Intensification of protein extraction from soybean processing materials using hydrodynamic cavitation

K E Preece a,⁎, N Hooshyar b, A J Krijgsman c, P J Fryer a, N J Zuidam c

⁎ Corresponding author.
E-mail address: kep704@bham.ac.uk (K.E. Preece).

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 alkaline aqueous conditions at high temperature to prepare soybase, 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, & Niranjan, 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 1000–5500 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).
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 soybase 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 (Donsì, 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, Raghavaraao, 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; Floury, Desrumaux, & Legrand, 2002; Poliseli-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, Mullivihill, Huppertz, & Kelly, 2015). A scale up study by Donsì 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; Floury et al., 2002; Kajiyama et al., 1995; Okamoto et al., 1990; Poliseli-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 × 10⁵g·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 (0; 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.
2.2.2. Okara solution treatment

Fresh okara solution, prepared as described in Section 2.1 (O, Fig. 1), was heated to 50 °C and stirred using a magnetic stirrer bar. This temperature was chosen due to the okara production temperature at pilot scale; when the milling was performed at 85 °C and diluted to 13.7% using room temperature water, a solution temperature of 50 °C was achieved. Once heated, the solution was added to the homogeniser for treatment, and a control sample was collected (0 passes). Care was taken to ensure particles were dispersed evenly when sampling okara solution. Once the solution was added to the sample hopper (Fig. 2), the solution was stirred to prevent particle settling. After each pass through the homogeniser, a sample was taken for analysis and further processing. The remaining solution was recirculated back through the homogeniser for up to 5 passes in total. The temperature was recorded before and after treatment.

2.3. Protein, oil and solid measurement methods and extraction yield calculation

To determine protein extraction yields, the protein content on a wet basis (w.b.) was defined in the pellets and supernatants using the Dumas method (Vario MAX CNS, Elementar Analysensysteme GmbH, Germany). L(+)-glutamic acid (VWR International BVBA, Belgium) was used as a standard sample and UHT milk (3.5% fat) (muva kempten, Germany) as a reference material. For soy samples, a protein conversion factor of 6.25 × N was utilised to determine protein content from the measured nitrogen content. From the protein concentrations and masses of streams, the protein extraction yield into the soybase could be calculated using Eq. (1).

\[
\text{Protein extraction yield} = Y(\%) = \left(\frac{S \cdot x_p}{S \cdot x_p + O \cdot x_o}\right) \times 100
\]  

Here \(S\) (soybase) and \(O\) (okara) represent the total weight of samples and \(x_p\) is the mass fraction of protein for each respective stream. These terms can be found labelled in Fig. 1. To analyse the effects of HPH on okara solution, it was necessary to consider the total protein extraction yield calculated using Eq. (2). Yield \(I(Y_I)\) refers to the primary extraction and centrifugation for the production of soybase and okara (calculated from \(S\) and \(O\), Fig. 1); yield \(II(Y_{II})\) corresponds to the okara solution treatment described.

Total protein extraction yield (\%) = \(Y_I + (100\% - Y_I) \times Y_{II}\)  

Table 1

Average composition of slurry and okara prepared using pilot plant facilities. Error represents standard deviation in production over 5 separate preparations using the facility.

| Component | Slurry (\%) | Okara (\%) |
|-----------|------------|------------|
| Protein   | 5.2 ± 0.1  | 5.4 ± 0.2  |
| Oil       | 2.8 ± 0.1  | 3.1 ± 0.3  |
| Moisture  | 87.2 ± 0.2 | 81.4 ± 0.5 |
| Other     | 4.8 ± 0.2  | 10.1 ± 0.6 |

Fig. 1. Process flow diagram of pilot plant preparation and HPH treatments of slurry and okara solution samples. The red box (→) shows the process for slurry homogenisation and the blue box (→) shows okara solution treatment, both carried out using a lab scale HPH. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 2. Schematic diagram of a 2-stage high pressure homogeniser.
In addition to the extraction yields, the separation efficiency (Eq. (3)) was derived to show the efficiency of deliquoring of okara during centrifugation. The availability of protein was also calculated using Eq. (4); this is a measure combining losses due to protein insolubility as well as intact cells, i.e. all losses that occurred as a result of extraction, not incurred by separation. In these calculations, it was assumed that the moisture content found in okara retained the same protein concentration \(x_{p,o}\) as the soybase.

\[
\text{Separation efficiency} (\%) = \left[ \frac{S_{X_{w,s}}}{S_{X_{w,s}} + O_{X_{p,o}}} \right] \times 100 \tag{3}
\]

\[
\text{Availability of protein} (\%) = \left[ \frac{S + O_{X_{w,s}}/x_{o}}{S + O_{X_{w,s}}/x_{w}} \right] \times 100 \tag{4}
\]

where:
- \(x_{w,s}\) Mass fraction of water in soybase
- \(x_{w,o}\) Mass fraction of water in okara
- \(x_{p,s}\) Mass fraction of protein in soybase
- \(x_{p,o}\) Mass fraction of protein in okara

Please note that the extraction yield (Eq. (1)) is equal to separation efficiency multiplied by the availability of protein.

Oil and solid contents were measured using a microwave moisture analysis system equipped with NMR for direct detection of fat content (SMART System5, CEM GmbH, Germany). Oil and solid extraction yields were also determined using Eq. (1), replacing the masses of protein, with the respective masses.

### 2.4. Particle size measurement

The particle sizes of soy slurries after extraction were determined using laser diffraction (Mastersizer 2000 Hydro S, Malvern Instruments Ltd, UK). To determine particle size distributions (PSDs), refractive indices of 1.33 and 1.45 were used for the water and the particles, respectively (Preece et al., 2015). Protein, moisture and particle sizes were measured in triplicate for each sample. The D(4,3) and D(3,2) values represent the volume weighted and surface weighted mean particle size, respectively. The D90 value gives an indication of the particle size under which 90% of the total particles fall below.

### 2.5. Rheology

An AR G2 rheometer (TA instruments, New Castle, DE) equipped with a sand blasted steel parallel plate (40 mm diameter) was utilised to study the effects of homogenisation on samples. All experiments were carried out at 20 °C with a gap width of 2500 μm. Flow curves were measured with increasing and decreasing shear rates in the range 0.1–200 s\(^{-1}\) over a time period of 2 min per sweep. The sweep of increasing shear rate was treated as a conditioning step and the shear viscosity measurements were recorded.

### 2.6. Confocal laser scanning microscopy (CLSM)

A Leica TCS-SP5 microscope in conjunction with DMI6000 inverted microscope (Leica Microsystems Inc., Germany) was used with the dye nile blue A (Janssen Chimica, Belgium) to visualise the effects of HPH treatment on soy slurries. One drop of dye stock solution (1% w/v nile blue) was added to 1–1.5 mL of sample and mixed well before adding the sample to the slide. For visualisation using nile blue, sequential scanning was employed to prevent the excitation laser occurring in the emission signals. Table 2 shows the scans utilised and the corresponding colours assigned to the emission channels.

### Table 2

Excitation and emission conditions when acquiring CLSM images using the dye nile blue.

| Sequential scan | Excitation wavelengths (nm) | Emission wavelengths (nm) | Illustrated colour in micrograph |
|-----------------|-----------------------------|---------------------------|--------------------------------|
| 1               | 488                         | 520–626                   | Green                         |
| 2               | 633                         | 662–749                   | Red                           |

### 3. Results & discussion

#### 3.1. Extraction yields

Soybeans were milled and the resulting slurry was separated using a decanter centrifuge to obtain the soybase and the okara fraction (\(S_i\) and \(O_i\), Fig. 1), as described in Section 2.1. To increase the protein extraction yield, HPH was applied to either the slurry or the okara solution at various pressures to determine an appropriate pressure for subsequent treatments. Fig. 3 shows the total protein extraction yield calculated using Eq. (2) as a function of HPH pressure after a single pass through the system. On first observation (Fig. 3), the total protein extraction yield increased with increasing HPH pressure for both samples after a single pass. Okara solution treatment included a primary extraction of protein during okara preparation (\(O_i\), Fig. 1), followed by subsequent dilution, HPH treatment and separation of insoluble materials, such as fibres, insoluble proteins and intact cells, if present. A pressure of 100 MPa (1000 bar) was chosen for all further experiments to ensure the optimal extraction yield of protein for both slurry and okara solution treatments was achieved. In the following sections, the resultant effects of the homogenisation treatment on the particle size, microstructure and rheology will be studied in order to explain the results from Fig. 3.

#### 3.1.1. Soy slurry

To locate the most optimal treatment conditions, an experiment was carried out to deduce the optimum number of passes through the homogeniser geometry at 100 MPa. For soy slurry, the homogeniser treatment was carried out at 80 °C to replicate the temperature straight from the pilot processing line. Fig. 4 shows the extraction yields of oil, protein and solids from slurry versus the number of treatments at 100 MPa. The control sample (0 passes) represents slurry heated to 80 °C and separated under the same conditions as the treated samples. The extraction yield of protein was approximately 65% without homogenisation. The optimum number of passes for the extraction of oil, protein and solids occurred for a single pass at the pressure investigated. Extraction yields improved by 21%, 16% & 12% for oil, protein and solids respectively after 1 pass through the homogeniser. After each subsequent pass through the homogeniser, a stepwise reduction in extraction of all components studied was observed.
3.1.2. Okara solution

The effects of homogenisation were also tested on okara, the waste stream from soybase production (OII, Fig. 1). The extraction yields of oil, protein and solids were calculated considering the okara treatment only (no primary extraction yield considered) (Fig. 5). From the control sample (0 passes), approximately 55% of oil and protein and 35% of solids were extracted. After 1 pass, oil protein and solid extraction yields were improved by 36%, 26% and 17% respectively. Unlike soy slurry treatment, the subsequent extractions did not lead to a reduction in extraction yield: a plateau in extraction yields was reached after 1 pass through the homogeniser (1 pass, 100 MPa), with no significant change for higher numbers of passes.

3.2. Separation efficiencies and availability of protein

To understand how the homogenisation treatment affected the extraction yield, the separation efficiency and protein availability were calculated. Protein extraction yield is a function of the availability of protein and the separation efficiency. Fig. 6 shows the effects of homogenisation treatment of both the slurry and okara feeds (Streams 4 and 8 (OII, Fig. 1), compared to a control sample with heating but without HPH treatment. Initially the availability of protein was considered: protein availability increased by approximately 18% and 30% (absolute values) after a single pass through the homogeniser of slurry and okara solutions, respectively. The increase in the availability of protein suggests that either intact cells were disrupted, or the solubility of protein was improved. After each subsequent pass of homogenisation treatment (2–5 passes), there was no change in the availability of protein for either slurry or okara solution.

Separation efficiency was affected in both slurry and okara solution homogenisation (boxes i and ii, Fig. 1). The largest effect of separation efficiency was observed for the slurry samples; after 1 pass, the separation efficiency was improved meaning less soluble protein was retained in the okara phase after homogenisation treatment (OII, Fig. 1). After subsequent passes, a large reduction in separation efficiency of the slurry was observed (13% after 5 passes). This reduction in separation efficiency of slurry provides good correlation with the stepwise reduction in extraction yields from soy slurry after more than 1 HPH pass as shown in Fig. 4. In contrast, the okara solution observed little change in the separation efficiency after homogeniser passes, suggesting a similar mass of soluble protein resided in the okara after each HPH treatment, compared to the control sample (OII, Fig. 1). This coincides well with the plateau in the extraction yield observed for okara solution after 1 HPH pass in Fig. 5.

3.3. Particle size measurements

To understand the effects of homogenisation, it was important to study the effects of treatment on the resulting sample characteristics. Particle size measurements were carried out for soy slurry and okara solution samples to study the effects of homogenisation treatment. Fig. 7 shows the effects of number of passes through the homogeniser on the resulting particle size of soy slurry. The control sample (0 passes) represents a soy slurry sample heated to 80°C, all samples had the same pre-treatment. For soy slurry heated to 80°C, the D90 value was approximately 760 μm with a D[4,3] of ca. 350 μm and D[3,2] of 15 μm. After one high pressure treatment at 100 MPa, the biggest change can be observed in the D90 value; a reduction to a value in the region of 100 μm was observed. This particle size reduction observed after a single pass can be attributed to homogenisation effects. A reduction in D[4,3] was also observed; however, the D[3,2] appeared to increase slightly after one pass.

The slurry sample without HPH treatment consisted of particles ranging from submicron to 1 mm, seen in the PSD (0 passes, Fig. 7B). The largest volume based reduction in particle size occurred after a single pass at 100 MPa compared to the control. The peak at 0.35–3 μm cannot be observed in any of the HPH treated soy samples, suggesting that oil droplets and other components, such as soluble proteins, reduced in volume (see also the Introduction section). After each subsequent pass of slurry, there was a small stepwise increase in the particle size. The distribution of particles in the size range 2–200 μm, observed for the slurry sample after a single pass increased in broadness to 2–350 μm after 5 passes at 100 MPa. In Section 3.2 Separation efficiencies and availability of protein, the availability of protein was not affected by multiple passes for slurry treatment (Fig. 6), suggesting protein
aggregation did not occur. In a previous study by Lopez-Sanchez et al. (2011), an increase in particle size was observed for tomato suspensions due to cell wall swelling caused by a single pass through a HPH at a pressure of 60 MPa. Soybean cell wall fibres could also swell similar to tomato cells after multiple passes through the HPH.

HPH treated okara solution was also analysed to examine the changes of particle size upon application of homogenisation. Fig. 8A shows the effects of the number of passes through the homogeniser at 100 MPa on the particle sizes of the okara solution. The initial particle sizes for okara solution were greater than those of the soy slurry. After 1 pass through the homogeniser geometry: all particle sizes were reduced when compared to the control sample (0 passes, Fig. 8A). Focusing on the particle size distribution of the control sample of okara solution (0 passes, Fig. 8B), there is a sharper, higher volume peak of particles in the range 100–1000 μm compared to the slurry control sample (0 passes, Fig. 7B). Generally, the initial distributions are similar in size ranges. An initial reduction in the larger volume particles is seen upon a single pass, the peak shifts to the range 10–200 μm. There was also a loss of particles with a size of 0.35–6 μm with any number of passes with the homogeniser, as was also observed for the slurry sample (Fig. 7B). This could be attributed to a reduction in size of the oil droplets. With each subsequent pass after a single pass, a small stepwise increase in the particles size was also observed, similarly to slurry treatment due to the swelling of fibrous materials.

For both slurry and okara solution treatments, homogenisation effects caused an initial reduction of the particle size after a single pass through the HPH at 100 MPa. After multiple passes through the homogeniser at 100 MPa, an increase in particle size for both samples was observed in comparison to their respective single pass samples. The resultant increase in particle size with multiple passes through the HPH can be attributed to swelling of the soybean cell wall fibres.

3.4. CLSM

To investigate the effects of homogenisation treatment on slurry and okara solution samples, CLSM was employed in the presence of nile blue for visualisation. Nile blue is a dye used to visualise apolar material (Preece et al., 2015). In the system settings oil appears green and other, less apolar materials, including protein and fibres, appear red. Initially, the microstructure of the control samples (0 passes) was investigated. Fig. 9 shows the typical structures observed in the soy slurry after

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**Fig. 7.** Soy slurry particle size variation (A) and PSDs (B) as a function of number of passes through the homogeniser geometry at 100 MPa. Each data point represents an average of 3 separate experiments carried out with different batches of slurry measured in duplicate; the error bars represent the standard deviation.

**Fig. 8.** The effect of number of passes through the homogeniser geometry at 100 MPa on the particle sizes (A) and PSDs (B) of okara solution. Each point represents an average of 3 different samples from 3 separate batches of okara, with error bars representing the standard deviation.

**Fig. 9.** Microstructural images of soy slurry without homogenisation, visualised using CLSM and the fluorochrome nile blue. Oil is highlighted in green and other, less apolar materials (including protein and fibres) are presented in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
milling of soybeans at pilot scale with a thermal pre-treatment of 80 °C. Droplets of oil, depicted in green, were found throughout the continuous phase of the sample, with sizes up to 12 μm in diameter. These are empty cell wall structures from where the contents have been extracted. After a homogenisation treatment at 100 MPa, a sample of slurry was visualised and the results are shown in Fig. 10. The representative micrographs show changes in many of the aspects of the slurry sample. The oil droplets, green in these images, are reduced significantly in size. It is near impossible to distinguish the individual droplets in the continuous phase. This supports the reduction in particle size and loss of the volume based peak at 1 μm to submicron sizes (Fig. 7B). Intact cells were not observed in any of the samples studied using CLSM, after one treatment at 100 MPa or for multiple passes. The fibrous structure observed in the control sample were reduced to shorter lengths with homogenisation treatment, which confirms the results seen in particle size examination (Section 3.3). The microstructure observed after 5 passes through the homogeniser using CLSM was similar compared to that seen after 1 pass. No aggregation of proteinaceous material could be visualised after 5 passes (data not shown).

The small increase in the particle size upon both slurry homogenisation (Fig. 7) and okara homogenisation (Fig. 8) might be due to limited protein aggregation (Toro-Funes, Bosch-Fusté, Latorre-Moratalla, Veciana-Nogués, & Vidal-Carou, 2015). After 1 pass, the protein extraction yield from slurry increased to 82% (Fig. 4). The D[4,3] was reduced to approximately 50 μm for all resultant samples, both slurry (53 ± 1 μm) and okara solution (59 ± 2 μm), after homogenisation. Apparently, most of the particles in this size range was still soluble or dispersible, and resided in the soybase during the extraction process. It has been shown previously using transmission electron microscopy (Rosenthal et al., 1998) and CLSM (Preece et al., 2015) that the size of hydrated soybean cotyledon cells vary in length from 70 to 80 μm and 20–30 μm in diameter. Assuming a spherical cotyledon cell, its average size is about 45–55 μm (average of length and diameter). The measured particle size data would suggest that homogenisation disrupted all intact cells (Fig. 7). That is indeed what has been confirmed by CLSM measurements in this study: after 1 pass through the homogeniser, no intact cells were present (see Figs. 9 and 10).

3.5. Rheology – flow behaviour

To understand the differences observed between the slurry and okara solution separation efficiencies, a study of the flow behaviour was conducted (Fig. 11). Focusing on the viscosity profiles of the control soy slurry (0 pass, Fig. 11A), it is possible to observe shear thinning behaviour: as the shear rate increased, the viscosity decreased. Upon 1 pass through the homogeniser, the slurry viscosity increased especially at the relatively lower shear rates. At shear rates above 3 s⁻¹, the viscosity of slurry decreased after a single pass. Upon 5 passes, the viscosity increased at all shear rates investigated compared to the control sample. This can be attributed to the change in the composition of the sample: intact cells are disrupted, and more intracellular components are solubilised into the soy slurry continuous phase. The drastic change in particle size after homogenisation treatment (Fig. 7) leads to the formation of a large number of smaller particles from a few number of larger particles. With a greater concentration of particles after homogenisation, particle-particle interactions play a greater role in the viscosity of the resultant sample.

Focusing on the okara solution viscosity profile (Fig. 11B), the first obvious difference compared to the slurry curves is the lower viscosity for all okara samples, treated and non-treated. The control okara

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**Fig. 10.** CLSM images of soy slurry after 1 pass through homogeniser at 100 MPa, highlighted using nile blue. Oil is highlighted in green & other apolar materials (including protein and fibres) are presented in red using Leica software settings. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Fig. 11.** Viscosity profiles of slurry (A) and okara solution (13.7%) (B) with various homogenisation treatments (100 MPa) including a control sample without homogenisation. Each graph shows a sweep with increasing shear rate followed by a downwards sweep.
solution (0 pass, Fig. 11B) behaved as a Newtonian fluid. Okara solution is a dispersion of a small volume of insoluble materials, such as intact cells and fibrous particles, dispersed in water. In the control for okara solution, there are less particle-particle interactions due to their low volume fraction. Particle sedimentation in the rheometer could be responsible for this behaviour observed in the okara solution control sample (0 passes). At shear rates below approximately 1 s⁻¹, a shallow plateau in the flow curve was observed. It is believed that this was caused by shear banding in the okara solution samples (Mewis & Wagner, 2012). This is an artefact and can be neglected. At a shear rate of approximately 100 s⁻¹, it was possible to see an increase in the dynamic viscosity for all of the okara solution samples; however, this is also an artefact caused by the presence of turbulence within the measurement, which is not assumed during the calculation of viscosity. This can also be neglected in the interpretation. Upon 1 pass through the homogeniser of the okara solution, the behaviour changed from Newtonian behaviour to shear thinning, with an increase of viscosity versus the control (0 passes). After 5 HPH passes, a viscosity increase was observed compared to the control at all shear rates investigated. Such an increase in viscosity could be beneficial for producing a low solids product, with a similar viscosity profile to soy slurry without homogenisation treatment.

The release of intracellular materials, such as proteins and smaller fibrous materials upon cell disruption could lead to enhanced particle-particle interactions and build-up of structure, thus increasing the viscosity (Fig. 11). This has also been observed previously by Lopez-Sanchez et al. (2011) for tomato cell suspensions. Focusing on the okara solution homogenisation (Fig. 11B), the lower viscosity of all samples compared to slurries was caused by a reduced solid content of the okara solution, i.e. 2.5 ± 0.1%. The control slurry sample (0 pass) consisted of 12.6 ± 0.1% solids in comparison, which accounts for soluble solids and insoluble solids, such as oil, protein, cell wall fibres and intact cells. As the particle size of the slurry slightly increased (see Fig. 7) and the viscosity increased (see Fig. 11) upon subsequent HPH passes, the slurry particles became increasingly difficult to separate from the bulk solution (see Fig. 6).

### 3.6. Energy input and productivity of HPH treatment

To understand the feasibility of scale up for this promising technology, it was necessary to calculate the energy efficiency in comparison to other technologies. Energy input was calculated using the following equation (Bylund, 1995):

\[
\text{Power input (kW)} = \frac{Q_{in} \times (P_1 - P_m)}{3600 \times \eta_{pump} \times \eta_{electric \, motor}}
\]

where \(Q_{in}\) is the volumetric flow rate, \(P_1\) and \(P_m\) refer to the pressure of homogenisation treatment and the inlet pressure respectively. The efficiency of the pump and electric motor were estimated as 75% (Peters, Timmerhaus, West, Timmerhaus, & West, 1968) and are denoted by \(\eta\) in Eq. (5). For the investigated homogenisation pressures (50–125 MPa), the energy inputs ranged from 0.01–0.05 kWh L⁻¹. Previously Preece et al. (2016) reported energy inputs in the range 0.004–0.12 kWh L⁻¹ using a lab-scale probe system for sonication of similar soybean processing materials. Maximum yields of 70% and 65% were achieved at 0.12 kWh L⁻¹ for ultrasound assisted extraction of protein from slurry and okara solution respectively (15 min ultrasound treatments, 65 W, 20 kHz) (Preece et al., 2016). The energy input during homogenisation was less than that quoted during sonication of soybean processing materials. The protein extraction yield is greater for materials treated with homogenisation at 100 MPa, 87% and 81% were found for slurry and okara solution respectively. The differences in extraction yields can be attributed to the disruption of intact cells during homogenisation treatment – no intact cells were visualised after 1 pass through the homogeniser using CLSM (Fig. 10), contrary to ultrasound.

For the implementation of high pressure homogenisation within industry, it is also vital to consider the energy density at each scale of treatment. Energy density for the lab-scale system has been calculated for each number of passes investigated in the experimental section (see Table 3). Extraction yields (see Figs. 4 & 5), show that a single pass of slurry or okara solution was able to reach maximal protein extraction yields, equating to an energy density of 740 MJ m⁻³. If this technique is considered for implementation at industrial scale, it is necessary to calculate the energy density for a suitable system. Processing at 72 MPa at a flow rate of 2500 L h⁻¹, it is possible to introduce 108 MJ m⁻³. Further studies are necessary to investigate the effects of homogenisation using a pilot-scale homogeniser and its energy density should also be considered.

Productivity is another important factor to consider when designing an industrial plant, giving an indication of the efficiency of processing. Fig. 12 shows the effects of number of homogeniser passes at 100 MPa on the productivity. The greatest productivity was found for 1 HPH pass at 100 MPa for both slurry and okara solution (Fig. 12). Comparing the productivity of slurry and okara solution after a single pass at 100 MPa, slurry treatment was found to be a more viable option. The low protein concentration in the resultant soybase after okara solution treatment caused lower productivity in comparison to slurry. After each subsequent pass for both slurry and okara solution, the productivity reduced below that of the control sample, without homogenisation treatment.

### 4. Conclusions

In conclusion, high pressure treatment (50–125 MPa) was found to improve the extraction of oil, protein and solids from soybean processing materials. The improvement for both slurry and okara solution treatment after one HPH pass was found to be a result of availability of protein and separation efficiency. The improvement in availability can be attributed to the reduction in particle size and cell disruption, as confirmed by particle size measurements and CLSM. A decrease in

![Fig. 12. Productivity versus number of passes through HPH at 100 MPa. Each data point represents productivity calculated from an average of 3 separate experiments, with error bars representing the standard deviation.](image-url)
separation efficiency was observed for slurry treatment with increasing number of homogenisation passes, resulting in a reduction of protein extraction yield contrary to okara solution treatment. This reduction can be attributed to a slight increase in particle size and increase in viscosity upon subsequent HPH passes. High pressure treatment based on the phenomena of hydrodynamic cavitation offers a more viable route of extraction intensification from soybean processing materials in comparison to ultrasound. Based on the productivity of the technology, the best scenario includes the use of HPH on slurry rather than okara solution, for 1 pass at 100 MPa. Further work is required to optimise this processing technology, including scale up to determine the viability for implementation at factory scale as well as sensory evaluation and storage stability of the resulting soy based products. To reduce energy costs, it is also beneficial to study further the effects of lower pressures than 100 MPa.

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