) gradually increased. Therefore, an overall decreasing trend was reflected in the particle size of the emulsion. The zeta potential in Fig. 4. B shows that the 260 W/L treatment group has the highest absolute value of the zeta potential compared with other treatment groups, while the 300 W/L treatment group shows a decrease in the absolute value of the zeta potential. Table 3 shows that with the increase of power density, the volume mean diameter of emulsion decreases, and the proportion of small particle size increases, while the large particle size is not obvious. Fig. 5 shows the creaming index of walnut emulsion under different processing power treatments. It can be seen that the processing power of 260 W/L can maintain 100% creaming index stability for 7 days. The creaming index of 260 W/L is

![Image](image-url)
3.3. Effect of ultrasonic frequency combination on the stability of walnut emulsions

Under the conditions of the ultrasonic time of 50 min, ultrasonic power of 260 W/L, and ultrasonic temperature of 40 °C, creaming and storage stability, particle size distribution, and zeta potential were used to screen the effects of e ultrasonic frequency combinations of 20/28, 20/40, 20/50, 20/68, 20/80, 28/40, 28/50, 28/68, 28/80, 40/50, 40/68, 50/68, 50/80, and 68/80 kHz on the emulsification effect of walnut emulsions.

The average particle size was shown in Fig. 6. A reflects the ultrasonic emulsification effect with different frequency combinations. The high-frequency combination resulted in a large average particle size and poor ultrasonic emulsification effect. The smallest average particle size was 1.0 μm at the 28/68 kHz frequency combination. Fig. 6. B shows that the absolute values of the zeta potential in the low-frequency combination were larger than those in the high-frequency combination, while the combination of 28/68 kHz has the highest zeta potential absolute value. The volume mean diameter varies greatly with different frequency combinations observed from Table 4, the 28/68 frequency combination has the smallest volume mean diameter. Fig. 7 compares the creaming index of walnut emulsion at different frequency combinations. Among the 15 frequency combinations, 20/50 kHz, 28/68 kHz, and 40/68 kHz had relatively better creaming stability. The 28/68 kHz combinations maintained a 100% creaming index for 7 days, but the 20/50 kHz and 40/68 kHz combinations only kept a 100 % creaming index for 6 days and 4 days, respectively. As can be seen from Fig. 7, the emulsion treated with 28/68 kHz can maintain a creaming index of 100% for 14 days without phase separation.

Low-frequency ultrasonic (20–100 kHz) is used to break the emulsion into small droplets using the strong shear force generated by cavititation in this frequency range [14,19,33]. Dual-frequency or multi-frequency ultrasonication can effectively enhance the cavitation effect compared with single-frequency ultrasonic. The ultrasonication cavitation effect is also different under different frequency combinations [34]. This is because single-frequency ultrasound generated obvious standing waves in the sonochemical reactor, whereas dual-frequency ultrasound reduced the generation of standing waves and boosted the sonochemical yield to some extent [35]. This explanation agrees with the research of Jun et al., which concluded that the particle size of egg white protein emulsion treated with 20/40 kHz continuous dual-frequency was greatly reduced [36]. According to Huang et al., the creaming stability index of emulsions of soybean protein isolate treated with ultrasonic 20/28 kHz dual-frequency was significantly improved [37]. Also, 25/80 kHz and 20/40 kHz combinations for modification and esterification had obvious effects [38,39]. In this study, the effect of dual-frequency ultrasonic emulsification of walnut emulsion was remarkable. The average particle size of emulsions was reduced to less than 5 μm after optimizing ultrasonic time and power density. The best combination of ultrasonic frequencies was 28/68 kHz, which could reduce the particle size to about 1 μm and the absolute value of zeta-potential was over 40 mV. The average particle size of walnut emulsion could be maintained for 7 days, which could maintain thermodynamic stability and had good stability [40]. The emulsion had high stability when the absolute value of the zeta-potential was greater than 30 mV [41]. This indicated that walnut emulsions treated with a dual-frequency of 28/68 kHz had a stable system that was comparable to nano-emulsions. It was of great significance for food emulsions. Walnut emulsions also demonstrated good emulsifying performance, with a 100% creaming index that could be maintained for 14 days without phase separation. Similar observations had been reported by Silva et al, who assessed that the ultrasonic treatment of annatto seed oil emulsion kept 24 h without stratification [42,43]. Shanmugam and Ashokkumar found that the ultrasonic treatment of flaxseed oil skim milk emulsion could be maintained for 7 days without stratification [24]. Qayum et al. also reported that α-whey protein emulsions treated by ultrasonic had no signs of separation after

### Table 3
Comparison of D(4,3), Dv50, and Dv90 value of walnut emulsions process at different power density. (*The results are average of two measurements and the error value varies from 0 to 0.017 μm.)

| Ultrasonic Power Density(W/L) | D(4,3) μm | Dv50 μm | Dv90 μm |
|-------------------------------|-----------|---------|---------|
| 0                             | 15.3      | 7.87    | 18.5    |
| 100                           | 10.3      | 9.26    | 21.1    |
| 140                           | 7.61      | 5.74    | 17.5    |
| 180                           | 7.45      | 5.64    | 17.1    |
| 220                           | 6.28      | 2.81    | 17.1    |
| 260                           | 6.56      | 3.18    | 17.0    |
| 300                           | 4.95      | 2.19    | 14.1    |

Fig. 5. Creaming stability of walnut emulsions processed for different power density: 100, 140, 180, 220, 260, and 300 W/L and visual observation of studied emulsions after 7 and 14 days upon storage at 4 °C.
14 days of storage at room temperature [44]. The addition of surfactant and polysaccharides to walnut emulsions can stabilize the emulsion system against the negative effects of ion concentration and temperature change. As expected, it was found that when coupled with the high-efficiency cavitation effect of dual-frequency ultrasonic treatment, walnut emulsions can maintain excellent stability.

3.4. Microstructure

Emulsion microstructure observations were also evaluated with high stability under slit ultrasonic treatment of 50 min, 260 W / L, and 28/68 kHz. Confocal images (Fig. 8. A) showed that oil droplets (green) and protein (red) in ultrasonic treatment were smaller and more dispersed than in non-ultrasonic treatment. Electron microscopy (Fig. 8. B) showed that droplet spacing without sonication was less than sonication at 28/68 kHz. There were more films in the emulsions without sonication, and the films were significantly reduced after sonication at 28/68 kHz. The average size of oil droplets without ultrasonic treatment was about 15 μm, while the ultrasonic treatment was about 2 μm, which was consistent with the results of particle size analysis.

It has been reported that UE is pretty beneficial to the stability of O/W emulsions, which can form smaller complex particles and have a narrow droplet size distribution [45]. Compared with UE emulsion, the original emulsions have obvious agglomeration formed by large droplets. Due to the unstable mechanism of the emulsion system, the oil droplets and protein particles are regularly gathered or precipitated by the Law of Stokes [46]. UE emulsions with smaller oil droplets aggregated at a slower rate and were, therefore, more stable [47,48]. Oil droplets without sonication were close to each other, and there was a thin film with surface tension, which would promote the aggregation of droplets and poor stability. After sonication, the film was significantly reduced and the aggregation between oil droplets was slowed down, leading to good stability of emulsions [49]. Moreover, because of the addition of surfactants, the surface tension of the emulsion was increased, and the stability of the emulsion was enhanced [50]. From the above, as expected, it was found that the oil droplets and proteins created by slit dual-frequency UE were small, uniformly dispersed with a slow aggregation rate. It is guaranteed that no phase separation occurs during the available shelf life.
4. Conclusions

Slit dual-frequency ultrasonic, as a green technology of walnut emulsions emulsification, has excellent treatment effect and good product stability, which make it suitable for industrial production of food emulsions. The optimal ultrasonic parameters of walnut emulsions were 50 min ultrasonic time, 260 W/L ultrasonic power density, and a 28/68 kHz ultrasonic frequency combination. The average mean volume diameter of walnut emulsions after treatment could be reduced to 2.05 μm, the absolute value of zeta potential reached more than 40 mV, and the creaming index was stable, which could be maintained for at least 14 days without phase separation. The laser scanning confocal microscope (LSCM) and transmission electron microscope (TEM) revealed the fine, uniform, and suitable long-term storage characteristics of UE emulsions. In the future, a pilot study can be carried out to expand the process scale and develop a new technology to produce food emulsions.

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CRediT authorship contribution statement

Ningning Ouyang: Investigation, Methodology, Data curation, Formal analysis, Validation, Writing – original draft. Haile Ma: Investigation, Methodology, Visualization, Writing – review & editing. Yanhua Ding: Software, Writing – review & editing. Feng Lu: Writing – review & editing. Lina Guo: Writing – review & editing. Xuei Zhang: Writing – review & editing. Chen Gu: Writing – review & editing.

Declaration of Competing Interest

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

Fig. 8. Confocal laser scanning microscopy (A) and transmission electron microscopy (B) images without sonication and after sonication at 28/68 kHz.
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