Journal of Food Engineering 206 (2017) 1–12

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Pilot-scale ultrasound-assisted extraction of protein from soybean processing materials shows it is not recommended for industrial usage

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ARTICLE INFO

Article history:
Received 31 August 2016
Received in revised form
15 December 2016
Accepted 2 February 2017
Available online 7 February 2017

Keywords:
Ultrasound-assisted extraction
Aqueous extraction
Pilot-scale
Soy protein

ABSTRACT

Unit operations to enhance protein extraction within the food industry are vital to improve current processes, especially for cost reductions and sustainability. Here a study of ultrasound-assisted extraction (UAE) from soy slurry and okara produced at pilot-scale and further processed using a lab or pilot-scale probe system is presented. Confocal imaging and particle size measurements were used to study the physical effects of UAE on these soy processing materials. Ultrasound at pilot-scale was infeasible for soy slurry, in contrast to lab-scale. UAE from okara solution significantly increased protein yield by 4.2% at pilot-scale ($p < 0.05$). Okara solution flow rate and okara concentration also significantly improved the protein extraction yield. During lab-scale sonication of okara solution, a greater energy intensity resulted in a higher yield of up to 40% after 15 min treatment. Considering total extraction yields at pilot-scale during soybase production, ultrasound is not considered viable for industrial processing.

1. Introduction

Soybeans are a source of 'complete' protein, providing the body with all of the essential amino acids that humans are unable to synthesise. Soybeans range in composition and their use is dependent on their desired function; for soymilk preparation, soybeans are chosen with a high protein content, compared to those utilised for oil extraction. In terms of resources: less energy, water and land is required to provide the world with sufficient protein from plant-based sources compared to animal-based (Aiking, 2011). For these benefits to be realised, the processing of raw materials to provide the final products needs to be efficient. During current soymilk processing plants, a significant portion of the available protein enters the waste stream currently utilised as animal feed (O'Toole, 1999). A more sustainable extraction of components is required using a green technology to make soymilk manufacture more profitable on social, economic and environmental levels.

The soybean microstructure is complex. Within the storage cells of the soybean, protein is organised in 5–20 μm protein bodies, surrounded by a cytoplasmic network containing oil bodies in the size range of 0.2–0.5 μm stabilised by proteinaceous oleosins (Rosenthal et al., 1998). In order to solubilise components inside the cells, the solvent needs to be in direct contact with those components, this is most easily facilitated by cell disruption. During soymilk production, soybeans are milled under hot (>80 °C), alkaline (pH 8+) conditions to solubilise protein, as well as inactivate the enzyme lipoxygenase and trypsin inhibitors (Vishwanathan et al., 2011). Insoluble materials are removed from the slurry using centrifugation; this gives two streams: soybase, the precursor for soymilk, and a waste stream, termed okara. The okara has been shown, using confocal laser scanning microscopy (CLSM) (Preece et al., 2015), to contain both intact cells and insoluble protein in the continuous phase. It would obviously be valuable to increase the process yield by breaking up a higher proportion of the cells.

Ultrasound has been widely studied in the food industry for aiding the extraction of components of interest from plant sources (Chandrapala et al., 2013; Chemat et al., 2011; Esclapez et al., 2011; Patist and Bates, 2008; Shirsath et al., 2012; Vilkhu et al., 2008). Ultrasound has been utilised and shown promise as a green technology within the field of extraction, reasons including reductions in extraction times, solvent use and more effective energy utilisation, as well as improving product quality (Chemat et al., 2017; Jacotet-Navarro et al., 2016; Li et al., 2013; Sicaire et al., 2016). The success of ultrasound is attributed to the cavitation phenomenon.

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http://dx.doi.org/10.1016/j.jfoodeng.2017.02.002

Conditions attributed to cavitation assist the solubilisation of materials into the liquid medium, enhancing the extraction. Upon asymmetric bubble collapse, liquid jets are formed which can disrupt cells upon contact with cell walls (Li et al., 2004; Shirsath et al., 2012), causing the release of intracellular compounds. A detailed overview of the mechanisms responsible for the enhancement of extraction yield from plant materials associated with ultrasound is described by Chemat et al. (2017). For ultrasound to be considered as a green technology, the greenhouse gas emissions resulting from the treatment should be lower in comparison to the greenhouse gases which are saved by the reduction in soybeans required.

Soy-based studies are present examining the effects of ultrasound on the extraction of various compounds. For protein and sugar extraction, one such study by Karki et al. (2010) showed the application of ultrasound (20 kHz, ≤2 min treatment) improved the extraction yields from hexane-defatted soy flakes at lab-scale. Protein functionality improvement from soy protein isolate (SPI) and concentrates (SPC) has also been reported with positive results in protein solubility and particle size reduction (Lee et al., 2016). Some studies on ultrasound-assisted extraction (UAE) from soy-based systems as the starting material exist, yet direct extraction from the soybean has largely been neglected. Preece et al. (2017b) showed that ultrasound (20 kHz, ≤15 min) improved protein extraction yield by up to 21% & 25% for directly from soybeans in a lab-scale system, through slurry treatment and okara treatment on samples prepared at lab-scale, respectively (Preece et al., 2017b). This research (Preece et al., 2017b) partially supported the patent of Wijngaard and Zuidam (2014), where improvements in extraction yields were only observed during okara solution sonication. During the preparation of soy-based beverages, direct extraction of proteins, oils and other alkali-soluble components by wet milling from soybeans is commonly used during factory-scale manufacture (Vishwanathan et al., 2011). UAE of soy protein has been studied previously (Moulton and Wang, 1982) under continuous conditions on pilot-scale, using defatted soy flakes as the starting material. However, this study was carried out more than 30 years ago, and key experimental data, such as particle size measurement and visualisation of the microstructure, were neglected. Direct extraction from soybeans are rarely studied at a lab-scale, and no pilot-scale studies were found in the literature.

Pilot-scale use of ultrasound has been documented for a limited number of food systems, other than soy protein extraction. Pingret et al. (2012) showed an improvement of 30% extraction of polyphenols was achievable using ultrasound-assistance (20 kHz, 40 °C, 40 min) versus conventional extraction from apple pomace in a 30 L tank. Another study focused on waste stream valorisation; phenolic compounds were extracted from maritime sawdust waste using UAE (25 kHz, 40 min) with an increased phenolic yield of 30% compared to conventional maceration on a pilot-scale (Meullemiestre et al., 2015). A lower recovery of capsaicinoids from chilli peppers on a pilot-scale (20 L tank) was obtained using UAE compared to hot maceration at industrial scale, although reductions in temperature and time were achieved (Boonkird et al., 2008).

Pilot-scale studies of UAE directly from soybeans has not been previously reported in the literature. Here the effects of ultrasound on the protein extraction yield during soybase production (pilot-scale) are shown using lab and pilot-scale probe systems. It was hypothesised that an improvement in extraction yield, as found before at lab-scale (Preece et al., 2017b), will be observed at pilot-scale as well due to the improved availability of of protein in the aqueous phase. A central composite design (CCD) was employed to examine the effects of okara concentration, okara flow rate and temperature of ultrasound treatment on the protein extraction yield at pilot-scale versus a conventional method for extraction. The optimum conditions are identified for the specific conditions tested and analysis of variance (ANOVA) will be employed to determine the significance of factors. Particle size measurements and an examination of the microstructure of the materials are performed to aid in identification of the mechanisms of ultrasound.

2. Materials & methods

2.1. Sample production

Soy slurry and okara were prepared from commercially available soybeans using pilot plant facilities (Unilever Research & Development, Vlaardingen). A process flow diagram and stream information can be seen in Fig. 1 and Table 1. Under these processing conditions, it was possible to prepare soy slurry and okara to test the effects of ultrasound. Soybeans (stream 3, Fig. 1) went through two wet milling stages to produce a soy slurry under alkaline conditions. The processing input consisted of 28 kg h^{-1} of soybeans treated with 175 kg h^{-1} of softened water and 0.2 kg h^{-1} of sodium bicarbonate. To prepare soybase and okara for subsequent treatment, the slurry was fed into a decanter centrifuge operating at a *g*-force-time of 1.5×10^5 *g*-s. Table 2 shows the average compositions of okara (Fig. 1, stream 8) and soy slurry (Fig. 1, stream 4) produced using the pilot plant processing equipment *without ultrasonic treatment*.

Fig. 1 also shows the process flow diagram for:

(i) & (ii) *Lab-scale ultrasonic treatment* of the okara and slurry from pilot-scale production (see section 2.2). Here, materials were transferred from the pilot plant and treated with a bench-scale 400 W probe previously used to study lab-scale extraction (Preece et al., 2017b).

(ii) *Pilot-scale ultrasonic treatment* of okara, where the okara was treated using a pilot-scale 2000 W probe (see section 2.3).

A schematic diagram of both the lab and pilot-scale probe systems can be seen in Fig. 2.

2.2. Laboratory–scale sonication

A bench-scale batch ultrasound probe system (Branson Sonifier 450, Branson Ultrasonics Corporation, Danbury, CT), (20 kHz, 65 W (output according to manual), 13 mm probe tip) was utilised to study the effects of ultrasound on slurry and okara solution samples produced in the pilot plant. The lab-scale probe is described schematically in Fig. 2A. Ultrasound treatment times from 0 min (control) up to 15 min were investigated to vary the energy input to the system. After the sample was treated for the desired time, the sample was immediately centrifuged at 4330 × *g* for 10 min.

The energy input was calculated using the equations reported by Bates and Patist (2010), which gives a unit independent of the scale of treatment for continuous and batch operation:

$$\textit{Energy input},\ W_{\text{input}} = \frac{P\ (\text{kW})}{Q\ (\text{L h}^{-1})} = \frac{P\ (\text{W}) \times t\ (\text{s})}{3.6 \times 10^6 \left(\frac{\text{J}}{\text{kWh}}\right) \times V\ (\text{L})} \tag{1}$$

where power (*P*) was calculated using the details from the supplier, and *Q* is the volumetric flow rate for continuous application. Power (*P*), time (*t*) and volume (*V*) were necessary to calculate energy based on batch operation. The volume for both slurry and okara solution treatments prepared at lab-scale was 100 mL. The calculation of the energy input for the lab-scale system was necessary in order to select the energy input range to be studied at pilot-scale.