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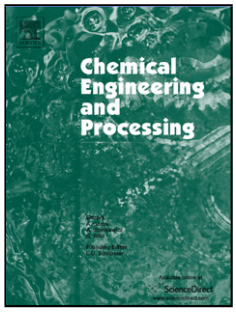
DOI:
10.1016/j.cep.2016.09.003



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PII: S0255-2701(16)30367-1
DOI: http://dx.doi.org/doi:10.1016/j.cep.2016.09.003
Reference: CEP 6859

To appear in: *Chemical Engineering and Processing*

Received date: 26-2-2016
Revised date: 20-4-2016
Accepted date: 6-9-2016

Please cite this article as: Katherine E.Preece, Nasim Hooshyar, Ardjan Krijgsman, Peter J.Fryer, Nicolaas Jan Zuidam, Intensified soy protein extraction by ultrasound, Chemical Engineering and Processing http://dx.doi.org/10.1016/j.cep.2016.09.003

INTENSIFIED SOY PROTEIN EXTRACTION BY ULTRASOUND

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Graphical abstract

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Highlights

- Ultrasound improves protein extraction yield during soymilk production.
- Ultrasound did not cause cell disruption.
- Solubility and separation efficiency are both accountable for improved yields.
- Particle size regime of 2–35 μm experienced greatest impact of ultrasound.
- Phytic acid stores were localised in protein bodies of dry soybeans using SEM.

Abstract

During soymilk production, aqueous extraction conditions are utilised resulting in suboptimal protein extraction yields. This research focuses on the intensification of extraction yields from soybeans using ultrasound and understanding the reasoning behind the results. Milled soybean slurry and okara samples were treated with ultrasound using a lab-scale probe system (20 kHz, 400 watts) for 0, 0.5, 1, 5 and 15 min. Ultrasound increased the protein, oil and solids extraction yield from soy slurry by ca. 10% after 1 min treatment, especially due to improved solubility and in a less extent to enhanced separation efficiency. Particles in the size range of 2-35 μm, corresponding to insoluble protein bodies in the continuous phase, were reduced in frequency but surprisingly not a stepwise decline in size upon ultrasound treatment, as shown by both laser diffraction and confocal laser scanning microscopy. No effects of ultrasound were observed on intact cells present in okara solution and soy slurries. Scanning electron microscopy could not reveal a hypothesised internal organisation of protein bodies within cells, although phytic acid stores were localised which have not been reported before. In conclusion, ultrasound has been identified as a technology with promise in soybean extraction systems where solubility requires improvement.

Keywords

- Soybeans
- Ultrasound-assisted extraction
- Acoustic cavitation
- Process intensification
- Confocal laser scanning microscopy

Nomenclature

S		Soybase mass
O		Okara mass
Y I		Primary extraction yield
Y II		Secondary extraction yield, resulting from okara treatment
x_i		Mass fraction of component i
$x_{i,j}$		Mass fraction of component i in stream j
i	p	Protein

		o	Oil
		w	Moisture
		s	Solids
	j	S	Soybase
		O	Okara

Abbreviations

CLSM – confocal laser scanning microscopy

EDX – energy dispersive X-ray spectroscopy

SEM – scanning electron microscopy

1. Introduction

Plant-based protein products are currently gaining much interest as a more sustainable alternative to animal-based protein products. One such product gaining popularity across the world is soymilk, due to its complete set of essential amino acids, cholesterol-lowering attributes and lactose free nature [1]. Soymilk production consists of aqueous extraction from soybeans using alkaline conditions at elevated temperatures, followed by removal of insoluble material to produce the resulting soybase. This soybase is then used as a precursor to produce soymilk by adding other ingredients such as sugar, gums, flavours, minerals and vitamins. The extraction of various components from soybeans is suboptimal; after extraction, a significant amount of protein resides in the insoluble fraction, termed okara. Thermal treatment during processing is often employed to reduce the activity of lipoxygenase; which, if left in its native state, results in off-flavour production [2]. Vishwanathan et al. [3] show that alkaline conditions (optimal pH 8+) gave enhanced protein solubility when compared to acidic conditions due to the proteins isoelectric points. Assistance during protein extraction from soybeans is supported by industry for reasons including: less expenditure on raw materials, less waste and lower costs associated with its subsequent treatment.

An alternative energy source that has been commonly studied for laboratory scale extraction assistance in the food industry is ultrasound [4-12]. The mechanism involved in enhancing extraction yields is attributed to the cavitation phenomenon. Upon the application of ultrasound, alternating mechanical

waves cause microbubbles located in the liquid medium to form and grow up to a sufficiently negative threshold pressure, where bubble collapse occurs [5]. As a consequence of bubble implosion, local physical effects may result in very high temperatures (5000 K) and pressures (2000 atm) [6]. Local regions of turbulence occur as a result of cavitation aiding mass transfer in solid-liquid extraction [7]. Many lab-scale studies claim that ultrasound can also enhance extraction yields of intracellular materials from vegetal tissue due to cell disruption [5;6;10;13-15]. This intensification of extraction yield caused by cell disruption is attributed to liquid jets of solvent resulting from asymmetric microbubble collapse [16].

More recently, this technology has begun to show promise for implementation at industrial scale [17;18]. Pilot scale studies have shown the positive effects of ultrasound on a number of food extraction systems. One such study by Pingret et al. [19] show the comparable results for ultrasound-assisted aqueous extraction of polyphenols from apple pomace at pilot-scale to those improvements observed at lab-scale. Boonkird et al. [20] showed the positive effects of ultrasound treatment on the extraction of capsaicinoids from chilli peppers at pilot scale. Within the food industry, there has been implementation of ultrasonic processing on an industrial scale for assistance during extraction from vegetal materials [10]. Ultrasound-assisted extraction has been regarded as a green extraction process, for reasons including reductions in processing times, energy consumption and enhanced rates of extraction [21;22]. These factors are of interest when considering protein extraction during soymilk production: protein that is currently used for low quality functions, such as animal feed, is made available for human consumption.

A key factor to be explored when considering the effects of ultrasound is the microstructure of the processing materials. It is important to understand the matrix from which the extraction occurs and the diffusion pathway by which protein can escape the solid, but so far little information is available in the literature about processed soybean microstructures. The soybean is composed of approximately 90% cotyledon cells, with the length range of 70-80 μm and a diameter of 15-20 μm once hydrated [23;24]. These cells contain protein bodies (5-20 μm) and a cytoplasmic network containing oil bodies (0.2-0.5 μm) stabilised by proteins termed oleosins [24]. The physical restraints for non-optimal protein

extraction yields upon preparation of soybase in an aqueous environment have been studied in previous work [25]. Barriers for extraction included intact cotyledon cells, aggregated protein bodies within the extraction medium caused by thermal treatment and a considerable amount of okara containing 80% moisture in which soluble proteins reside [25].

Earlier studies of soy-based systems investigating ultrasonically-assisted extraction have been shown to improve extraction yields or to enhance the functionality of components [26-32]. Fukase et al. [24] investigated the effects of ultrasound on soybeans that underwent defatting using ether prior to protein extraction. The ultrasound-assisted extraction from defatted soybean flakes yielded 50% more protein versus the control sample (no US) after 10 min treatment at ultrasonic pressure of 106 kPa in an aqueous system [24]. Another system showed the extraction of oil from soybeans using hexane was enhanced by 20% with the application of ultrasound for 30 min (20 kHz) compared to a control sample [31]. However, there are very limited studies investigating the effects of ultrasound on aqueous extraction from soybean as the starting material, without pre-treatment. One study by Fahmi et al. [33] investigated the effects of ultrasound treatment (35 kHz, up to 60 min) on soy slurry protein extraction from pre-soaked soybeans. The protein extraction was intensified: the protein content of soymilk increased by 6.3% [33].

This study investigates the effects of ultrasound-assisted extraction, after initial grinding of soybeans at elevated temperatures. We hypothesise that ultrasound assistance will improve the extraction yields of protein, oil and solids due to increased cell disruption, as discussed above. Ultrasonic treatment of both the soy slurry and okara solution is investigated and extraction yields, solubilisation and separation efficiencies will be discussed. In addition, confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) has been used to study the microstructure of the soybeans, to understand the target of ultrasound in our soybean extraction system.

2. Experimental

2.1 Slurry preparation

Preece et al. [25] describe a method for aqueous extraction to produce soy slurry and okara for subsequent treatments. Figures 1 (A) and (B) show the process schematically. Firstly, ('Milling 1' in Figure 1(A) and (B)) commercially available soybeans were ground in demineralised water at a ratio of 1:6 (w/w) and at 80°C using a commercial blender (Varoma Thermomix, Vorwerk, Germany) for 10 min (stepwise levels 2-8). Then the ground soybeans were treated ('Milling 2') with a high shear mixer (Silverson L4RT, Silverson Machines International, UK) for 20 min (stepwise 3000-6500 rpm) to produce a slurry with a volume-weighted mean diameter ($D_{4,3}$) of less than 300 μm.

2.2 Okara solution preparation

The slurry was centrifuged (4330 × *g*, 10 min) to produce soybase *S* (supernatant) and okara *O* (pellet). The solid content of okara was measured using methods described in Section 2.4. A solution of 2.85% solids was then made by diluting the okara with demineralised water.

2.3 Ultrasonic treatment

After sample preparation, 100 g of sample (slurry or okara solution) was weighed and added into in a water bath at 50 ± 1°C whilst stirring at 200 rpm using a magnetic stirrer bar (25 mm in length, 10 mm in diameter cylindrical bar with central ring). Once the sample achieved the desired temperature, the sample was either subjected to sonication or held at 50°C in the water bath (control). Various times of exposure to ultrasound were investigated to understand the effects of sonication on extraction.

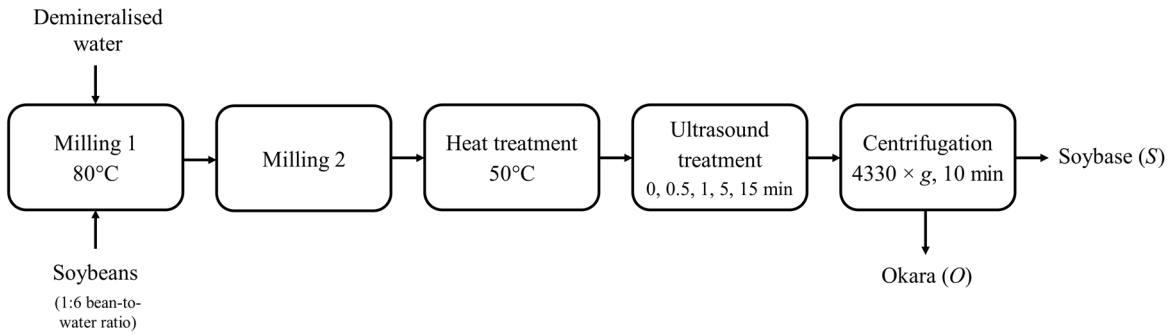


Figure 1A. Schematic diagram of preparation and treatments applied to slurry during processing.

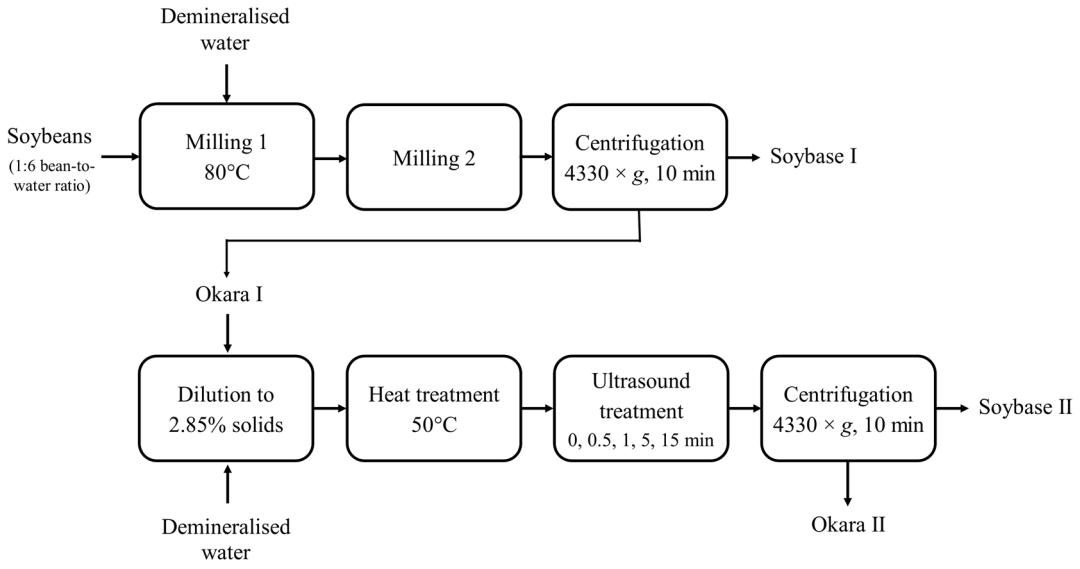


Figure 1B. Schematic diagram of okara preparation and subsequent treatments.

Sonicated samples were treated with an ultrasonic probe (Branson Sonifier 450, Branson Ultrasonics Corporation, Danbury, CT), (400 watts, 20 kHz, output level 7 which translates to a power output of 65 W, 13 mm probe tip) for various time periods; 0.5 min, 1 min, 5 min & 15 min. In the data presented, no temperature control was employed during ultrasonic treatment, however the temperature was recorded prior and post treatment (Table 1). From the recorded temperature increase, it was possible to calculate the actual ultrasonic energies and power inputs introduced using calorimetry: $Q = mC_P\Delta T$, where Q is the energy input as heat (J), m is the mass of sample (kg), CP is the specific heat capacity (assumed to be that of water, (4181 J kg-1 K-1) [22]) and ΔT is the temperature change (Table 1). The power input was calculated by dividing the energy input by the treatment time (in s), assuming that all energy was transferred to heat energy in the system. Those powers quoted are less than those detailed by the probe manufacturer as 65 W, which might indicate that especially at high temperatures some of the heat energy was transferred from the system into the environment.

Table 1. Temperature increase reported during ultrasound (US) treatment of soy slurry and corresponding energy and power input calculated using calorimetry.

US treatment time (min)	Start T (°C)	End T (°C)	ΔT (°C)	Energy input (J)	Power input (W)
0	49	-	-	-	-
0.5	49.7	49.9	0.2	84	3
1	50.2	54.8	4.6	1923	32
5	50	80.5	30.5	12752	43
15	50.2	90.4	40.2	16808	19
15 (No US)	49.5	49.8	0.3	-	-

After reaching the desired process time, the samples were immediately centrifuged (4330 × *g*, 10 min) to prevent further extraction occurring. Pellets and supernatants were weighed and analysed to determine extraction yields.

2.4 Protein & solids content determination

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 equation 1.

$$\text{Protein extraction yield} = Y\ (\%) = \left[\frac{S \cdot x_{\mathrm{p,s}}}{(S \cdot x_{\mathrm{p,s}} + O \cdot x_{\mathrm{p,o}})}\right] \times 100 \quad (1)$$

Here *S* (soybase) and *O* (okara) represent the total weight of samples and x_p is the mass fraction of protein. To analyse the effects of ultrasound on okara solution, it was necessary to consider the total protein extraction yield calculated using equation 2. In this equation the nomenclature is that shown in Figure 1(B); yield I refers to the primary extraction and centrifugation for the production of soybase and okara; yield II corresponds to the okara solution treatment described.

$$\text{Total protein extraction yield (\%)} = Y\,I + (100\% - Y\,I) \times Y\,II \quad (2)$$

In addition to the extraction yields, the separation efficiency (equation 3) was derived to show the efficiency of deliquoring of okara during centrifugation. The solubility of protein was also calculated using equation 4. In these calculations, it was assumed that the moisture content found in okara retained the same protein concentration ($x_{p,s}$) as the soybase, so that ($O \cdot x_{w,o} \cdot x_{p,s}$) is the amount of protein in the water fraction of the okara.

$$\text{Separation efficiency (\%)} = \left[\frac{S \cdot x_{p,s}}{\left(S \cdot x_{p,s} + (O \cdot x_{w,o} \cdot x_{p,s})\right)}\right] \times 100 \quad (3)$$

$$\text{Solubility of protein (\%)} = \left[\frac{S \cdot x_{p,s} + (O \cdot x_{w,o} \cdot x_{p,s})}{S \cdot x_{p,s} + O \cdot x_{p,o}}\right] \times 100 \quad (4)$$

Note that the extraction yield (equation 1) is equal to separation efficiency multiplied by the solubility of protein.

Fat 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 equation 1, replacing the masses of protein, with the respective masses.

2.5 Particle size analysis

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, refractive indices of 1.33 and 1.45 were used for the water and the particles, respectively [19]. Protein, moisture and particle sizes were measured in triplicate for each sample.

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 ultrasound 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

209 nile blue, sequential scanning was employed to prevent the excitation laser occurring in the emission
210 signals. Table 2 shows the scans utilised and the corresponding colours assigned to the emission
211 channels.

212 **Table 2. Excitation and emission conditions when acquiring CLSM images**

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

213

214 **2.7 Cryo-scanning electron microscopy (cryo-SEM)**

215 A soy bean was cut into 2 pieces using a razorblade. One piece was placed in an aluminium sample cup
216 and plunged into liquid nitrogen. The sample was then cryo-planed using a cryo-ultramicrotome
217 (Ultracut UCT EM FCS, Leica Microsystems Inc., Germany), to obtain a freshly prepared cross-section.
218 The sample was freeze-etched for 2 min at -90°C to reveal the microstructure and then sputter coated
219 with platinum (120 s) in order to obtain a better image contrast. Samples were imaged using a Zeiss
220 Auriga field emission SEM (Carl Zeiss Microscopy GmbH, Germany) at -125°C and an accelerating
221 voltage of 3 kV. The microscope was equipped with an energy dispersive X-ray spectroscopy (EDX)
222 unit; therefore, it was possible to chemically characterise regions visualised using the microscope.

223

3. Results & Discussion

3.1 Extraction yields

3.1.1 Soy slurry treatment

To understand the mechanisms of ultrasound on the soy slurry matrix, it was necessary to determine the extraction yields for the components of interest. Extraction yields were calculated from measurements of oil, protein and solid contents of samples after treatment. Figure 2 shows the effect of ultrasound treatment time versus extraction yield for the treatment of soy slurry. Ultrasound was shown to improve the extraction of oil, proteins and solids vs. the control sample. After 1 min treatment time, protein and oil extraction yields had improved by approximately 10% versus the 0 time point. It was shown that there was no benefits to perform ultrasound-assisted extraction for more than 5 min as the maximum yields had been achieved. A control sample was also analysed at 15 min to show the thermal treatment with stirring was not responsible for the increases in extraction yields observed. An improvement in extraction yields was also observed for control samples; however, not as much as those observed for respective ultrasound treatments.

Temperature control was not employed for the data shown within this study. Without temperature control, 15 min ultrasonic treatment caused the temperature of the solution to increase by 40.2 ± 0.8°C. In a separate study, the effect of temperature was determined by controlling the temperature of the sample using a jacketed vessel cooled using counter-current flow of water at 20 ± 1°C. The protein extraction yields for US-treated samples (0.5-5 min, without temperature control) yielded insignificant differences when compared to US-treated samples with temperature control (data not shown). Considering the effects of ultrasound with temperature control for the 15 min US-treated sample, the protein extraction yield was approximately 5% lower (absolute value) when the temperature of the sample was held at 50°C.

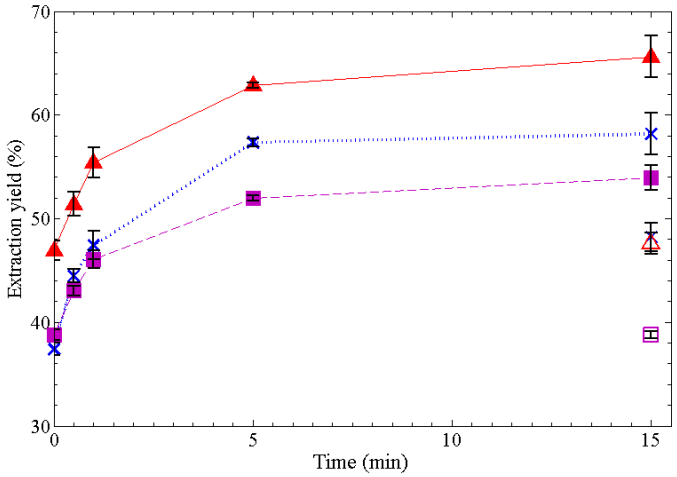


Figure 2. Improvement of extraction yields of slurry, oil (▲), protein (X) and solids (■) at various sonication times. Non-filled shapes correspond to control samples with corresponding component labels. Each data point is an average of three separate experiments and the error bar represents its standard error.

3.1.2 Okara solution treatment

It has been previously reported that ultrasound has a significant effect on the extraction yield of protein from okara during soymilk production [34]. Total extraction yield refers to the addition of initial extraction yield during soybase production (soybase I and okara I production in Figure 1B) plus the extra materials which solubilised after subsequent okara treatments. Figure 3 shows that an increase in protein extraction yield upon ultrasound treatment was indeed achievable in comparison to the control samples, recorded for each time point in this instance. Total oil and solid extraction yields were also intensified during ultrasound treatment. In contrast, the total extraction yields from the control samples (no ultrasound) remained unchanged during all time periods. In this study, no temperature control was employed during the ultrasonic treatment of okara solution. It was previously shown that the 80°C thermal treatment included with the milling of the soybeans in water (detailed in section 2.1) for okara preparation affected the protein extraction yield [25]. Based on the limited effect of temperature control shown for the slurry data, the effect of subsequent thermal treatments after sample preparation was considered to be negligible.

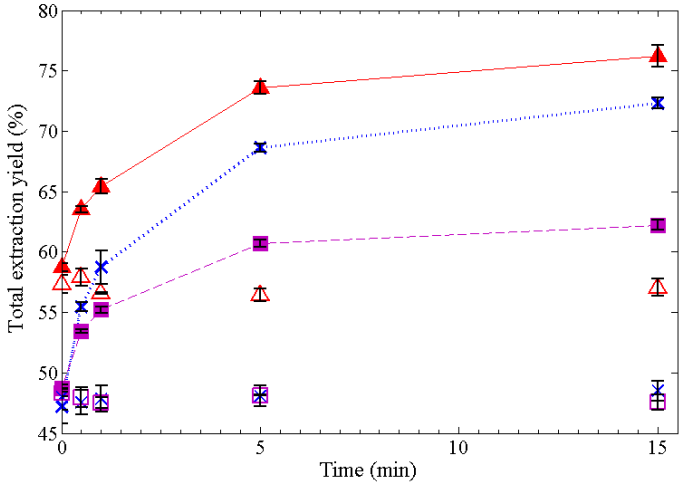


Figure 3. Effect of ultrasound treatment when applied to okara solution. Oil (▲), protein (X) and solids (■) extraction yields are presented. Non-filled shapes are corresponding control samples. Data points are averages of three separate experiments. Error bars represent standard error of the mean from 3 separate experiments.

3.2 Protein solubility and separation efficiencies

During treatments of soy slurry using ultrasound, it was possible to identify whether the solubility of protein was attributed to the increase in protein extraction yield and/or in the separation efficiency (deliquoring of okara). For the control sample, it was observed that the solubility and separation efficiency of protein were approximately 60% and 65%, respectively. As can be seen from Figure 4, the solubility of protein had the greatest impact on the protein extraction yield during ultrasound treatment. Separation efficiency was also positively influenced (to a lesser extent) by increasing ultrasonic treatment time; less water was present within the waste stream (okara), reducing the amount of soluble proteins entrapped within the matrix.

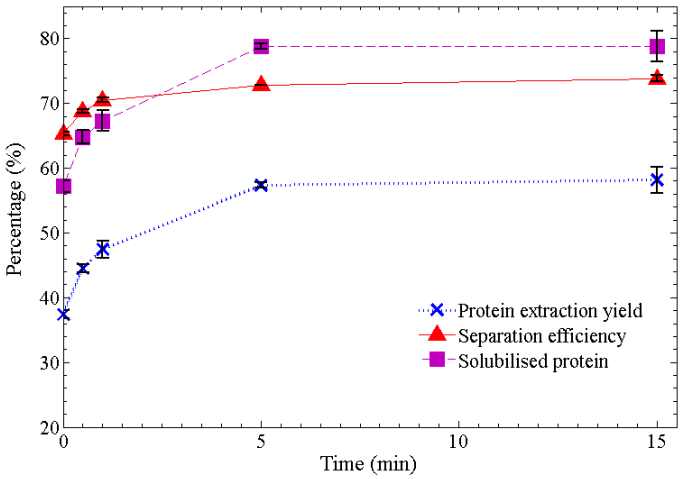


Figure 4. Protein extraction yield, solubility of protein and separation efficiency as a function of ultrasonic treatment time on soy slurry. Error bars represent standard error from 3 separate experiments.

3.3 Particle size distribution

During ultrasound treatment, it has been well-documented that a reduction in particle size is observed for many systems [5;11;14;35]. During the present study, particle size distributions of the treated samples were recorded and are shown in Figure 5A. The control slurry sample (0 min) showed a bimodal distribution of particles in the size range 2.5-2000 µm. The peak in the range of 2.5-35 µm is caused by the insoluble protein bodies located in the continuous phase of the sample, which have been visualised previously under the same processing conditions [25]. The larger size particles include fibres, intact cells and seed coat materials. Upon treatment with ultrasound, the peak in the 2.5-35 µm range (Figure 5A) containing insoluble protein bodies was visibly reduced after 0.5 min and a stepwise reduction was observed with increasing treatment time. Interestingly, no stepwise peak shift to smaller sizes was observed. Using the Malvern Mastersizer software, it was possible to perform a number transformation on the particle size data, resulting in a plot of number fraction versus particle size (Figure 5B). The greatest number of particles in the control sample (0 min) were within the size range of 2.5-30 µm. The number-based particle size distribution confirms that ultrasound caused the particles to disintegrate; even after 0.5 min ultrasound treatment, the number of particles shifted to a smaller particle size (in the range 0.3-1.1 µm). Particles within this size range can be found within the soybase after centrifugation.

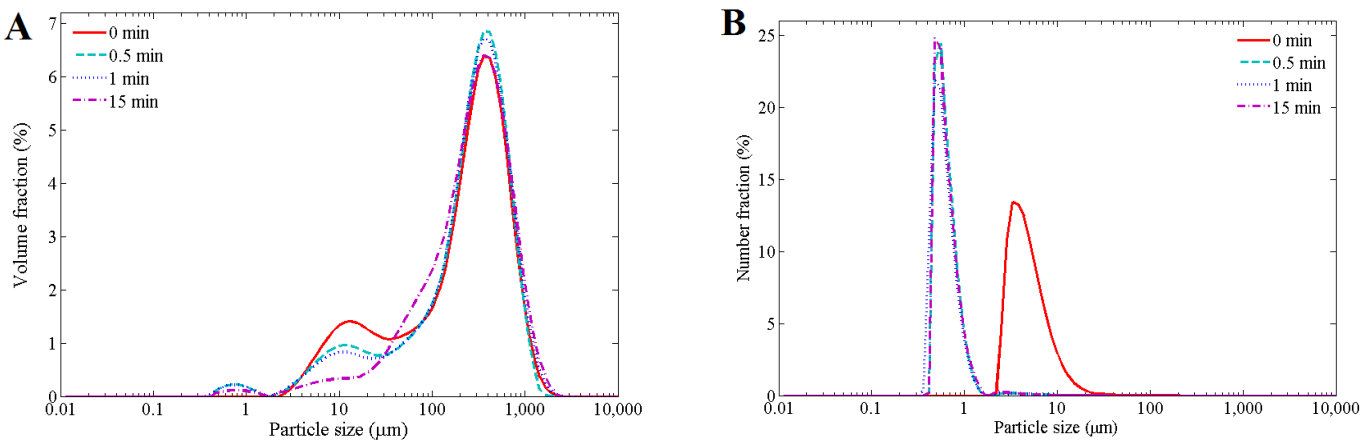


Figure 5. Particle size distributions of soy slurry after ultrasound treatment for 0 min (control), 0.5 min, 1 and 15 min based on volume fraction (A) and number fraction (B).

The instrumentation used to determine the particle size was based on laser diffraction technology. During the determination of the size, the particles are estimated to be spherical, which was not the case for this system, as confirmed by laser scanning confocal microscopy (detailed in section 3.4). Particle size measurements are thus to be used for comparisons to one another, and not as absolute values.

3.4 CLSM

Confocal laser scanning microscopy was employed to observe the visual effects of the ultrasound treatment. To highlight the apolar features in the soy samples of interest, nile blue was employed. Nile blue can be excited by two excitation wavelengths; emission at shorter wavelengths (520-626 nm) was highlighted green in this study, visualising oil. Longer wavelengths (662-749 nm) of emission are depicted in red and correspond to protein and fibrous materials. Figure 6 shows typical images that were observed by CLSM. Within the control micrograph (i.e. the unprocessed material) intact cells were seen, each containing complete protein bodies, shown as red within the cells (Figure 6A). Protein was also present in the continuous phase of the sample with the same size range observed within the intact cells (again coloured red in this instance). Submicron oil droplets were also observed in the continuous phase (highlighted in green). With increasing ultrasonic treatment time, the presence of the protein bodies free in solution reduced in concentration. Interestingly, no reduction in particle size of the protein bodies was observed, which correlates with the particle size distribution data (Figure 5). Intact cells were observed throughout all samples, even after 15 min of treatment (Figure 6E). If the cells are intact,

the materials present within are unavailable for extraction. Protein bodies within the intact cells were not affected by the ultrasound treatment; CLSM visualises intact cells containing protein bodies including after 15 min US treatment (Figure 6E). Upon the application of ultrasound at a frequency of 20 kHz, it is proposed that transient cavitation will be the main cause of effects in a liquid system [4]. Liquid jet formation occurs as a result of asymmetric bubble collapse during transient cavitation and this phenomena is independent of the frequency of applied ultrasound [5]. In the soybean slurry system, the cell wall disruption force was apparently much higher than that supplied with liquid jet impingement on the cell wall surface, as no change in the number of intact cells was observed via CLSM. The force required to overcome the energy holding together the insoluble protein must be lower than supplied with liquid jet impingement.

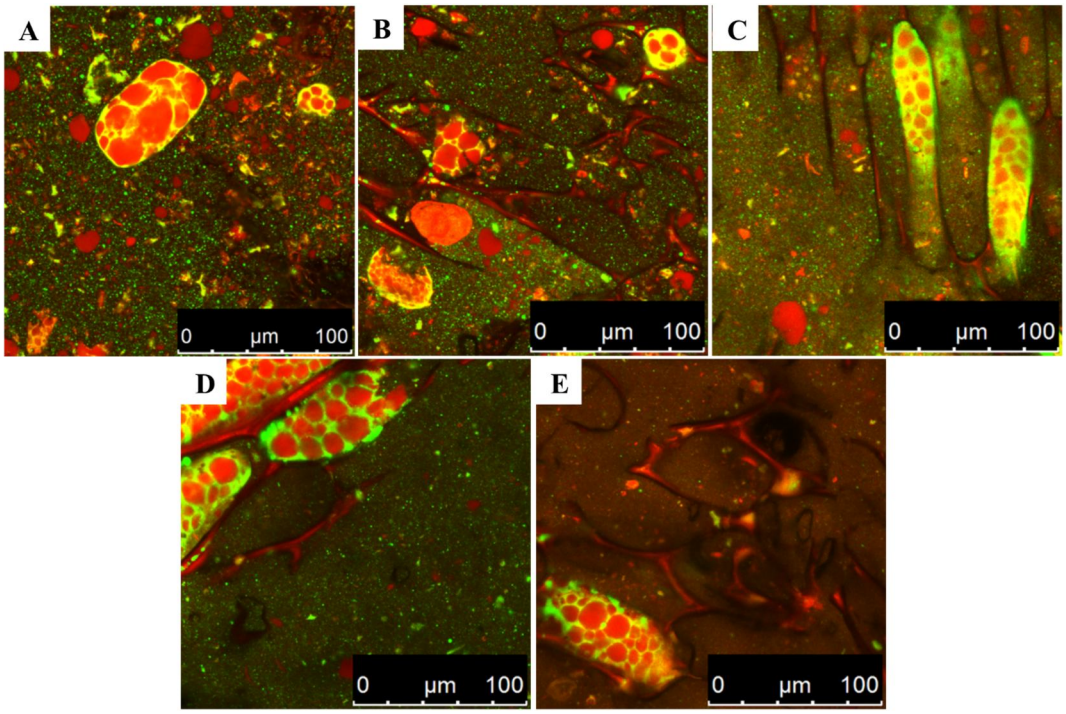


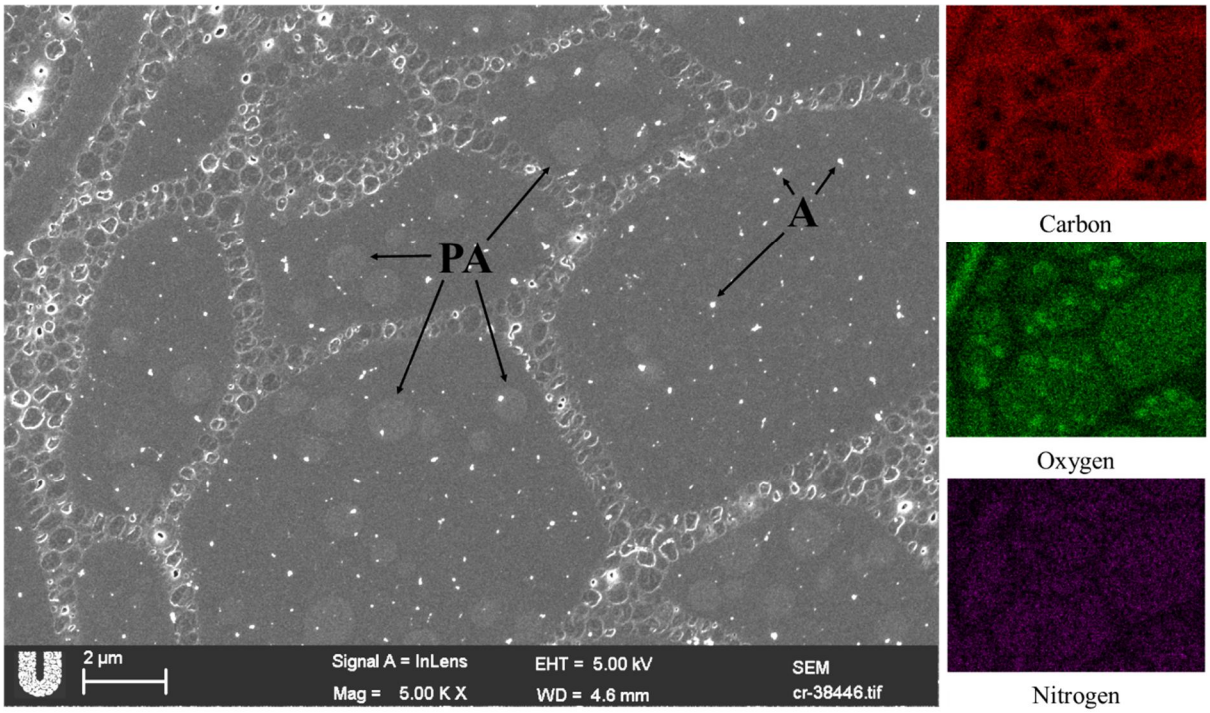
Figure 6. CLSM images of soy slurry after various ultrasound treatments visualised with nile blue A: (A) Control (0 min); (B) 0.5 min; (C) 1 min; (D) 5 min; (E) 15 min. Oil is presented in the green channel and other apolar material such as agglomerated protein, protein entrapped within intact cells and fibres are highlighted in red.

3.5 Cryo SEM-EDX

Surprisingly, no stepwise reduction in particle size of the protein bodies was found upon ultrasound treatment during soybase production (see Figures 5 and 6). We hypothesise that internal compartments within the protein bodies of the soybean are responsible for this 'all or nothing' effect, and that

ultrasound is either able to disrupt this internal organisation holding these internal compartments completely, or not at all. In a study by Krishnan et al. [36] compartmentalisation of protein bodies within rice seeds (*Oryza sativa* L.) was indeed shown. Storage proteins made in the endoplasmic reticulum within plant cells may accumulate in the form of smaller protein bodies primarily into so-called protein storage vacuoles by autophagy [37]. The limiting membrane of the sequestered protein bodies is then digested by vacuolar enzymes, resulting in aggregated, larger protein bodies (those visible in Figure 6).

SEM-EDX was utilised to investigate the structure and composition of the protein bodies within dry soybeans (Figure 7). The Figure shows protein bodies surrounded by oil bodies, which were lighter in appearance. The protein bodies ranged in size (2.4-13.5 μm), which fall in the lower part of the size range quoted in the literature of 2-20 μm, derived from imaging hydrated samples by transmission electron microscopy [24]. Bright white spots are artefacts arising from cryo-planing during sample preparation and sample transfer (labelled on Figure 7). It was possible to visualise spherical features within the protein bodies, these show as a lighter grey signal and annotated in Figure 7. EDX analysis (insets to Figure 7) showed these were carbon-deficient, oxygen-rich spherical structures within the protein bodies; EDX also clearly shows the difference in oxygen and carbon composition between the protein and oil bodies. Nitrogen was difficult to observe using EDX analysis due to its low abundance throughout the soybean; therefore, little difference in spatial arrangement was not apparent in the signal (Figure 7). These spherical structures (annotated on Figure 7 as PA) are most likely phytic acid, which act as a store of phosphorus and other cations during germination of the soybean [38;39]. Magnesium was also present within these external structures (data not shown), which is explained by the chelating ability of the phytic acid. The hypothesised compartmentalisation of soybean protein bodies was not observed in Figure 7.



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365 **Figure 7. SEM image of dry soybean with examples of phytic acid (PA) and artefacts (A) annotated. Scale bar represents 2**
366 **µm. Red, green and purple colour channels correspond to carbon, oxygen and nitrogen signals, respectively, during EDX**
367 **analysis.**

368 ## 4. Conclusions

369 Soymilk production consists of aqueous extraction from soybeans, followed by removal of insoluble
370 materials. The conventional extraction of various components from soybeans is suboptimal. The effect
371 of ultrasound on separation and extraction has been studied. Ultrasound intensifies the extraction of
372 valuable components from soybeans, leading to improved yields of protein, oil and solids of ca. 10%
373 after 1 min treatment. It is important to understand the effects of ultrasound on the aqueous extraction
374 system for its industrial application during soymilk production, which has not been extensively covered
375 within recent literature. The microstructural analysis undertaken in this study indicates improved
376 solubility as the main cause of the improved yields upon ultrasound treatment, and not cell disruption
377 as is frequently stated in the literature. The amounts of particles in the size range of 2.5-35 µm, most
378 likely protein bodies, were found to reduce for all ultrasound treatments investigated in an 'all or
379 nothing' effect as no intermediately sized features were observed.

Acknowledgements

The authors would like to thank the Engineering and Physical Science Research Council (EPSRC) for partially funding this project, which was funded through the EPSRC-Centre for Doctoral Training in Formulation Engineering. Caroline Remijn (Unilever Research & Development, Vlaardingen) is gratefully acknowledged for performing the SEM-EDX part of this study. Mr Clive Marshman, Dr Kylee Goode and Dr Phil W. Cox (University of Birmingham, UK) are also acknowledged for their input within this project.

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