



Intensification of protein extraction from soybean processing materials using hydrodynamic cavitation



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ABSTRACT

High pressure homogenisation (HPH) has been investigated for its potential to aid the aqueous extraction of protein and other components from soybeans. HPH treatments (50–125 MPa) were applied to soy slurry and okara, the diluted waste stream from soybean production. Extraction yields of oil, protein and solids were calculated, and the feasibility of the technology was assessed. The most productive HPH treatment investigated improved extraction yields of protein up to 82% with a single pass of soy slurry at 100 MPa. In comparison, a maximal protein extraction yield of 70% has been achieved previously using ultrasound at lab-scale for 15 min (20 kHz, 65 W according to manual, 13 mm probe tip) (Preece et al., in press). Results showed a particle size reduction upon HPH and disruption of intact cells, confirmed via confocal laser scanning microscopy. Multiple HPH passes of soy slurry caused an increase in dynamic viscosity and a small increase in particle size probably due to cell wall swelling, resulting in decreased separation efficiency and consequently a reduced extraction yield. HPH offers extraction assistance, with more promising results reported in comparison to ultrasound-assisted extraction of soybean processing materials.

Industrial relevance: Improvement of current soybean processing is desirable on an industrial level to better use available raw materials and reduce waste production. This study shows the effects of a technology already widely employed in industry for other benefits, such as fine emulsion production and microbial cell disruption. High pressure homogenisation was carried out on a lab-scale on soybean processing materials which were prepared in a pilot plant, with similar feed compositions to those produced at an industrial scale.

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1. Introduction

Protein is an important nutrient to be considered when studying food production for human consumption, with major pressure to provide nourishment for an increasing population. The use of vegetable proteins like soy instead of animal derived protein sources is a rapidly increasing consumer trend. Extraction of protein and other soybean components from milled soybeans may happen under alkali aqueous conditions at high temperature to prepare soybean, the soybean extract further processed to soymilk or tofu. After the extraction, insoluble materials are removed from the extract typically by decanting, and the fibrous waste stream, termed okara, is utilised as animal feed (Preece et al., in press). This process requires attention as the current yield in factories is relatively low (50–60%); improved production methods may yield a greater mass of protein for human consumption.

The majority of the soybean structure (90%) is made up of cotyledon cells, ranging in length from 70 to 80 μm and 15–20 μm in width

(Rosenthal, Pyle, & Niranjana, 1998). Within the cotyledon cells, the majority of protein is organised in protein bodies that are typically 2–20 μm in diameter (Preece et al., 2015). Oil is located within the cytoplasmic network in oil bodies stabilised by low molecular weight proteins termed oleosins (Rosenthal et al., 1998). These oil bodies are smaller in size than protein bodies with sizes in the range 0.2–0.5 μm . The main barrier for the extraction of intracellular components of interest is the cell walls. Other limitations include insolubility of materials and entrapment in the continuous phase of the insoluble waste stream (Preece et al., 2015).

Cavitation is a process responsible for the success of some extraction assistance process technologies (Gogate, Tayal, & Pandit, 2006). The phenomena of cavitation include air void formation within a treated sample, growth of the voids and their potential violent collapse. Upon microbubble collapse, local regions of high pressure and temperatures result in the regions of 1000–5000 atm and 500–15,000 K, which can aid the extraction process (Gogate & Kabadi, 2009). Another result of cavitation is void collapse near a solid surface: leading to local regions of high shear resulting in solubilisation and also cell disruption (Sutkar & Gogate, 2009).

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Ultrasound, a processing technology based on acoustic cavitation, has been shown to enhance the extraction of protein and other components during the processing of soybean materials. Ultrasound improved the extraction of protein by up to 19% upon 15 min treatment of okara solution with a lab probe system (Preece et al., *in press*). The material was examined using confocal laser scanning microscopy (CLSM); improved solubility was found to be the main factor enhancing the yield, not cell disruption (Preece et al., *in press*). Unfortunately, when ultrasound was applied at pilot plant scale it was not feasible to give the soy slurry a treatment equivalent to that possible at lab scale. Pilot scale ultrasound treatment of okara was shown to increase protein extraction yield by only 4.2% compared to control samples (Preece, Hooshyar, Krijgsman, Fryer, & Zuidam, 2016). Other parameters, including okara solution flow rate and okara concentration, also had a significant impact on the protein extraction yield. During the lab scale sonication treatment an approximately 300× greater energy intensity was experienced by the samples, compared to the pilot scale sonication. Considering the minimal total extraction yields for soybean production at pilot scale, ultrasound was not considered viable for industrial processing. It was found that the remaining protein within the okara was within intact cells (Preece et al., 2016). Therefore, a processing technology that targets intact cells might be more beneficial.

Hydrodynamic cavitation is widely accepted as a technique for cell disruption of microbes and microalgae (Lee & Han, 2015; Save, Pandit, & Joshi, 1997), as well as for the recovery of intracellular enzymes (Gogate & Pandit, 2008). It can be achieved using a high pressure homogeniser (HPH) at pressures above 35 MPa (Donsi, Ferrari, Lenza, & Maresca, 2009). HPH has been employed in the food industry for large scale microbial cell disruption, as well as for other purposes, such as emulsification (Gogate, 2011). Extraction with assistance from high pressure has been studied for several food systems with promising results, such as carotenoid extraction from tomato paste waste (Xi, 2006) and phenolic acids extraction from potato peel (Zhu et al., 2016), as well as oil extraction from microalgae for use in biodiesel production (Dumay et al., 2013; Lee, Lewis, & Ashman, 2012; Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007; Xi, 2006).

High pressure treatment has also been applied to a number of soy based systems. Typically pressures of greater than 300 MPa have been studied for the formation of soy protein gels (Apichartsrangkoon, 2003; Kajiyama, Isobe, Uemura, & Noguchi, 1995; Okamoto, Kawamura, & Hayashi, 1990). These studies did not include hydrodynamic cavitation; only the effects of high pressure achieved using a pressure cell were investigated. Some studies of the effects of HPH on soy protein, focusing on the microbial stability of products and the production of fine emulsions rather than on extraction, have been published (Cruz et al., 2007; Flourey, Desrumaux, & Legrand, 2002; Polisel-Scopel, Hernández-Herrero, Guamis, & Ferragut, 2012).

For the implementation of HPH for extraction in industrial scale processes, a number of factors have to be considered, including energy consumption, instrument geometry and wear, and productivity (Dumay et al., 2013). Many examples of the use of HPH within the food industry are available, yet current applications focus on the structuring of products, such as fine emulsion production. Creaming, which is an unwanted phenomenon seen in the dairy industry, is one such example for the possible industrial use of HPH (Tobin, Heffernan, Mulvihill, Huppertz, & Kelly, 2015). A scale up study by Donsi et al. (2009) showed that the scale of HPH operation did not influence microbial cell disruption at a given pressure. This gives confidence for the scalability of HPH for use in extraction at an industrial scale, if positive results are achieved at lab scale for extraction.

Extraction of protein from soybeans has been reported previously in the literature as discussed above (Apichartsrangkoon, 2003; Cruz et al., 2007; Flourey et al., 2002; Kajiyama et al., 1995; Okamoto et al., 1990; Polisel-Scopel et al., 2012); however, there are no studies describing the effects of HPH on soybean processing materials and extraction yields. Here we show an investigation of the extraction yields of oil,

protein and solids with high pressure treatment compared to the industrial control sample, as well as the availability of protein and separation efficiency on soybean processing materials. Particle size measurements, flow behaviour and an investigation into the microstructure using confocal laser scanning microscopy (CLSM) are carried out to identify the mechanisms of HPH.

2. Materials & methods

2.1. Sample preparation

Slurry and okara were freshly prepared in the pilot plant facilities at Unilever R&D Vlaardingen. A process flow diagram can be seen in Fig. 1. Commercially available soybeans (Stream 3, Fig. 1) went through two wet milling stages to produce a soy slurry (Stream 4, Fig. 1) 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, which resulted in a soybean-to-water ratio of 1:7 (w/w) (water content of soybean was considered). To prepare soybase and okara for subsequent treatment (streams 7 and 8, respectively), the slurry was fed into a decanter centrifuge operating at a *g*-force-time of 1.5 × 10³g-s. Before homogenisation, the okara was diluted approximately 7 times (13.7 wt.%) with demineralised water on the day of homogenisation and stirred using a magnetic bar. For each homogenisation study, a fresh 1 L solution was made from okara stored below 5 °C for no longer than 6 days. The composition of slurry (Stream 4, Fig. 1) and okara (Stream 8, Fig. 1) can be seen in Table 1.

2.2. High pressure homogenisation (HPH) treatment

Fig. 1 shows the process flow diagram for experiments conducted on:

- (i) Slurry prepared as above (Stream 4, Fig. 1), and
- (ii) Okara prepared using decanter centrifugation (O₁; stream 8, Fig. 1),

to identify what effects of HPH can be identified on both materials.

All HPH treatments were conducted using a homogeniser, PandaPLUS 2000 (GEA Niro Soavi S.p.A., Parma, Italy), equipped with 2 stages as shown schematically in Fig. 2. During the homogenisation treatments, the 2nd stage was always adjusted to 10 MPa using a manual hand wheel actuator on the equipment, and then the pressure was increased to the required total pressure by the 1st stage, using the 1st hand wheel. The approximate flow rate for demineralised water of 150 mL min⁻¹ (9 L h⁻¹) was recorded prior to each experiment using the homogeniser, with a lower limit set to 142.5 mL min⁻¹. The soy sample was introduced through the feed hopper of the homogeniser. A sample of approximately 100 mL was taken after each pass through the homogeniser for analysis. For the control samples (0 passes), the samples were heated to their relevant temperatures and stirred; however, they were not passed through the homogeniser.

2.2.1. Slurry treatment

For each trial using slurry (Stream 4, Fig. 1), 1 L was heated to 80 °C and stirred using a magnetic stirrer bar. This temperature was chosen to replicate the conditions which would be found during processing in a factory after the milling process. Once the desired temperature was reached, a control sample was taken and the remaining slurry was introduced into the homogeniser, which was preheated using boiling water. For each treatment, the soy slurry was passed through the homogeniser and a sample was collected for analysis and further processing. The remaining slurry was added into the homogeniser for subsequent treatment, up to a maximum of 5 passes in total. The temperature was recorded before and after treatment.

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