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Impact of ultrasonic treatment on an emulsion system stabilized with soybean protein isolate and lecithin: Its emulsifying property and emulsion stability

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ABSTRACT

The present study aims to investigate the impact of ultrasonic treatment on the emulsifying property and emulsion stability of an emulsion system stabilized with soybean protein isolate (SPI) and lecithin. Ultrasonic parameters used were ultrasonic powers of 150, 300, and 450 W and ultrasonic durations of 12 and 24 min. Emulsifying properties of emulsions were all improved with different extents after ultrasonic treatments. The emulsion treated at 150 W & 24 min showing the best emulsifying property and emulsion stability than the rest. However, the higher ultrasonic power of 450 W gave negative effects on emulsion stability, with increased particle size and decreased absolute ζ-potential values due to protein aggregation. Prolonged ultrasonic duration from 12 to 24 min resulted in a more stable emulsion under the ultrasonic power of 150 W. However, for ultrasonic powers of 300 and 450 W, the additional ultrasonic energy from prolonging ultrasonic duration from 12 to 24 min generated negative effects to emulsion stability.

1. Introduction

Soybean protein is an attractive food ingredient due to its high nutritional value, and its desirable flavour in foods (Ma et al., 2015). The specific surface properties of proteins allows them to be adsorbed at oil-water interfaces, forming a thick protective layer which reduces interfacial tension. Therefore, they act as effective emulsifiers by ensuring oil droplets remain in a stable condition in an aqueous continuous phase (Imura et al., 2015). However, emulsions prepared by soybean proteins are sensitive to changes in ionic charge, which limits its application in food processing and production (Nguyen et al., 2014). Cao et al. (2015) reported that the stability of the emulsion was compromised once the system pH approached around pH 4.8, which is the isoelectric point of soybean protein.

Lecithin, a type of a zwitterionic surfactant, is one of the most effective natural emulsifiers, and is extensively used in reducing the interfacial tension of emulsions (Mottola, Vico, Villanueva, & Fanani, 2015). Upon interacting with lecithin, soybean protein exhibits different surface activity. Scuriatti, Tomás, and Wagner (2003) and Mantovani, Cavallieri, Netto, and Cunha (2013) reported that the addition of lecithin improved the stability of emulsions consisting of soybean protein and whey protein, respectively, even though the system was near the isoelectric point of the proteins. Comas, Wagner, and Tomás (2006) also found that the addition of lecithin enhanced the stability of emulsions prepared by native or denatured soybean protein isolates, sunflower oil, and water. The hydrophobic interaction was found to play a key role in maintaining the interactions between proteins and phospholipids by incorporating the proteins into lecithin vesicles and micelles (van Nieuwenhuyzen & Szuhaj, 1998). Recently, Kasinos et al. (2013) and Li, Li, and Guo (2014) further concluded that lecithin and soybean protein could interact through electrostatic

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and hydrophobic interactions, which provide desirable changes to the conformation of protein, and therefore result in improved emulsifying ability.

Ultrasound can be classified into low and high power ultrasound according to its frequency range (Awad, Moharram, Shaltout, Asker, & Youssef, 2012). Low-power ultrasound is always used in ensuring food quality and safety, while high power ultrasound is applied to modify functional properties of different foods, including improving the stability of emulsions (Chemat & Khan, 2011). Kaltsa, Gatsi, Yanniotis, and Mandala (2014) reported that in an emulsion system containing a combination of whey protein isolate and xanthan, the droplet size of the oil phase was significantly reduced by either increasing ultrasonic power or the duration of ultrasonic treatment. Similarly, an emulsion system using coconut milk protein was found to have a reduced mean droplet size and improved stability after ultrasonic treatment (Lad & Murthy, 2012). However, few studies have investigated the change in the physicochemical and functional properties of proteins after ultrasonic treatments (Chandrapala, Zisu, Palmer, Kentish, & Ashokkumar, 2011). Moreover, to the best of our knowledge, no other study has investigated the emulsifying property and emulsion stability of SPI and lecithin-stabilized emulsions after ultrasonic treatments.

Thus, this work aims to evaluate the effects of ultrasonic power and duration on the properties of emulsions prepared using SPI and lecithin. Ultrasonic power of 150, 300, and 450 W were adopted for two ultrasonic durations (12 and 24 min). The results of this work would be useful in providing a novel and improved method of producing soybean protein based emulsions.

2. Materials and methods

2.1. Materials

Soybeans were purchased from Heilongjiang Agriculture Co. Ltd., Harbin, China. Lecithin, with a content of acetone-insoluble material (phosphatidylcholine, PC) > 95%, was obtained from Sigma-Aldrich (St. Louis, MO, USA). Sunflower oil (COFCO Co. Ltd., Harbin, China) was purchased from a local shop. Nile Blue dye and Nile Red dye were purchased from Sigma-Aldrich, Wicklow, Ireland. All other chemicals were of analytical reagent grade and deionized (DI) water was used throughout.

2.2. Emulsion preparation

SPI were prepared according to the method of Speroni, Añón, and de Lamballerie (2010). Defatted soybean flour was dispersed in DI water (1:10, w/v), and the pH of the dispersion was adjusted to 8.0 using NaOH (2 mol/L). The solution was subjected to alkaline extraction with continuous magnetic stirring for 2 h and then centrifuged at 10,000*g* and 4 °C for 30 min. The sediment was discarded and the supernatant was collected by adjusting the pH to 4.5 using HCl (2 mol/L) and then centrifuging at 6000*g* and 4 °C for 30 min. The obtained precipitate was dialyzed against DI water for 48 h at 4 °C before neutralization using NaOH (2 mol/L), and then was freeze-dried for further use.

SPI was mixed well with lecithin at a ratio of 10:1 (w/w) before dispersing in 0.05 M phosphate buffer solution (1.1%, w/v), followed by mixing at room temperature for 2 h using magnetic stirring. Sunflower oil was then added to the prepared slurry at a ratio of 1:3 (v/v), followed by homogenization at ambient temperature using an Ultra-Turrax T18 homogenizer (ANGNI Co. Ltd., Shanghai, China) at 20,000 rpm for 1 min. The freshly-prepared emulsion was then subjected to the following studies.

Table 1
The parameters of different ultrasonic treatments.

Sample number	A	B	C	D	E	F	G
Ultrasonic power (W)	0	150	300	450	150	300	450
Ultrasonic duration (min)	0	12	12	12	24	24	24

2.3. Ultrasonic treatments

Freshly-prepared emulsions were subjected to ultrasonic treatment using an ultrasonic processor (NingBo Scientz Biotechnology Co. Ltd., Ningbo, China) with a 0.636 cm diameter titanium probe. An aliquot of 30 mL of the emulsion was poured into a 50 mL flat bottom breaker surrounded by a double-walled cooling water jacket, which helped to keep the temperature consistent. The parameter of ultrasonic treatments was shown in Table 1. The ultrasonic treatments were performed at output intensity of 0, 150, 300, and 450 W, which cover the often used ultrasonic powers. The ultrasonic treatment duration was set to 12 and 24 min, considering few study evaluated the two sonication durations.

2.4. Determination of emulsifying properties

The emulsifying properties of control and ultrasound-treated emulsions were measured according to the method of Li, Huang, Peng, Shan, and Xue (2014) with slight modifications. After ultrasonic treatments, 50 μL of emulsions were immediately sampled and diluted 100 times using 0.1% sodium dodecyl sulfate (SDS). The absorbance of the emulsion at 500 nm was recorded immediately (A_0) and after 10 min (A_{10}) using a spectrophotometer (SHJH Co. Ltd., Shanghai, China). The emulsifying activity index (EAI) and emulsion stability index (ESI) were computed using Eqs. (1) and (2), respectively.

$$\mathrm{EAI}\left(\mathrm{m}^2/\mathrm{g}\right) = 2 \times \mathrm{T} \times \frac{A_0 \times N}{10000 \times \theta \times L \times C} \quad (1)$$

$$\mathrm{ESI}\ (\mathrm{min}) = \frac{A_0}{A_0 - A_{10}} \times (T_{10} - T_0) \quad (2)$$

where T equals to 2.303; A_0 is the absorbance at 0 min; N is the dilution factor (100); θ is the proportion of the oil phase (0.25); L is the thickness of the cuvette (1 cm); C is the concentration of SPI (g/mL); A_{10} is the absorbance at 10 min; T_0 represents 0 min; and T_{10} represents 10 min.

2.5. Creaming stability measurement

Creaming index (%) represents the creaming stability of an emulsion, whereby a lower creaming index indicates a lesser extent of phase separation (Zisu, Schleyer, & Chandrapala, 2013). Control and ultrasound-treated emulsions were placed inside hermetic tubes and stored at room temperature for up to 7 days. The magnitude of creaming was measured once a day by measuring the height of the clear liquid layer at the bottom (H_C) and the height of total emulsion (H_E). Creaming index can therefore be calculated using Eq. (3).

$$\text{Creaming index}\ (\%) = (H_C/H_E \times 100) \quad (3)$$

2.6. Confocal laser scanning microscopy

Proteins can be stained by Nile Blue dye, emitting green light. Oil