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Regular Article

Functionalizing soy protein nano-aggregates with pH-shifting and mano-thermo-sonication

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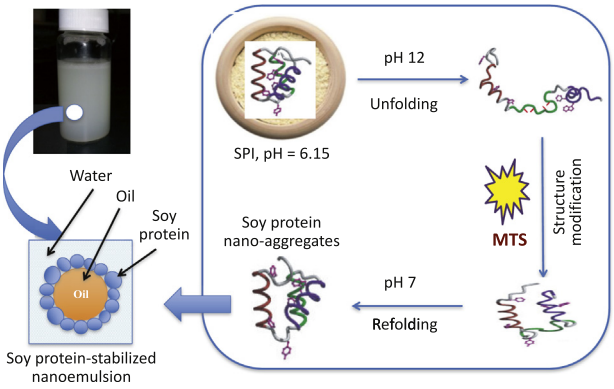
GRAPHICAL ABSTRACT



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ABSTRACT

Plant protein-mediated nano-delivery systems have gained increasing attention in the food and pharmaceutical industries in recent years. Several physical and chemical methods for improving the functional properties of plant proteins with respect to the native forms have been proposed. This study presents a new approach, which combines pH-shifting and mano-thermo-sonication (MTS) to produce soy protein nano-aggregates with significantly improved functional properties. Soy-protein isolate (SPI) was treated with pH-shifting at pH 12 or in combination with MTS and high-pressure homogenization (HPH). Response Surface Methodology was used to find the optimal conditions (50 °C, 200 kPa, and 60 s) for the MTS. The combination of pH-shifting and MTS resulted in spherical SPI aggregates of the smallest size, 27.1 ± 1 nm, as shown by transmission electron microscopy. The SPI nanoaggregates were used to prepare oil-in-water nanoemulsions with canola oil, which exhibited good stability over 21 days at 4 °C. In addition, the pH 12-MTS samples had resulted in the highest protein solubility, lowest turbidity, free sulfhydryl and disulfide bonds, surface hydrophobicity, antioxidant activity, and rheological and emulsifying properties than the other samples.

1. Introduction

Proteins are widely used in the food industry as foaming and emulsifying agents [1]. They stabilize oil-in-water emulsions by forming an interfacial membrane on which the adsorbed proteins unfold and rearrange their secondary and tertiary structures to

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expose hydrophobic residues to the hydrophobic phase [2]. Plant proteins are low cost, non-toxic, natural, biocompatible, and biodegradable polymers. In recent years, they have drawn increasing attention from the food and pharmaceutical industries as an alternative to animal proteins due to increased consumer concerns over the safety of animal-derived products (e.g., prion diseases) [3]. Among plant proteins, soy proteins have been widely studied for numerous applications [4–7]. Soy protein is an ample byproduct of the soybean oil industry. Whole soybeans can be processed into a number of products such as roasted soy nuts, soy flours, soybean oil, defatted flakes, soy-protein concentrates, and soy-protein isolates (SPI) [8]. The main storage protein in soybean are globulins, which are mostly insoluble in water. The poor solubility of globulins in soy makes it a less effective emulsifier than dairy proteins, such as casein [9].

A number of attempts have been made to modify the native structures of soy proteins in order to improve their functional properties, including high-pressure and heat combined treatments [10,11], extreme pH treatment [12], high-pressure processing (HHP) [13], pulsed electric field (PEF) [14], enzyme hydrolysis [15], subcritical water treatment [16], and ultrasonication [17]. Among the chemical treatments, pH-shifting is a relatively new method that adjusts the pH of a protein solution to extreme basic or acidic conditions to unfold the protein, followed by changing the pH back to neutral to refold the protein. This unfolding-refolding process was reported to effectively modify the protein functional properties [18,19]. *High intensity ultrasound* or *power ultrasound* refers to sonic waves with frequencies higher than sound audible to the human ear, with sound intensities in the range of 0.1–1 W/cm^2 [20]. The mode of action of ultrasound-induced protein-structure modification is often attributed to acoustic cavitation. The physical forces produced by cavitation, such as shear forces produced by micro-streaming and normal impingement from the water jets at the solid-liquid interfaces, help to break down the protein particles or aggregates in dispersions [19]. Ultrasound treatment was reported to enhance protein solubility and surface hydrophobicity, and modify protein subunits [21,22]. Conventional ultrasonic modification of protein functionality is performed at relatively low cavitation intensities. Consequently, relatively long treatment times, i.e. 15–30 min for soy proteins [17] and 20 min for whey protein [23], are required to produce meaningful changes in protein structures. There is a need to reduce the treatment time of ultrasound processes, thereby increasing throughput, lowering costs, and making their applications practical.

Ultrasonication in combination with low hydrostatic pressure and low heat, a process termed mano-thermo-sonication (MTS), has been reported to enhance acoustic cavitation activity [24]. Significant process enhancement by MTS has been reported in microbial inactivation tests. For instance, it took 15.9 min to achieve a 5-log cycle (99.999%) reduction in the population of *E. coli* K12 in apple cider, a benchmark set by USFDA for microbial inactivation in juices, in an ultrasound-alone treatment [25]. The time was reduced to 1.4 min when an MTS treatment at 400 kPa and 59 °C was applied to the juice to obtain the same 5-log reduction [26]. However, MTS has not been reported for use in protein modification. This study documented the first attempt to utilize MTS to enhance the functional properties of soy-protein isolate. Furthermore, we combined pH-shifting with MTS to further improve the efficacy of the treatment. The MTS was applied when the protein was unfolded by an extremely basic pH treatment, as the open structure of the protein made it more susceptible to the ultrasound treatment. The pH-shifting and MTS combined treatment was optimized with Response Surface Methodology, and compared with high pressure homogenization (HPH). The physicochemical, interfacial, and emulsifying properties of the treated soy protein were evaluated.

2. Materials and methods

2.1. Soy protein isolate (SPI)

Soy protein isolate (SPI, Pro-Fam® 955, pH 5.0–5.5) was provided by Archer Daniels Midland Company (ADM, Decatur, IL, USA). The Pro-Fam® 955 contains 90% soy protein on dry basis and is a water washed intact protein (without hydrolysis with proteases).

2.2. pH-shifting, high pressure homogenization, and MTS

Mano-thermo-sonication treatment was conducted using a laboratory scale MTS system (Fig. 1). In continuous mode, the protein dispersion in a sample bottle placed in ice was pumped by a peristaltic pump (750 W, Model 7523-20, Masterflex, Vermon Hill, IL, U.S.A) into a custom-made sono-reactor. The treated samples were collected from the two outlets of the sono-reactor and cooled down for analysis. A preliminary test was performed to determine the optimal MTS treatment conditions. In MTS tests, 9 g of SPI were dispersed in 300 mL DI water by agitation with a stirrer for 30 min, and the dispersion was pumped through the MTS unit. The MTS treatment time (or residence time of the sample in the sono-reactor) was determined by the flow rate (mL/s) and the volume (L) of the treatment chamber. The MTS parameters include 3 temperatures (40, 45, and 50 °C), 3 sonication times (30, 45, and 60 s) and 3 absolute pressures (100, 200, and 400 kPa).

Five treatments were applied to modify SPI: MTS (at optimal conditions), pH shifting, pH 12-MTS, HPH, and pH 12-HPH (Table 1). In the pH alone treatments, three grams of SPI was dissolved in 100 mL deionized (DI) water and stirred at room temperature for 30 min to get the protein dispersions. The pH of the protein dispersions was adjusted to pH 12 with 2 M sodium hydroxide (NaOH). Then, the sample was stored at room temperature for 1 h, followed by adjusting the pH back to pH 7 with 2 M hydrochloric acid (HCl). In the pH 12-MTS treatment, the SPI dispersions were adjusted to pH 12 with 2 M NaOH and then treated by MTS (50 °C, 200 kPa and 60 s) before the pH was adjust to neutral. For the pH 12-HPH treatment, the SPI dispersions were adjusted to pH 12 with 2 M NaOH and then treated by HPH followed by changing the pH of the dispersion to pH 7. Neutralized protein dispersions were centrifuged (1200 *g* and 20 °C) for 15 min and the supernatants were collected as soluble soy protein for further experiments. The HPH treatment was conducted using a high-pressure homogenizer (APV two stage homogenizer; SPX Flow Technology, Denmark) at 8000 psig for 3 min with a sample size of 500 mL containing 15 g of SPI.

2.3. Protein solubility

Soluble protein content was determined with a Bio-Rad Protein Assay based on the method of Bradford [27]. Bovine serum albumin (BSA) (Bio-Rad 500-0007) was used as the standard. Dye reagent was prepared by diluting 1 part of dye reagent concentrate (Bio-Rad 500-0006) into 4 parts of DI water, and filtered through Whatman #1 filter paper. Diluted dye reagent was added to soluble SPI. Protein concentration of soluble SPI was measured using a spectrophotometer (Lambda 1050 UV/VIS/NIR Spectrometer, PerkinElmer, Waltham, MA, USA) under the wavelength of 595 nm [27]. Protein solubility was calculated as the percentage of the soluble protein content in the supernatant over the total protein added in the dispersion as shown in Eq. (1).

$$\text{Recovery of soluble protein}(\%) = \frac{\text{Protein concentration in soluble SPI}}{\text{Initial protein concentration}} \times 100 \quad (1)$$