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Whole soybean protein extraction processes: A review

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ABSTRACT

Soybeans are an important raw material for those seeking vegan, lactose-free products, such as soymilk and tofu. The aim of this review article is to provide an overview of aqueous extraction of protein and other desirable components from whole soybeans. Firstly, a discussion over the microstructure of the soybean is held, including a summary of protein localisation and properties. A detailed review of common whole soybean extraction process is then given, along with extraction process parameters and process intensification steps that can improve yield. A novel extraction model is presented, based on a mass balance of the water phases. The extraction model reveals separation as the main limitation for protein recovery during aqueous soy protein extraction due to the high amount of okara waste stream and its high moisture content.

Industrial relevance

Typically, the extraction of protein from an intermediate soy-protein ingredient is studied at lab-scale. Within industry, aqueous extract from whole soybeans is commonly used for making consumer products containing both soy protein and soybean oil, and this has been the focus of this review. Key extraction process parameters are presented and challenges of each extraction step are given for the whole soybean extraction process. A novel model for determining the separation efficiency has been presented, which is useful for many other extraction systems that contain components of interest in high amounts of waste stream.

KEYWORDS

Aqueous extraction; Microstructure; Soymilk; Cavitation

HIGHLIGHTS

Understanding the microstructure of raw materials is vital for enhancing yields.

Processing parameters influencing protein extraction have been discussed.

By-product for soybase production, okara, contains protein in the water phase.

A model using liquid to solid ratio was presented to calculate separation efficiency.

ABBREVIATIONS

CLSM	Confocal laser scanning microscopy
d.b.	dry basis
HPH	High pressure homogenisation
SEM	Scanning electron microscopy
SPC	Soy protein concentrate
SPI	Soy protein isolate
TEM	Transmission electron microscopy
w.b.	wet basis

NOMENCLATURE

S		Soybase mass
B		Soybean mass
W		Water mass
O		Okara mass
x_i		Mass fraction of component i
$x_{i,j}$		Mass fraction of component i in stream j
i	p	Protein
	w	Moisture
j	s	Soybase
	b	Soybean
	w	Water
	o	Okara

1. INTRODUCTION

Plant-based protein is more sustainable than animal-based protein when comparing fossil fuel usage, land use and water consumption (González *et al.*, 2011). With the human population projected to increase to 9.5 billion by the year 2050 (Reynolds *et al.*, 2015), a greater portion of the nutrients required for human nutrition will be supplied by plant-based sources. The first generation plant-based protein source for human consumption has been the soybean. Reportedly the consumption of soybeans can be dated back as early as 3rd century BC in China (Huang *et al.*, 2008). The consumption of soy has gained popularity in the western world over recent decades due to:

- increased knowledge of the consumer and drive for a healthier lifestyle
- increased prevalence of lactose intolerance
- improved processing of soybeans with reduced off-flavour (Debruyne, 2006)

The increased consumption of soy-based products leads to the incentive for more sustainable soybean processing, by reducing the carbon footprint and/or greenhouse gas emissions compared to current processes. Not only is sustainability a motivation for industry, but financial gain is also made possible through improved utilisation of the raw material. Table 1 shows an overview of the typical oil and protein contents of soybean and the main soy-derived commercially available ingredients. The summarised production methods for these soy-ingredients can be seen in Figure 1.

Table 1: Typical variations in protein and oil contents on a wet basis (w.b.) for soybean and some of its commercial ingredients. *Values derived from Riaz (2006) and own observations.

Product	Typical protein content (w.b. %)	Typical oil content (w.b. %)
Soybean	40	20
Soybase	4-5	2
Soy flour	40	20
Defatted soy flour/flakes	44-54*	0.5-1*
Soy protein concentrate (SPC)	65-70*	Trace*
Soy protein isolate (SPI)	85-90*	Trace*

Defatted soybean flakes are common by-products from oil extraction, the most common component utilised from the oilseed. Soybeans are crushed in a roller mill and then the oil is extracted using a solvent, typically hexane. Hexane-based processing can lead to the production of greenhouse gases and concerns regarding safety due to the flammable nature of the solvent (Rosenthal *et al.*, 1996). The remaining solvent within the soybean matrix is removed via heat evaporation. Mechanical extraction can also be employed; however, compared to solvent extraction, the oil yield is not as lucrative. Defatted soy flour refers to the same material as defatted soybean flakes, but with a finer particle size. It can be used as a feed, yet more value can be created when the proteins are extracted from it. To produce soy protein concentrate (SPC), defatted soybean flakes are added to alcohol or water to remove carbohydrates. Soy protein isolate (SPI) contains a higher protein content than SPC (see Table 1) due to the removal of insoluble carbohydrate and dietary fibres via an intermediate, acidic precipitation step.

A less commonly used extraction route is the whole soybean extraction (far left process in Figure 1). The aqueous extract of whole soybean extraction is called soybase and it is mainly used for making consumer products containing both soy protein and soybean oil. Products like soymilk, soy-fruit beverages and tofu are produced by adding various ingredients to the soybase, such as flavours, gums, stabilisers, minerals, vitamins, sugars, fruit juices and/or

coagulating agents in case of tofu. At industrial scale, the use of soybase may result in consumer products with better sensory properties and might be a commercially more attractive route than first isolating soy protein and soybean oil separately and then blending them together at a later stage. However, a large quantity of protein, oil and other components exit the process in the waste stream (30-40% of total protein, depending on the exact conditions). The waste stream, referred to as okara in the field, is typically utilised as animal feed (Li *et al.*, 2012).

This review article provides an overview of the latest insights in whole soybean extraction processing and the emerging technologies employed to aid especially protein extraction. Firstly, the soybean composition and microstructure is discussed, as insights from these can be gained into extraction processes. Then an overview of these most common processes is given, as well as a more detailed discussion of the challenges and opportunities of each extraction step. Finally, a novel extraction model will highlight the location of greatest losses of protein during soybean processing. Findings in this area of research are also beneficial for the advancing generations of other plant-based protein sources, such as pea, canola and lupin, as well as many other extraction systems that contain components of interest in high amounts of waste stream.

2. SOYBEAN COMPOSITION AND MICROSTRUCTURE

The composition of soybeans can vary; for the production of soy-based products, such as soymilk and tofu, a strain of soybean containing relatively high amounts of proteins should be selected. Other criteria for soybean selection include the colour and sensory properties of its extract in the final consumer product. The composition of a typical soybean for producing soymilk on a wet and dry basis can be seen in Figure 2 (Imram *et al.*, 2003). Soybeans used for soybean oil production are commonly richer in oil and lower in proteins (about 40 and 20%, respectively). Soybeans are considered as the most important legume as they are one of few vegetal materials containing all of the essential amino acids required for human development. Dairy alternative products prepared from soybeans are not only selected for their high protein content, but also for their lack of cholesterol and lactose.

For optimal extraction of components from soybeans, it is vital to understand the structure located within the soybean. There are a number of structures which make up the soybean: the hull, the hypocotyl axis and predominantly cotyledon cells (Campbell *et al.*, 2011). Figure 3 shows an image of soybeans and their microstructure after pre-soaking. The main constituent of the soybean, cotyledon cells, are organised within the bulk in a space-filling arrangement. Cotyledon cells are 70-80 µm in length and 15-30 µm in width (Campbell & Glatz, 2009; Rosenthal *et al.*, 1998). Hydration may cause the cells volume to increase.

The cell wall of the soybean cotyledon is comprised of a series of polysaccharides, which are often cross-linked with proteins and phenolic compounds (lignin) (Ouhida *et al.*, 2002). The primary cotyledon cell wall contains pectins, hemicelluloses and microfibrils of cellulose cross-linked with proteins (Campbell *et al.*, 2011). There is a secondary cell wall within the primary wall containing cellulose and hemicelluloses also capable of binding to proteins. Cells are held together by adhesive substances found in the middle lamella, the extracellular

space between cells, and contain pectins, glycine and hydroxyproline-rich proteins (Campbell *et al.*, 2011; Kasai *et al.*, 2003).

2.1 SOYBEAN OIL

Oil consists of approximately 88.1% triglycerides, 9.8% phospholipids, 1.6% unsaponifiable components and 0.5% free fatty acids (Salunkhe *et al.*, 1992). The majority of oil is located in oil bodies (oleosomes) within the cotyledon cells (Waschatko *et al.*, 2012). Oil bodies are found within the cytoplasmic network of the cells and are stabilised by small molecular weight proteins termed oleosins (Rosenthal *et al.*, 1998), which make them more hydrophilic and easy to extract aqueously. The oil bodies typically vary in size from 0.2-0.5 μm (Campbell & Glatz, 2009). Figure 4 shows a SEM image of a dry soybean. Oil bodies are observed in this micrograph, as well as other components of interest, most notably protein bodies and phytic acid (Preece *et al.*, 2017b).

2.2 SOY PROTEINS

The majority of proteins are organised in protein bodies of the cotyledon cells, labelled in Figure 4. According to Preece *et al.* (2017b), the protein bodies within the cotyledon cells were found to be in the size range 2.4 to 13.5 μm when examined using SEM without sample hydration. These values fell on the low side when compared to values recorded using TEM of 2 to 20 μm on hydrated soybeans (Rosenthal *et al.*, 1998). It has been reported previously (White *et al.*, 2013) that protein bodies swell upon hydration with water at neutral pH to double their original size, confirming these findings.

There are two major storage proteins that account for typically 60-80% of the total soybean protein: the globulins glycinin (11S) and β-conglycinin (7S) (Murphy, 2008). At neutral pH and ambient temperature, glycinin (11S) is a hexameric complex comprised of acidic and

basic polypeptides linked by disulphide bridges to provide a molecular weight in the range 320-375 kDa (Lakemond *et al.*, 2000). β-conglycinin (7S) contains three major subunits (β, α and α') reportedly with sizes of 50, 67 and 71 kDa, respectively (Maruyama *et al.*, 1999). Globulins are, by definition, only 100% water soluble in a salt solution (Kapchie *et al.*, 2012).

Other metabolic protein sources within the soybean include oleosins (8-20% of the total protein) for oil body stabilisation, trypsin inhibitors (0-1.7% of the total protein) and enzymes such as lipoxygenase (LOX) (Murphy, 2008). Trypsin inhibitors are a group of proteins present within the soybean that cause negative effects on human digestion. Trypsin and chymotrypsin are digestive enzymes located within the gastrointestinal tract with which trypsin inhibitors form very stable complexes with (Savage, 2003). Trypsin inhibitors are commonly denatured by heat inactivation during the extraction process ((Kwok *et al.*, 2002; Van Der Ven *et al.*, 2005); e.g., 90% inactivation after 5-10 min at 121°C at pH 6.5). High temperature treatment may also denature LOX, an enzyme activating the oxidation of polyunsaturated fatty acids and formation of fatty acid hydroperoxides, which are turned into volatile off-flavours. A balance is recommended between the inactivation of trypsin inhibitors and LOX and the detrimental effects of heat treatment, especially as protein denaturation will cause a reduction in the protein extraction yield (Rosenthal *et al.*, 1998). As soy protein is a complex mixture containing various proteins, it has different denaturation temperatures. β-conglycinin and glycinin, the main storage proteins, possess denaturation temperatures of about 68°C and 86°C, respectively (Peng *et al.*, 2016). Solubility of soy proteins also depends on their isoelectric points (pI), which is on average at pH 4.5 (Campbell *et al.*, 2011; Kinsella, 1979); so most soy proteins are soluble at pH values below 3 and above 6.

3. WHOLE SOYBEAN EXTRACTION PROCESSES

3.1 COMMON PROCESSES

The traditional process for soybase preparation used in the Orient, which is still used for the preparation of soybase in the home or by street vendors today, includes the following steps (Kwok & Niranjan, 1995):

- Soaking of the soybeans
- Grinding in cold water
- Filtering
- Cooking at 93-100°C for 30 min

This method could be modified by grinding in hot water, which has the advantage of LOX inactivation. Today such processes are adapted to make soybase at larger scale, either in a batch or continuously. As illustrative examples, some of these processes are shown in Figure 5. Process 1 is also known as the Tetra Pak Alwin™ process (Imram *et al.*, 2003) and is shown in more detail in Figure 6. Soybeans are ground at about 85°C and pH 8, and then the okara is removed by decanting and the soybase is further heated to 141°C and vacuum treated. It is quite similar to the classic Illinois process, although this was originally done with overnight soaking of soybeans (Nelson *et al.*, 1976). Process 2 is a process in which the soybeans are first dehulled and the soybean slurry is heated to 141°C and vacuum treated prior to decanting. Although a limited amount of protein is lost due to dehulling, it has the advantage that less okara will be produced during the extraction process and therefore slightly higher extraction yields can be achieved in comparison to process 1 (see section 4). Another advantage of process 2 is that the okara has more exposure to heat, and it is therefore more microbiologically stable and useful for other food applications after immediate drying. Process 3 is an example of grinding soybeans at ambient temperature, in contrast to the other

processes. This is commonly utilised by ProSoya, for example (Gupta, 2014). An advantage of cold grinding is that the proteins are not denatured at this stage and can solubilise well into the aqueous phase (see also section 3.3). However, the oxygen levels should be kept low otherwise LOX may cause off-flavour production. An alternative is to use LOX-free soybeans, but these are more expensive than standard soybeans. Heat treatment is still necessary at a later stage in Process 3 to denature trypsin inhibitors and create some microbiological stability of the soybase until further usage. Please note that these three processes are used here as examples and that some elements of one process can be used in one of the other process as well.

The sections below discuss each extraction step and important process parameters in more detail.

3.2 MECHANICAL DISRUPTION OF SOYBEAN CELLS

Traditional methods for producing homemade soymilk, such as 'nama-shibori', include pre-soaking of the soybeans prior to mechanical disruption (Toda *et al.*, 2007). Yet in most industrial processing plants, this is not employed due to the generation of off-flavours (Kwok & Niranjan, 1995). Blanching of soybeans is sometimes utilised by manufacturers to eliminate off-flavours in the final product (Peng *et al.*, 2017); however, this causes denaturation and aggregation of the protein. Soybeans are ground by either dry or wet milling to disrupt intact cotyledon cells to make protein and other components available for extraction. Dry milling can be achieved with, e.g., six-roller mill equipped with proper cooling, in which three sets of two rolls are used with one roll in each pair rotating faster than the other. For optimal dry milling, the particle size of the flour should be smaller than that of intact cells (see Section 3.3.1). Wet milling is often done at elevated temperatures (≥80°C) to eliminate the effects of the LOX. Two to three mills might be used in sequence, such as disk

mill, colloid mill and/or high pressure homogeniser. Swelling of soybean cells occurs upon hydration, therefore the maximal particle size for optimal extraction is greater for wet milling than for dry milling. New mills are grinding better than ones that have worn, and this affects the final yield of the extraction process directly.

3.3 SOLUBILISATION OF COMPOUNDS

The solubilisation of compounds can be split further into a number of steps: diffusion of the solvent into the plant matrix, solubilisation of cellular components, transport of the solutes to the exterior of the solid matrix and transport of the solutes from the matrix surface to the bulk medium. The rate determining step for mass transfer is most likely the solubilisation of intracellular components and their transfer to the surface (Jung *et al.*, 2011). Solubilisation of protein is influenced by several processing parameters, which impact the extraction of intracellular components into the bulk phase:

- particle size of the matrix (milling efficiency)
- pH of the solvent
- ionic strength of the solvent
- temperature of the solvent
- solubilisation time
- protein concentration of the resultant soybase

In the following sections, these main parameters are discussed in more detail, assuming there is enough solvent to solubilise or disperse the soy ingredients. In sections 3.4 and 4, we will explain that the liquid-to-solid ratio affects the separation efficiency and is therefore discussed there.

3.3.1 Particle size

Lower particle sizes of the plant matrix results in high extraction yields of protein and oil from flour (Rosenthal *et al.*, 1998; Russin *et al.*, 2007; Vishwanathan *et al.*, 2011a, 2011b). This is related to the available surface area for interaction with the solvent, or the fact that more cell walls were then disrupted by the milling, favouring cell content release. Decreasing the average particle diameter of soy flour from 223.4 to 89.5 μm, increased the protein recovery from 40 to 52% (Russin *et al.*, 2007). The maximum recovery of protein by extraction was achieved from a fine fraction of particles with a particle size below 75 μm; 97% and 93% from soybean flour and okara flour, respectively (Vishwanathan *et al.*, 2011a). The effects of particle size during wet milling of soybeans to produce soybase has also been reported (Vishwanathan *et al.*, 2011b). Particle size (364 to 182 μm) was found to have an inverse relationship with protein recovery (78.8 to 89.3%) (Vishwanathan *et al.*, 2011b). Similar results were also obtained using a stone grinder and a colloidal mill (Vishwanathan *et al.*, 2011b). These sizes quoted are slightly greater than those discussed previously relating to the size of intact cotyledon cells in Section 2 and to those measured during dry processing. This can be attributed to the particles swelling to larger sizes when in contact with water compared to the dry milling counterpart. Laser diffraction techniques to measure the particle size can also overestimate the size of structures; the technique cannot differentiate between a mass of intact cells and a cage-like structure of cell walls with the contents extracted. Campbell and Glatz (2009) argued that only broken soybean cells in the outer layer of soybean particles can release their content. Hence only soybean particles with a diameter as large as one or two soybean cells may fully release their content (if broken), as particles with larger diameters will have soybean cells which are not in the outer layers. The model presented by Campbell and Glatz (2009) assumes that cotyledon cells are spherical with a length of 55 μm (an average of 30 and 80 μm, see section 2) and that the processed soybean

particles from which contents are extracted are also spherical. So one may estimate a minimal particle diameter for full release from soybean particles of 110 μm (= 2 broken cells × 55 μm), although the structures of processed soybean microstructures we observed by confocal laser scanning microscopy (CLSM) did not fit with this model (Preece *et al.*, 2015).

3.3.2 Effect of pH and ionic strength

There is a good correlation between extraction yield and solubility (see section 2.2) of soy protein as function of pH. The highest extraction yields from either milled soybeans or okara are observed at either low or high pH values (<3 and >6), and the lowest around the pI of the majority of soy proteins at 4.5 (Ma *et al.*, 1996; Rosenthal *et al.*, 1998; Vishwanathan *et al.*, 2011a). Interestingly, oil extraction yields follow the same pH correlation as the protein extraction yields, although oil itself cannot be (de)protonated. This can be explained by the properties of the surface active proteins that stabilise oil droplets within the aqueous extract (emulsions). In general a pH of about 8 – 8.5 is chosen for extraction. Although the solubility may be improved at higher pH values, the functional properties of the proteins may then be impaired because of disaggregation and hydrolysis of the proteins. The extraction medium can be alkalised using sodium bicarbonate ($NaHCO_3$). This also increases the ionic strength, which also contributes to the solubility of soy protein (see section 2.2).

3.3.3 Effect of temperature

Proteins in their native state are more soluble than those that have been denatured. Therefore, the extraction temperature should ideally be kept below the soy protein denaturation temperature (<70°C, see also section 2.2) (Deak & Johnson, 2007; Rosenthal *et al.*, 1998). However, increasing the temperatures aids in breaking the protein-carbohydrate complex, leading to an improved protein yield (Kasai & Ikehara, 2005). Other benefits of thermal treatments also include the inactivation of LOX and trypsin inhibitors. Thus, a sacrifice must be made for improved solubility versus denaturation of certain protein components.

Preece *et al.* (2015) visualised the effect of thermal treatment at 80°C and compared it to wet, ambient extraction (<42°C) at lab scale. Upon 30 min wet, ambient milling of soybeans, it was possible to extract 26% more protein (absolute value) in comparison to the thermal equivalent (80°C, 30 min). CLSM (Figure 7) confirmed aggregation of protein bodies (depicted in green) in- and outside of intact cotyledon cells (assigned purple in microscope software) upon extraction at 80°C. Interestingly, we could not visualise such aggregated protein bodies when the soybean extraction was performed at pilot plant scale, which might be caused by its much shorter milling time (Preece, *et al.*, in press).

The release of protein from okara was found to be more sensitive to temperature in comparison to flour and wet milling of soybean (Ma *et al.*, 1996). Increasing the temperature from 25 to 90°C resulted in a progressive increase in the extraction of protein. This can be explained through the thermal processing previously experienced by the okara proteins during soybase production, leading to their denaturation and retarded release.

3.3.4 Effect of solubilisation time

The effect of incubation time of soy flour on protein extraction yield has been studied previously at lab-scale (Rosenthal *et al.*, 1998). After a period of approximately 10 min at 50°C, the protein yield plateaued at 75% for non-heat treated flour and 25% for heat treated samples. The rapid release rate of protein can be attributed to a short diffusion path offered by the fine flour, and follows first-order kinetics (unpublished Unilever results). Similar release rates as the ones for proteins were found for oil by Rosenthal *et al.* (1998) and us, which might be explained by the fact that most proteins are present on the surface of oil droplets (emulsions). Higher temperatures and smaller particles may result in faster release rates.

3.3.5 Protein concentration

Proteins above a concentration of 5-6% (w/w) in soybase tend to aggregate and sediment (unpublished Unilever results), and therefore the liquid-to-solid ratio commonly used for whole soybeans is often around 6:1 or 7:1 w/w (40% protein in soybean / 7 = 5.7%). Although higher amounts of water may result in relatively more yield (see section 4), both the protein throughput and the protein concentration will be lower. The latter is important for further product formulation; e.g., one needs a soybase with a concentration well above 4% if one would like to make a soymilk with 3% protein as other ingredients need to be incorporated as well.

3.4 SEPARATION AND EFFECT OF SOLID-TO-LIQUID RATIO

For the removal of insoluble ingredients, typically filtration or centrifugal separation techniques are employed, with decanting the more frequent choice due to its continuous operation. For the production of soybase, where the product contains a variety of soy-based components, no further separation of streams is required. However, if it is essential to separate the fractions further, such as the oil phase, water-soluble and insoluble materials, a 3-phase decanter would be more suitable. Successive extraction like double decanting, using a water-to-okara ratio of about 1:3 (w/w, equal to a solid content of ca. 5%), achieve higher yields (about 10-15%, depending on exact conditions). Please note that insolubility is here defined as ingredients which do not end up in the soybase, as some small and/or light solids can still end up in the soybase (e.g. denatured proteins, oil droplets, etc.). Insoluble ingredients are separated based on their density compared to the solvent in a decanter, resulting in a ‘milky’ soybase and the waste material okara. Particle size of insoluble particles also influences their separation during the recovery of components. Based on the principle of Stokes’ law, smaller particles are more difficult to separate from the bulk solution.

Okara (see Figure 8) has a surprisingly high moisture content of approximately 80% (Preece *et al.*, 2015). We propose that this high moisture content is responsible for most of the protein losses during whole soybean extraction, especially as the amount of okara produced is quite high. It is about two times more in weight than hulled soybeans in a process similar to the one shown in Figure 6, and for tofu production a soybean/okara ratio of 1:1.2 (w/w) has been reported (Li *et al.*, 2012). Surprisingly, others have not reported this cause for the low yield. We also hypothesise that the efficiency of the separation step is highly dependent on the liquid-to-solid ratio (also called water-to-bean ratio) because of the presence of okara. This is in contrast to what has been reported before by others, who argue that the use of more water results in more protein solubilisation (see below and Ma *et al.*, 1996; Rosenthal *et al.*, 1998; Sari *et al.*, 2015; Vishwanathan *et al.*, 2011a). However, we propose that, after a certain minimum needed to solubilise soy ingredients, the use of more water results in better separation efficiency as relatively less water stays in the okara. Therefore, we propose a new model in section 4.

3.5 ENSURING SENSORIAL QUALITY AND A SAFE PRODUCT

Soybase is heated to denature LOX and to hinder the effects of trypsin inhibitors (section 2.2), which cause digestive problems, if left untreated. Either steam injection or steam infusion can be used to increase the temperature to 121°C or higher. This unit operation may cause some denaturation or dissociation of proteins (Johnson & Synder, 1978), browning of the product by Maillard reactions, and cooked flavours (Kwok & Niranjan, 1995). Afterwards the soybase enters a vacuum tank for deodorising. The drop in pressure (from about 2 to 0.6 bar) and temperature (from about 121 to 80°C) facilitates off-flavour removal by flashing. The thermal and vacuum treatment can be carried out before or after separation of insoluble materials, which influences the properties of both soybase and okara.

The obtained soybase is then chilled but not sterile, although the microorganisms that survived the steam injection process are unlikely to grow during short-term storage. Furthermore, oxidation may take place when exposed to air in the processing facility and this is the main reason that soybase should be processed quickly.

3.6 PROCESS INTENSIFICATION OPTIONS

To aid in the extraction of protein and other components from soy, a number of other unit operations can be employed.

3.6.1 Enzyme-assisted extraction

Enzyme-assisted extraction using protease was found to improve both the extraction of oil and protein from soy flour (Rosenthal *et al.*, 2001; Sari et al., 2015). The effects of protease were also studied with positive effects in combination with membrane recovery by De Moura *et al.* (2011) and enhanced protein solubility from full fat extruded flakes (De Almeida *et al.*, 2014). Protease may improve the solubility of soy proteins, especially when they are denatured and/or aggregated. In general the smaller the proteins, the better their solubility. In addition, protease may cleave proteins from cell wall materials. Cellulases have also been used in an attempt to degrade cell walls, although the results were limited (Kasai *et al.*, 2003, 2004; Rosenthal *et al.*, 2001). The main issues with cellulases are that most of them are not food-grade and that the food-grade enzymes are most active at pH 5; at this pH soy proteins tend to aggregate since their pI is 4.5 (see section 2.2). One enzyme that has shown promise in previous research (Rosset *et al.*, 2014) is Viscozyme L, a multi-component carbohydrolase containing arabanase, cellulose, hemicellulose and xylanase. Viscozyme L resulted in a protein yield improvement of 23% at pH 9 from defatted soy flakes versus a control sample upon a 30 min incubation at a 1:20 solid-to-liquid ratio at 60°C (Rosset *et al.*, 2014).

Enzyme treatment times are usually long (30 min – hours), and expenses of both enzyme and processing are relatively high. One should also realise that optimal conditions for enzyme activity might not be similar to the optimal conditions of whole soybean extraction (e.g. pH of 8 and high temperature, see above).

3.6.2 Cavitation-assisted extraction

Cavitation is a phenomenon which has been widely studied within the food industry (Knorr *et al.*, 2004), with improvements attributed to enhanced mass and heat transfer and cell disruption.

3.6.2.1 Ultrasound-assisted extraction

Ultrasound-assisted extraction has been studied at lab (Preece *et al.*, 2017b) and pilot scale (Preece *et al.*, in press) for enhancing the extraction of protein from soybeans, based on cavitation. Improvements in protein extraction yields of 11% and 12% were found for soy slurry and okara ultrasound treatments (20 kHz, 1 min, 50 °C) respectively from materials prepared at lab-scale. Ultrasound caused the disruption of aggregated protein in the aqueous phase, confirmed by CLSM (Preece *et al.*, 2017b). When okara was prepared at pilot-scale, the improvement in protein extraction yield was less significant (4.2%), therefore was not recommended for industrial scale-up. Rationales for limited effects of ultrasound at pilot-scale include: less protein present within the okara for subsequent extraction with ultrasound due to shorter milling times and smaller energy intensities of the probe system versus the lab-scale system (Preece *et al.*, in press).

3.6.2.2 High pressure homogenisation

High pressure homogenisation (HPH) is another unit operation based on cavitation which can improve extraction yields from soybean processing materials (Preece *et al.*, 2017a). Improvements in yields were found to be a result of disruption of all intact storage cells, with a maximum total protein yield of 94% reported for okara solution with a single pass through a homogeniser at 100 MPa (Preece *et al.*, 2017a). Debruyne (2006) mentioned that homogenisation can cause a negative effect on the separation efficiency. However, this was only the case after multiple passes of okara solution or soy slurry through the homogeniser at 100 MPa (Preece *et al.*, 2017a). Additional investigations are required to assess the scalability

of this promising result obtained using a lab-scale homogeniser as well as the sensory and physico-chemical properties of the final soy-based product. Blockages of the homogeniser also needs to be avoided and, if not prevented, could result in halting the production of soybase.

3.6.3 Double decanting

Decreasing the moisture content of okara will improve the separation yields (see also next section). Washing of the okara results in an increase in the extraction of 10% or more extra soybase at an industrial scale (Debruyne, 2006). Options to get drier okara may include the use of centrifugal separation techniques using a higher *g*-time than the standard decanter (e.g. using a Sedicanter® (Flottweg Separation Technology, 2016)).

3.6.4 Belt press

Another option might be the addition of an expression device such as a belt press (also available from Flottweg or other companies) at the end of the current process, which may reduce the water content of okara from 80 to 65-70%. Please note that this is equal to an about 40% weight reduction. Direct treatment of okara is desirable versus re-diluting the waste stream. In this example filter press, okara is fed between two belts, the press belt and filter belt. The drive rollers cause these belts to turn, feeding the okara in between the press rollers that apply pressure to the okara. The filter belt contains holes that are designed to allow the filtrate to squeeze out into the collection area. The hole size will require investigation to achieve a similar solid content as the soybase, as well as being sufficiently large enough to not be frequently blocked with solids. If necessary, a vacuum can be applied to the filter bed to draw through more of the filtrate. This new soybase should be analysed for composition and sensory perception, in comparison to decanted soybase. Filter pressing is commonly utilised during wastewater treatment; e.g. raw sludge slurry containing 55% solids has been dewatered to 95% solids in previous reports (Chen, *et al.*, 2002). Unfortunately, the design of

the belt press is quite open to air in the processing facility and therefore hygienic control may not be easy.

4. A NOVEL MODEL FOR EXTRACTION

Previously a model was derived by Rosenthal *et al.* (1998), which described their results very well, relating the yield to the concentration in the 'solid' phase using a partition coefficient. The use of more water (higher liquid-to-solid ratio) results in more protein dissolution and therefore higher extraction yields. The difference in protein concentration of solvent versus biomass is widely proposed as the driving force for protein going into solution and should explain that more yield is achieved at higher liquid-to-solid ratios (see e.g. Sari et al., 2015). Some of our own data could be fitted well to the model of Rosenthal *et al.* (1998), especially when performed at ambient temperature when protein aggregation did not occur (see Preece *et al.* (2015) for more information). The protein extraction yield could also be predicted well using another mechanistic model developed by Campbell and Glatz (2009) and measured volume-weighted mean diameter values (D[4,3]) (see section 3.3.1). However, this model did not fit with the processed soybean microstructures we observed by CLSM (Preece *et al.*, 2015). We propose that, after a certain minimum of water needed to solubilise soy ingredients, protein dissolution in the aqueous phase is not the limiting factor in most cases. It is also hard to see how extracted protein is going back to the solid phase in beans (as is suggested in the equilibrium model by Rosenthal *et al.* (1998)). Moreover, especially at high temperature of 80°C and above at which whole bean extraction is commonly performed, soy proteins are mainly present (75-100%) in an aggregated, dispersed form with a particle size of about 0.1-1 micron in the liquid phase (soybase) (unpublished Unilever data).

Therefore, we would like to propose a new model. The protein extraction yield can be defined by the amount of protein in the soybase divided by the total amount of protein in both the soybase and okara; this yield is a function of both protein availability and separation efficiency as well:

$$Yield\ (\%) = \left[\frac{S \cdot x_{\mathrm{p,s}}}{\left(S \cdot x_{\mathrm{p,s}} + O \cdot x_{\mathrm{p,o}}\right)}\right] \times 100$$

$$= Protein\ availability\ (\%) \times Separation\ efficiency\ (\%) \qquad (1)$$

where:

S mass of soybase

O mass of okara

$x_{\mathrm{p,s}}$ mass fraction of protein in soybase

$x_{\mathrm{p,o}}$ mass fraction of protein in okara

Protein availability is here defined as the % of proteins that can be solubilised or dispersed in the extraction medium, here water. It is affected by the mechanical disruption of cells and solubilisation of compounds, whereas separation efficiency deals directly with the removal of solid materials. We hypothesise that the main losses in soy protein extraction yield are especially due to the high amount of okara and its water content, containing available proteins. Often the separation efficiency is not optimal. Of course, losses may also occur if the milling step is not optimal. After separation, okara still contains a large volume of water (about 80% w/w, see Figure 8) and commonly about 1-2 times more okara is produced than soybeans used in whole soybean extraction (see section 3.4). We propose that this high amount of water and the large amount of okara to be separated are the fundamental reasons why separation is more efficient at a higher liquid-to-solid ratio. This is illustrated by the equations and figure below.

$$S = B + W - O \qquad (2)$$

where:

B dry mass of soybeans

W mass of water (either added or already in the soybeans)

The mass here or below can be in weight (kg or ton), and can also be expressed per time (mass flow rate) when using a continuous set-up, without accumulation.

Assuming that the available proteins are equally distributed over the aqueous phase of soybase and the aqueous phase of okara, the separation efficiency can then be calculated by taking the ratio of the water phase in the soybase and the total water phase (in both soybase and okara):

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

where $x_{\text{w,s}}$ and $x_{\text{w,o}}$ are the fractions of water in the soybase and okara, respectively. Please note that the protein concentration is not present in equation 3, so it is applicable to soybeans with different protein contents as well as to partially solubilised protein conditions. In other words, the difference in protein concentration of solvent versus biomass, as widely proposed as the driving force for protein going into solution (see e.g. Ma *et al.*, 1996; Rosenthal *et al*., 1996; Sari *et al*., 2015; Vishwanathan *et al.*, 2011a), does not play a role here.

The liquid (L)-to-solid (S) ratio can be expressed as:

$$\frac{L}{S} = \frac{W}{B} \quad (4)$$

Rearrangement and addition of equation 4 into equation 2 and then 3 results into:

$$\text{Separation efficiency (\%)} = \frac{\frac{L}{S} \times B - O}{\frac{L}{S} \times B + B - O + \left(O \times \frac{x_{\text{w,o}}}{x_{\text{w,s}}}\right)} \times 100 \qquad (5)$$

Figure 9 below shows that the separation efficiency becomes more efficient when more water is used. Again, this is not due to high protein solubility, but there is simply more aqueous soybase compared to (the water in) the okara phase at relatively higher water contents.

In the figure above we have also plotted the measured protein yields of whole soybean extraction performed at our pilot plant in Unilever Research & Development facilities, Vlaardingen, as well as the separation efficiency calculated using equation 5 (no correlation factor used here). The experimental data agrees very well with the theoretical lines. Interestingly, the calculated separation efficiencies using real flow rate data of the different masses from pilot-scale experiments are most often just slightly above the measured protein yield data (0.5-6% greater, see Figure 9). This indicates that very little protein was unavailable for extraction in our pilot-scale experiments; about 92-99% was available (see equation 1). Indeed we have found by CLSM that the milling process in the pilot plant was very efficient as no aggregated protein bodies outside cells and not many intact cells were found during soybean experiments at pilot-scale, in contrast to soybean extraction performed at lab scale (Preece *et al.*, in press). During our lab scale extraction from whole soybeans (Preece *et al.*, 2015), the yields were lower due to observed protein aggregation or inefficient milling. However, a similar trend in yields as a function of liquid-to-solid ratio can still be observed and one may then calculate the yield by multiplying the separation efficiency with the amount of protein that is available for extraction (from 0-100%) according to equation 1. Unfortunately, such high yields as shown in Figure 9 are not common at factory scale, probably due to quick wear of the mills resulting in lower % of available protein.

The model above assumes that the protein or other ingredient concentration is equal in all phases, especially in the soybase and okara phases. This may not completely be true. Despite the very good agreement between theory and experimental data, we have measured that the protein content was often greater in okara than in the soybase during this pilot study (0.96 times at L/S of 1:6 to 1.3 times at L/S at 1:11 or higher). Both the protein concentration in the soybase ($x_{p,s}$) and in the okara ($x_{p,o}$) decreased with higher L/S ratio, but not with the same magnitude.

We also derived yield data from the study by Rosenthal *et al.* (1998) and plotted these in Figure 9 as well. Again, the theory and practical data agree very well with each other, although Rosenthal *et al.* (1998) obtained these with a very different system, with extraction from soybean flour at 50°C and pH 8 at lab-scale for 15 min whilst stirring at 200 rpm. They did not report amounts of okara and did not explain with their model the lower yield obtained at 80°C. One should also not neglect the differences in the scale of centrifuge used; Rosenthal *et al.* (1998) used a bench top centrifuge, whereas the measured protein yield represents a pilot-scale operation, using a decanter centrifuge. However, similar separation efficiencies (ca. 70%) were obtained when comparing the decanter centrifuge used by Preece *et al.* (in press) and a bench top centrifuge operated at 4330 × *g* for 10 min.

The effects of okara mass (O) and okara moisture content ($x_{w,o}$) are also shown in Figure 9. Reducing the moisture content from 80%, typically achieved using a decanter centrifuge, to 65% improved the separation efficiency. A higher separation efficiency is also achieved if a mass reduction of okara production is attained. In section 3.6 we discussed the use of belt press, which can reduce $x_{w,o}$ from 80 to 65% and O by 40% (e.g. soybean/okara ratio (B/O) from 2.3 to 1/1.4, w/w). Figure 9 shows that an improvement of yield by 13-18% might then be achieved at L/S values of 7-5, respectively.

Please note that equation 5 can also be expressed with a solid-to-liquid ratio (instead of liquid-to-solid ratio) in a similar way, and this will give an almost linear plot that is very comparable to the results published by Rosenthal *et al.* (1998). Please also note that the extraction yield of oil and solids follow a similar dependency as function of liquid-to-solid ratio, although their values are not the same as the protein yield (Preece *et al.*, 2017a, 2017b; Rosenthal *et al.*, 1998).

As we argued above, a good fit with theory and experimental data is not a full proof of our model. The model is simple but not perfect, as shown by the measured difference in protein concentration in soybase and okara. However, we based our model on the soybean microstructures seen upon processing (see Figure 7 and previous article (Preece *et al.*, 2015)) and highlight for the first time (as far as we know) that the well-known L/S ratio effect on protein extraction yield is caused by the amount of okara and its moisture content.

5. CONCLUSIONS

In this review, aqueous extraction from whole soybeans has been detailed, with a focus on protein extraction. During a whole soybean extraction process, soybeans are first ground, and the intracellular components are then extracted into the medium followed by separation of the okara waste stream. The availability of a component depends on particle size of the plant matrix after grinding, the properties of the aqueous medium used (pH, ionic strength, temperature) and the incubation time. High temperature is needed to inactivate LOX and trypsin inhibitors, so a sacrifice is required when considering processing conditions for soybean processing between protein solubility and inactivation of anti-nutritional proteins. The amount of water used influences the separation efficiency and therefore the yield. This could be explained by a novel model based on the mass balance over the water phases. The best process intensification options to consider might be the use of high pressure homogenisation and ways to obtain drier okara (e.g. decanters with higher centrifugal force, belt press). Less promising options seem to be the use of enzymes or ultrasound. The best, total extraction process for obtaining the final product might be a compromise between the optimal conditions of each individual step. Both microstructural control and process science should play a role in the design of an optimal process. Aspects like sensorial quality and throughput (which affect costs) should be taken into account as well.

The current review focuses on whole soybean extraction processes, but we believe that some of these insights and also the model can be utilised for the study of other soy protein extraction processes (like SPC or SPI, see Figure 1). Extraction of other plant protein (such as pea, canola and lupin) and many other extraction processes from biomass in which the moisture content of the waste stream is high and entraps a notable amount of the desired component can also benefit from understanding the discussed information.

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FIGURE CAPTIONS

Figure 1. Simplified scheme of processes applied to soybeans to produce common soy ingredients. Please refer to the text and e.g. Riaz (2006) & Imram *et al.* (2003) for more information about these ingredients.

Figure 2. Composition of a soybean preferably chosen for soymilk production on a dry and wet basis. Data from Imram *et al.* (2003).

Figure 3. Image of soybeans (about 5 mm in size) and scanning electron microscopy (SEM) image of a pre-soaked soybean. Within this micrograph, the seed coat (hull) and swollen cotyledon cells containing protein bodies can be observed.

Figure 4. SEM micrograph of soybean cell without prior pre-soaking (Preece *et al.*,2017b). Various components are labelled on the image: oil bodies (OB), protein bodies (PB), phytic acid (PA; spherical structures) and artefacts (A; white dots) can be seen. Scale bar represents 2 microns.

Figure 5. Examples of common, large-scale processes for soybase preparation. Typically, soybeans are dry cleaned first to remove dirt and damaged soybeans. Process 1 describes the Tetra Alwin process from Tetra Pak (Imram *et al.*, 2003, see also Figure 6); process 2 shows a variation in the order of processing with steam injection prior to the decanter; and process 3 is an example of an airless, cold grinding extraction process, utilised by ProSoya (Gupta, 2014).

Figure 6. Tetra Alwin® Soy process line (courtesy of Tetra Pak; published with their permission) is an example of a commercially available for the continuous production of soybase directly from soybeans (Imram *et al.*, 2003). It is the same as Process 1 in Figure 5. Numbers of the unit operations are: 1) Grinding of soybeans by mills; 2) heating of water; 3) pH adjustment to 8 and increase of ionic strength by addition of sodium carbonate (optional); 4) separation by use of a decanter; 5) double decanting (optional); 6) CIP unit, cleaning in place; 7) steam injection to increase temperature to 121°C or higher; 8) spiral to increase holding time; 9) flashing into a deodorisation tank under reduced pressure; 10) cooling unit to cool to about 5°C.

Figure 7. CLSM image of soy slurry visualised using acridine orange after grinding soybeans at 80°C. The green colour of the protein bodies indicates that these are relatively hydrophobic and thus denatured, as protein bodies inside cotyledon cells after grinding at ambient temperature are purple and not seen outside these cells.

Figure 8. Photograph of okara produced at Unilever Research & Development facilities, Vlaardingen. It contains a surprisingly high water content of about 80%, most likely due to remaining, although most often broken, cell wall structures in which water becomes bound to via hydrogen bonding (see also the CLSM image of okara shown by Preece *et al.*(2015)).

Figure 9. Separation efficiency or yield as a function of liquid-to-solid ratio. The separation efficiency lines were calculated using equation 5, assuming a mass of okara is either 1.4 or 2.3 times the mass of soybeans used. Also plotted in this figure are measured protein yields from a continuous soybean extraction process under alkaline conditions at 85°C (like Process 1, see Figure 5 or Figure 6) at pilot scale at Unilever R&D Vlaardingen (●). The slurry, at an operating flow rate of 200 kg h^{-1}, was fed into a decanter centrifuge operating at a *g*-force-time of 1.5×10^5 *g*-s. Details of the pilot plant set-up can be found in the research by Preece et al., (in press). Separation efficiencies were also calculated using equation 5 and real data, i.e. measured flow rates of soybeans (corrected for their moisture content), water and okara (**X**). On average the okara to dry bean flow rate ratio during the pilot plant experiments was 2.3 and the $x_{w,o}$ was approximately 0.8. Finally, the yields as obtained from Rosenthal *et al.* (1998) are also plotted here (▲).

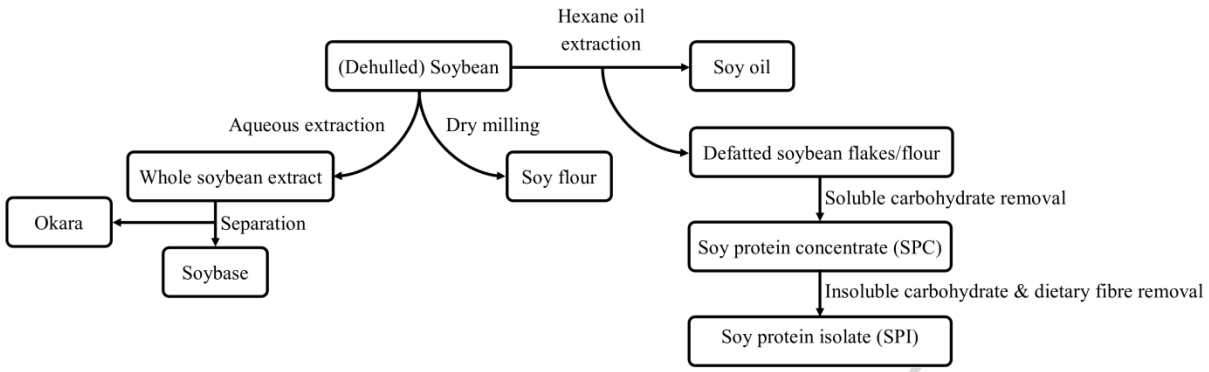

Figure 1

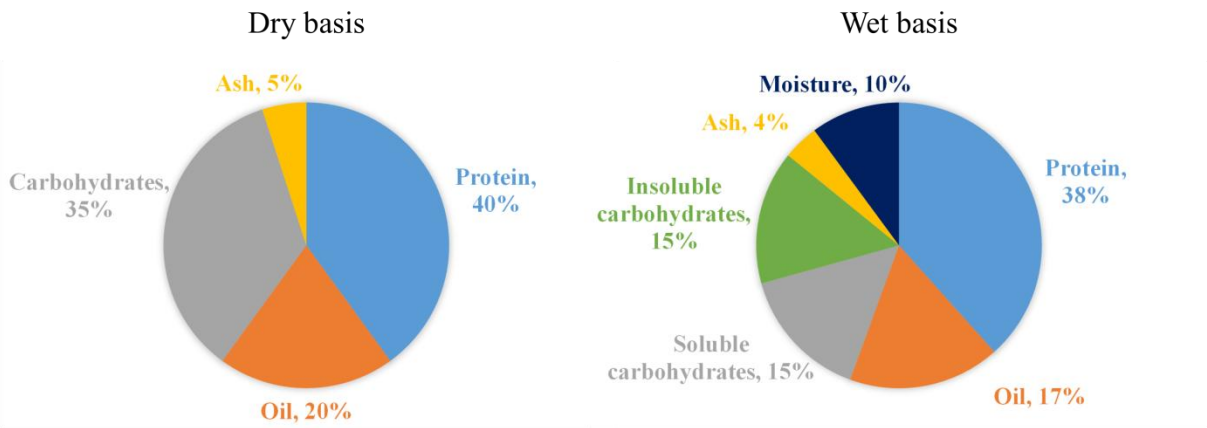

Figure 2

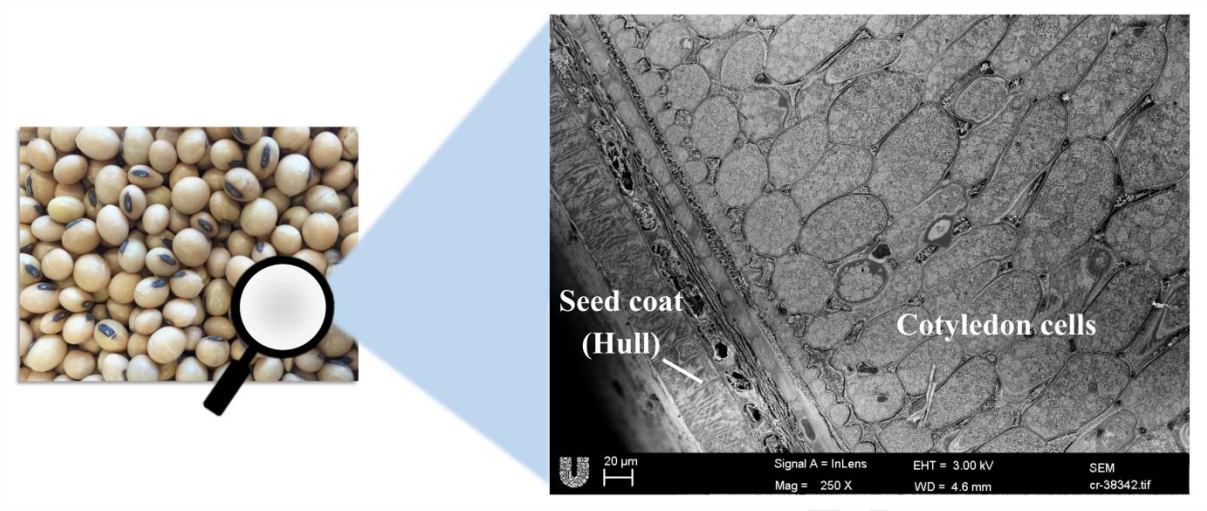

Figure 3

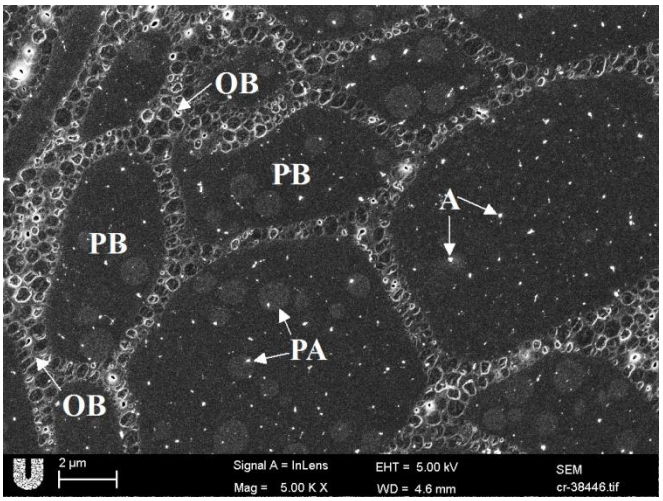

Figure 4

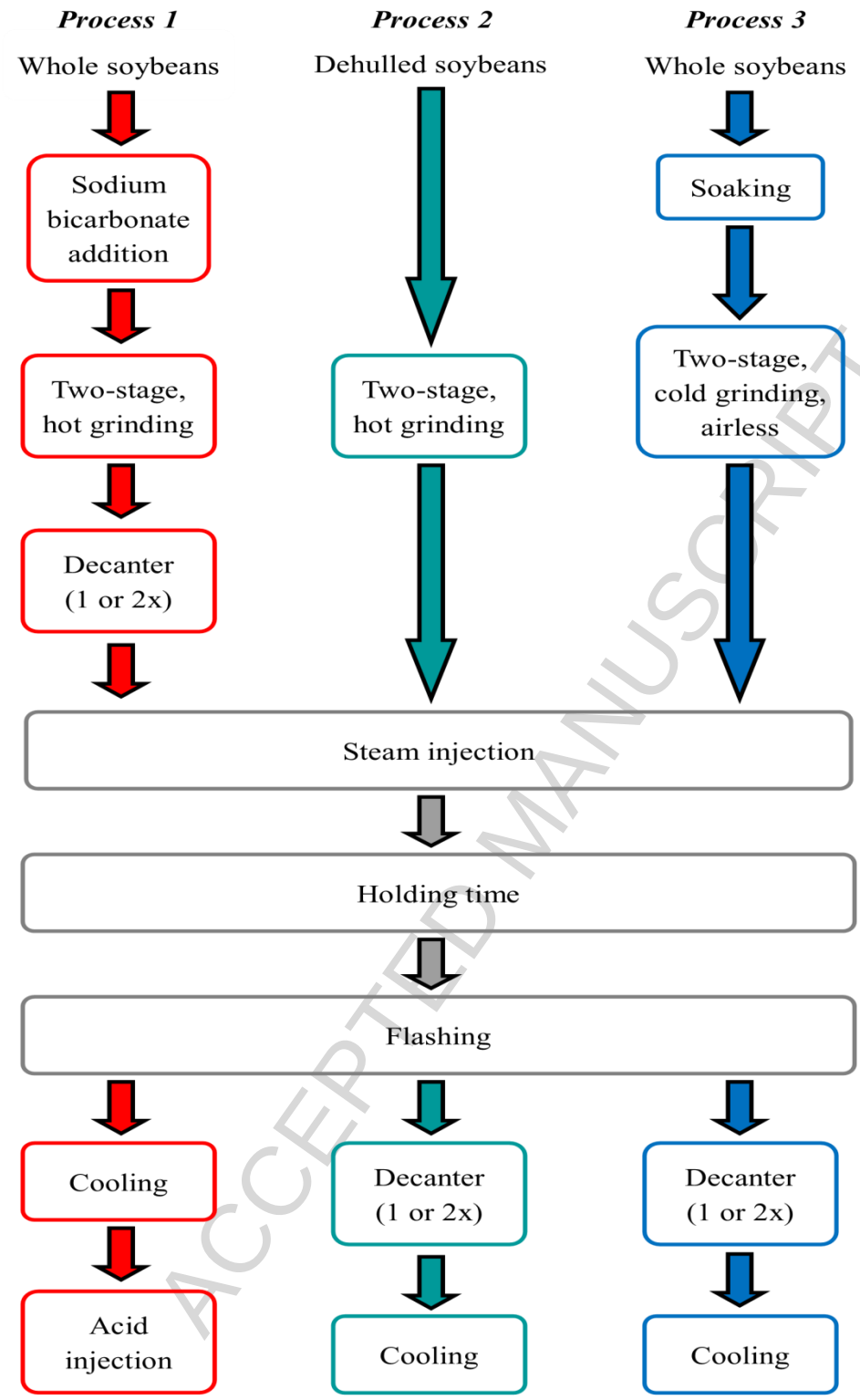

Figure 5

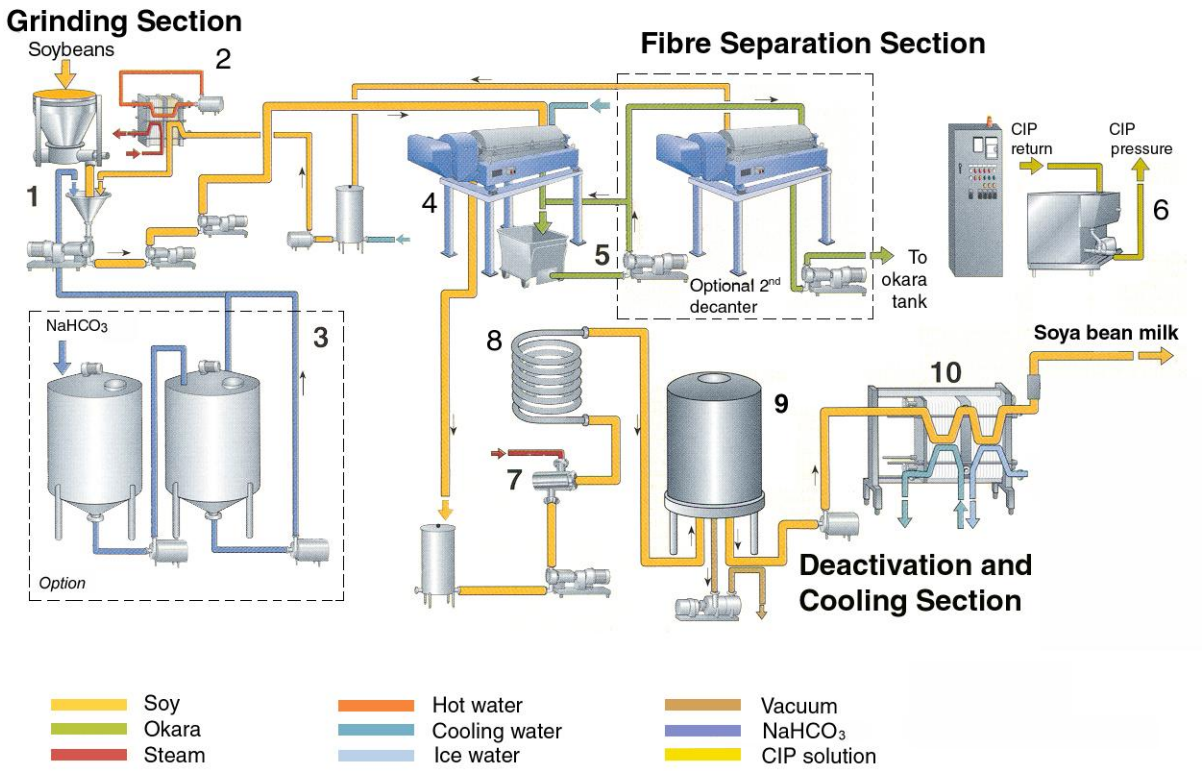

Figure 6

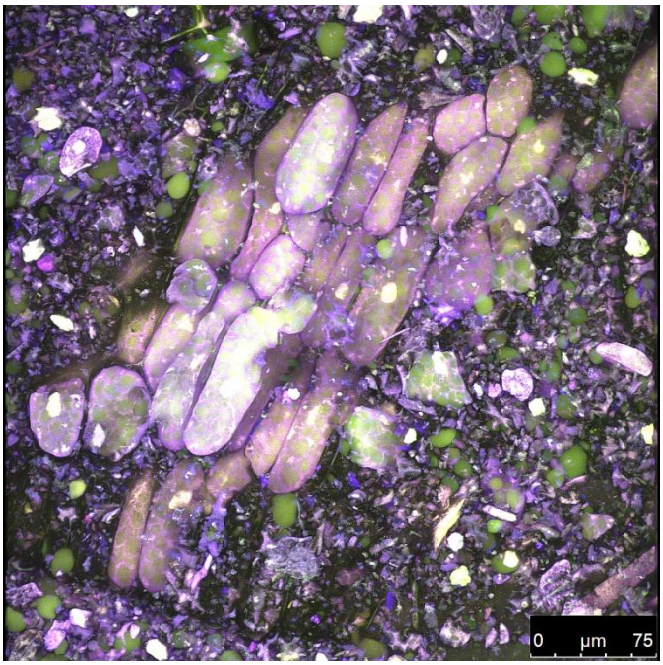

Figure 7

Figure 8

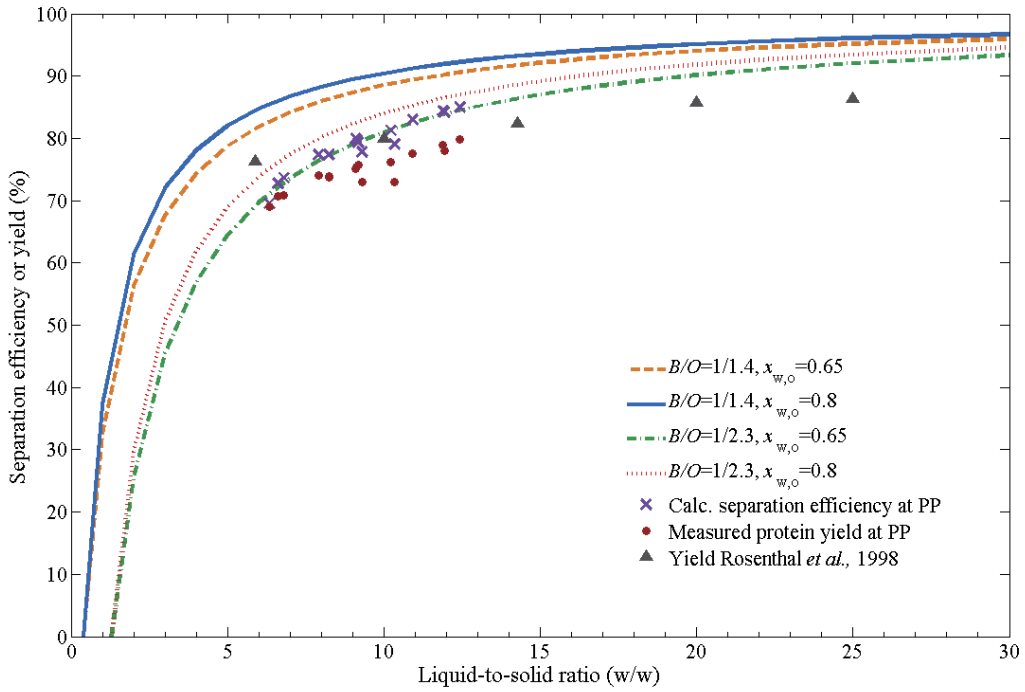

Figure 9