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Use of high-power ultrasound during soy protein production and study of its effect on functional properties of soy protein isolate

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Use of high-power ultrasound during soy protein production and study of its effect on functional properties of soy protein isolate

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ABSTRACT

A comprehensive review focusing on advantages of high-power ultrasound in extraction, emulsification, crystallization, defoaming and modification of functional properties of food proteins was conducted to understand the ultrasonic mechanisms, important process parameters and components to consider for scale-up. The effect of high-power ultrasound on the overall extractability of soy proteins, total sugar, and soy protein isolate yield and functional properties were evaluated. It was hypothesized that the pretreatment of defatted soy flakes with high-power ultrasound prior to soy protein extraction would enhance the extractability of the protein and sugar molecule. Because of the cavitation phenomenon occurring during ultrasound treatment, change in protein native state was expected to occur, which would affect the functional properties of the soy protein isolate. Defatted soy flakes dispersed in water were sonicated for 15, 30, 60 and 120 seconds using a bench-scale ultrasound unit. The ultrasonic amplitude was varied from 0, 21, 42, 63 and 84 μm_{pp} (peak to peak amplitude in µm). The power densities were 0.30, 0.87, 1.53 and 2.56 W/mL representing, very low, low, medium and high-power, respectively. Scanning electron microscopy of sonicated samples showed the structural disruption of soy flakes cell wall. The defatted soy flakes particle size was reduced nearly 10-fold following ultrasonic treatment at high-power settings. Treatment at high-power for 120 seconds gave the highest increase in total sugar release and protein yield, which was of 50 and 46%, respectively, when compared to non-sonicated sample (control). These conditions also gave the highest soy protein isolate yield increase, which was of 34%. To determine effect of temperature increase occurring

during sonication, the ultrasonic pretreatment of the defatted soy flakes was carried out with and without temperature moderation.

The heat generated during sonication had no significant effect on protein and sugar release from defatted soy flakes. Functionality of soy protein isolate obtained from defatted soy flakes treated for 30, 60 and 120 seconds at 0.30 and 2.56 W/mL was assessed. The sonication power and the sonication time both impacted significantly the soy protein isolate functional properties. The sonication of defatted soy flakes for 120 seconds at the high-power level improved the soy protein isolate solubility by 34% at pH 7.0, while decreasing emulsification and foaming capacities by 12 and 26%, respectively, when compared to soy protein isolate obtained from untreated defatted soy flakes. Rheological behavior of the soy protein isolate was also modified with significant loss in consistency coefficient due to sonication. Some of these results could be explained by the loss of the protein native state with increased sonication time and power.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

Soybeans are one of the most important bean sources in the world, providing a source of edible oil and a source of high quality proteins for both feed and food applications. While soybeans have certain anti-nutritional aspects such as presence of protease inhibitors, phytic acid, and flatus-producing oligosaccharides, that need to be overcome, they also contain several components, such as isoflavones, saponins and soy proteins that confer them some health benefit attributes, when consumed as part of a healthy diet. Soy proteins can also be used in non-food applications including the production of biodegradable plastics and paper coatings and sizing (Johnson et al., 1992). Besides their inexpensive cost compared to animal proteins, the versatile use of soy proteins can be attributed to the myriad of functional properties that native or modified soy proteins can confer to a food product (Deak and Johnson, 2007; Johnson et al., 1992). Particularly, soy proteins are used for their unique foaming and emulsification properties, solubility and apparent viscosity. Only about 5% of the soy proteins are used for food applications, and therefore there is a potential growing market for the food applications of soy proteins. One of the drawbacks of soy protein isolate production from defatted soybean meal, a by-product of the oil industry, is the incomplete aqueous protein extraction leaving half of the proteins with the fiber fraction. Increasing the protein extractability and developing soy protein foods and ingredients with unique functionality would certainly contribute to their increasing use by the food industry. The work proposed in this dissertation aims to contribute to this goal by providing knowledge on

effects of ultrasound pretreatment of defatted soybean flakes, which is a non-traditional technology that could benefit to the soy industry. Ultrasound is defined as sound waves of frequency > 20 kHz and it produces cavitation effects thereby producing hydrodynamic shear forces in the aqueous phase, which facilitates disintegration of particles (Khanal et al., 2007). High-power ultrasound has been recently reported as an alternative method to improve extractability of intracellular compounds from plant cells (Hromádková et al., 2002; Li et al., 2004; Wu et al., 2001) and to modify the functional properties of proteins (Jambrak et al., 2008; Krešić et al., 2008).

The low yield of protein obtained during the alkaline aqueous extraction of defatted soybean flakes can be attributed to the location of the protein inside the cotyledon cells (Kasai and Ikehara, 2005). Thermal, enzymatic and chemical modifications have been applied to soybean in order to modify the cell wall structure, resulting in a moderate increase in protein extractability (Choi et al., 2006; Jung et al., 2006). In addition, some of these treatments severely degraded protein functionality (Panyam and Kilara, 1996). There is therefore a need to develop alternative extraction processes based on physical and mechanical treatment that can enhance the extractability of protein and sugar from defatted soybean flakes without adversely affecting their functionality.

One of our central hypotheses was that high-power ultrasound by producing cavitation effects and thereby generating macro-turbulence, particle fragmentation, mass and heat transfer, dissolution of cell content and diffusion through the cell-wall in aqueous phase, could enhance the extraction yield of protein and sugar. Because these changes in the protein structure due to ultrasonic effect could affect the functionality of soy protein isolates,

establishment of the functional properties of the proteins recovered from sonicated defatted soybean flakes was also performed.

Four main research questions are addressed in this dissertation. First, do sonication time and sonication power (amplitude) have an effect on bench-scale aqueous extraction of protein and sugar from defatted soybean flakes? Secondly, can the temperature control during sonication affect the extractability of protein and sugar? How do sonication power and sonication time affect the soy protein isolate yield? And, finally how do sonication power and sonication time affect the functionality and structural properties of soy protein isolates.

1.2 Dissertation organization

This dissertation is organized into six different chapters. The first chapter is a general introduction which includes research hypothesis and objectives for further research approach. The chapters 2, 3 and 4 are prepared as manuscript for publication in various international journals. The second chapter reviews the various aspects of high-power ultrasound application in food processing. It is entitled as “Innovative Application of High-power Ultrasound in Food Processing: A Review” and will be submitted to the *Journal of Food Engineering* for publication. Third chapter and fourth chapter have been published in the *Journal of Food Engineering* and *Journal of American Oil Chemists’ Society* in July 2009 respectively. Third chapter is focused on the use of high-power ultrasound prior to soy protein extraction to simultaneously enhance protein and sugar release in soy extract, while fourth chapter discusses the effect of pretreating defatted soy flakes with ultrasound on soy protein isolate yield and functional properties. The fifth chapter focuses on process

economics of the dissertation. General conclusions and recommendations for this dissertation are contained in chapter six. It is noted that the figures, tables and equations are embedded within the texts of each chapter and cited references are added at the end of each chapter.

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CHAPTER 2: INNOVATIVE APPLICATION OF ULTRASOUND IN FOOD PROCESSING: A REVIEW

A paper to be submitted to *Journal of Food Engineering*

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2.1 Abstract

The effects of ultrasonication in slurries, such as particle fragmentation, heat and mass transfer, and dissolution, create many potential application of this technology in food processing. The recent research advances show that ultrasound has become an efficient tool for large-scale commercial applications, as improved equipment design with higher efficiency, larger capacity and new tooling design has been developed. This review focuses on advantages of ultrasonication for extraction, emulsification, crystallization, homogenization, defoaming, food cutting and modification of the functional properties of protein.

Keywords: High-power ultrasound; Food process; Cavitation

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2.2 Introduction

The application of high-power ultrasound as a processing aid has been explored in various industrial sector for many years. The first recorded ultrasound application was in 1917, when ultrasound was used for the estimation of depth of water by Langevin (Mason, 2003). Although the technology was conceived during the years preceding World War II, the industrial application of ultrasound only started in the 1960s (Brown and Goodman, 1965; Frederick, 1965). Ultrasound is now widely used in multiple areas including medical imaging, sonochemical processing (Mason & Lorimor, 1988), cleaning of surfaces, and many other applications. The medical imaging uses high frequency (2-10 MHz) low- energy (up to 10 Wcm^{-2}) diagnostic ultrasound, while low frequency (20-100 kHz) high-energy (10-1000 Wcm^{-2}) ultrasound is used in sonochemical processes. Low-power ultrasound is non-destructive so it is widely used as an analytical technique in food industry for assessment of physicochemical properties of food such as texture, composition, structure, and flow rate (McClements, 1995).

The application of high-power ultrasound in food industry is relatively new and has not yet been fully explored. However, various applications have been identified with great potential for future development, e.g. crystallization, drying, degassing, extraction, filtration, homogenization, meat tenderization, oxidation, sterilization, cutting and freezing (Floros and Liang, 1994; Gennaro et al., 1999; Li and Sun, 2002; Mason et al., 1996; McClements, 1995).The recent developments in ultrasound generation techniques, as well as increased understanding of cavitation phenomenon has attracted the interest of scientific community to examine the use of high-power ultrasound as an alternative tool in modifying the physical

and chemical properties of food macromolecules such as food proteins and carbohydrates as well as other physical effects, such as cutting, mixing and defoaming. The objectives of this review are to discuss the current developments in the application of high-power ultrasound in assisting extraction of food macromolecules along with the description of the ultrasonic mechanisms, important process parameters and components to consider for scale-up.

2.3 Mechanism of ultrasound

Ultrasound is defined as sound waves of frequency > 18-20 kHz. When ultrasound is applied to a liquid, it imposes an acoustic pressure in addition to hydrostatic pressure. In an infinite medium, the acoustic pressure (P_a) of sound wave is typically considered to be a sinusoidal wave and dependent on frequency (f), time (t) and maximum pressure amplitude of the wave ($P_{a,\,max}$), which can be written as: $P_a = P_{a\,max}\, sin2\pi ft$ [1]

$P_{a,\,max}$ is directly proportional to power input of the transducer. At low ultrasound intensity, the pressure wave induces motion and mixing within the liquid (Leighton, 1998), while at high intensities, the sound wave propagates into the liquid medium creating alternating compression and rarefaction cycles as shown in Figure 1 (Zheng and Sun, 2006).

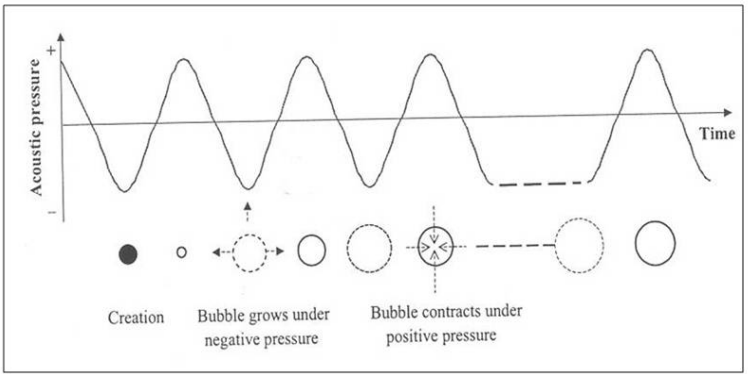

Figure 1: Motions of bubbles during cavitation

Source: *Zheng and Sun, 2006*

The negative pressure in the rarefaction cycle causes liquid to fracture, which ultimately results in the formation of small bubbles or cavities (Ashokkumar and Grieser, 1999; Gong and Hart, 1998). During the subsequent compression and rarefaction cycle, bubbles contract and expand resulting in an increase in bubble size over each ultrasound cycle (Zheng and Sun, 2006). After a number of compression and rarefaction cycles, the bubbles attain a critical size in which sonic energy is unable to keep the vapor phase inside. As a consequence during the following compression cycle, vapor suddenly condenses and bubbles implode (Mason et al., 1996). The implosion of cavitation bubbles results in many physical, mechanical and chemical effects due to generation of macro-turbulence, high-velocity inter particle collisions and perturbation in micro-porous particles of the material treated resulting in the acceleration of eddy diffusion and internal diffusion. Macro-turbulence has been used to enhance the heat and mass transfer in many processes (McClement, 1995). The physical effect that cavitation provides have been used to accelerate the extraction kinetics and to enhance the extraction yield of intracellular materials (Ma et al., 2008; Shah et al., 2005; Wu and Li, 2003). The improvement in extraction performance of ultrasound-assisted extraction achieved in food processing can probably be attributed to diffusion through the plant cell walls, disruption and washing out of the cell contents, reduction in particle size of the vegetal material as enhanced by ultrasonic cavitation. The disruption of tissue surface structure of caraway seeds and soybean flakes due to cavitation was revealed by microscopic examination (Chemat et al., 2004; Haizou et al., 2004). A recent study on the sonication of bacterial cell (*Escherichia coli*) has shown that the cavitation at cell surface has the ability to produce holes through the cell wall (Ugarte-

Romero et al., 2006). In addition to physical effect, the cavitation phenomenon of ultrasound also generates chemical effects in the liquid media by the formation of highly reactive radicals such as $H^{\bullet}$ and $OH^{\bullet}$ when the sonication medium is water (Henglein, 1993). This chemical effect of ultrasound can be advantageous or disadvantageous depending upon the processes. Formation of $OH^{\bullet}$ radicals produced during cavitation may affect the quality of some food substances. For example, a study by Ashokkumar et al. (2008) observed the decrease in antioxidant capacity of cyanidin-3-glucoside to approximately one-fifth of the original value after 4 h of sonication. In contrast, these radicals can also be used to enhance the functionality of food ingredients. The study by Ashokkumar et al. (1999) showed that sonication of phenol leads to hydroxylation in the *ortho*-, *meta*- and *para*- position suggesting that sonochemically generated $OH^{\bullet}$ may be used to enhance the antioxidant properties of compounds from plant sources such as flavonoids.

2.4 Factors affecting the cavitation phenomenon

2.4.1 Pressure

Applied external pressure and solvent vapor pressure are two major influencing factors of the cavitation phenomenon. Increasing the external pressure increases the cavitation threshold by dissolving the suspended gas molecules, and thus, the number of cavitation bubbles are reduced (Muthukumaran et al., 2006). More importantly, increase in applied pressure increases the pressure in bubbles increasing the energy during their collapse. Thus, cavitation bubbles collapse is enhanced, which ultimately increases the sonochemical

effect (Lorimer and Mason, 1987). The energy required to produce cavitation in a solvent of low vapor pressure is relatively high because there is less mass transfer into the bubble. Thus a more volatile solvent will support cavitation at lower acoustic energy and produce vapor-filled bubbles.

2.4.2 Intensity

Ultrasonic intensity is the power dissipated per unit of surface area of the sonotrode and defined in W/cm^2 (Patist and Bates, 2008) and ultrasound energy is of the integral of the dissipated power as a function of time. The time of ultrasonic exposure is inversely proportional to the flow rate through the ultrasonic devices (L/h). In general, an increase in ultrasonic intensity increases the sonochemical effect (Luque De Castro and Capote, 2007); however, the amount of energy that can be applied to any system is limited.

2.4.3 Temperature

The vapor pressure, surface tension and viscosity of liquid are affected by the temperature of liquid (Muthukumaran et al., 2006). An increase in temperature decreases the cavitation threshold. Increase in temperature also facilitates the cavitation and increases the number of cavitation bubbles at a low acoustic intensity. However, the sonochemical effects are reduced at these conditions; because the higher number of cavitation bubbles will act as a barrier to sound transmission. Thus, in order to maximize sonochemical effects, experiments should be conducted at low temperature or using a solvent of low vapor pressure. The study of Xu et al. (2000) showed that the extraction of flavonoid from bamboo can be improved

using ultrasound assisted extraction at low temperature rather than hot-water extraction at 80 °C. While Rosangela et al. (2007) reported the improvement in mass yield of caffeine and palmitic acid from leaves of *Ilex paraguariensis* in methanol solvent.

2.4.4 Solvent viscosity

The cavitation threshold increases with viscosity. One way of decreasing the viscosity would be increasing the temperature to a certain limit, which would allow for violent collapse. But as mentioned in the earlier section, if a temperature is reached beyond the optimum limit, the vapor pressure increases and ultimately sonochemical effects will be diminished by the dampening effect at the tooling face due to high vapor pressure. Thus, there is an optimum temperature at which the viscosity is low enough to generate violent collapse and to avoid the dampening effect by high vapor pressure.

2.4.5 Applied frequency

It is known that cavitation bubbles require an extremely short but definite time to form at sites of rarefaction that have nucleation sites. Thus, as ultrasonic frequency is increased, the duration of low pressure phase shortens and intensity of ultrasound has to be increased to maintain an equivalent amount of cavitation energy in the system. For example, the duration of the rarefaction cycle reduces from 25 μs to 0.025 μs when frequency is increased from 20 kHz to 20 MHz (Luque De Castro and Capote, 2007).

2.4.6 Gas and particulate matter

Any dissolved gases or minute gas bubbles in the liquid act as nuclei for the formation of cavitation bubbles. Thus, in any process where the cavitation collapse is the primary factor in causing the sonochemical effect, degassing would be counterproductive.

Because the overall process output is affected by the various process parameters, it takes time and effort to scale and fine-tune the process with the goal to achieve the maximum result with a minimum amount of energy.

2.5 Applications

A wide range of ultrasonic mechanisms and treatment conditions provide multiple food application opportunities for the food industry as summarized in Table 1.

Table 1: List of high-power ultrasound applications in food industry

Application	Mechanism
Extraction	Increase in mass transfer and release of plant cell materials
Emulsification	High shear microstreaming
Defoaming	Pressure waves causing bubble collapse
Crystallization	Nucleation and modification of crystal formation
Modification of functional properties of protein	Changes in the structure and conformation of proteins

2.5.1 Extraction

The traditional extraction method of organic compounds from plants or seeds is based on the adequate combination of solvents, heat, pH, and or/agitation. The treatment of the material to be extracted by high-power ultrasound prior to extraction can significantly

improve the extractability of the intracellular material for example improvement in soy protein isolate yield by 34% was obtained when high-power ultrasound was applied prior to protein extraction from defatted soybean flakes (Karki et al., 2009a). The enhancement in extractability of plant material can be attributed to the propagation of ultrasonic pressure and resulting cavitation phenomena. The energy released due to the collapse of cavitation bubbles promotes a higher penetration of the solvent into the cellular material and improves the mass transfer to and from the interfaces (Knorr, 2003; Li et al., 2004; Vinatoru, 2001).

Additionally, the extraction process can be improved further at high ultrasonic intensities due to disruption of cellular and sub-cellular components and the softening of the cell membranes caused by the periodic oscillation of acoustic pressure (Jayasooriya et al., 2004). An ultrasonic method for polysaccharide extraction from the herbal plant *Salvia officinalis* L. has been developed and increased yield by 18% relative to conventional polysaccharide extraction method (Hromádková et al., 1999). This increase was attributed to an increased accessibility and diffusion of the carbohydrate polymer from the cell wall. More recent studies have shown that the ultrasonic disruption of buckwheat hulls for hemicelluloses components (Hromádková and Ebringerová, 2003) and sugarcane bagasse for celluloses (Sun et al., 2004) not only accelerated the extraction process but also preserved the molecular and structural properties of these compounds. The increase in hemi-cellulose extraction from sonicated buckwheat hull was attributed to the destruction of the cell wall and breakage of the links between lignin and hemicelluloses (Jing et al., 2004).

Ultrasound has also been used for the extraction of proteins and enzymes from the cells. For example, ultrasound-assisted extraction has been found to be effective in extracting

insulin from pancreatic tissues and rennin from calf stomach in a shorter period of time as compared to the conventional extraction processes (Kim et al., 1989; Zayas, 1985; Zayas, 1986). This increase in extraction efficiency and reduction in extraction time can be attributed to cavitation, which occurs within the tissue resulting in dispersal of tissue, destruction of the cells, intensive blending and acceleration of diffusional osmotic processes (Kim et al., 1989; Zayas, 1985; Zayas, 1986).

The food grade oil industry has also applied the ultrasound-assisted extraction to maximize production efficiency and reduce extraction time (Babaei et al., 2006). The benefit of using ultrasonic pretreatment before extracting oil from soybean and extracting carvone and limonene from caraway seeds have been reported (Chemat et al., 2004; Haizhou et al., 2004). These improvements were again attributed mainly to destruction of the cell wall and increased cell permeability along with the effects of solvent vapor pressure and surface tension on cavitation intensity. Ultrasound-assisted extraction has also been used for the production of herbal and bioactive compounds such as carnosic acid from rosemary (Albu et al., 2004), anthraquinones from roots of *Morinda citrifolia* (Hemwimol et al., 2006), tartaric acid from red and white variety grapes (Palma and Barroso, 2002), and aromatic compounds from different plant sources (Cocito et al., 1995; Vila et al., 1999).

Effect of high-power ultrasound to enhance protein and sugar release from defatted soybean flakes was studied by Karki et al. (2009b). The study reported the increase in sugar and protein yield by 50% and 46%, respectively, when sonicated for 2 min at a high power level, i.e. 2.56 W/ml. The increase in yield was attributed to the cavitation phenomenon and resulting effect such as size reduction, increased mass and heat transfer.

Ultrasound has also been combined to other processing technologies such as supercritical fluid extraction. This combination was used for the extraction of gingerols from ginger (Balachandaran et al., 2006), almond oil from seeds (Riera et al., 2004) and significantly enhanced the extraction rate and extraction yield by 30% in both studies.

2.5.2 Emulsification

Emulsion is a heterogeneous system consisting of two immiscible liquids, one of which (the dispersed phase) is intimately dispersed into the other phase (the continuous phase) in the form of small droplets. They usually occur naturally in the plant and animal kingdom, e.g. rubber tree latex and milk respectively (Luque De Castro and Capote, 2007). However, to produce an emulsion by mixing two immiscible liquids, additional energy is required and also the resultant emulsion is usually unstable. High-power ultrasound is efficient in creating emulsions and this application has been one of the earliest used in the food industry. The mechanism for ultrasound-assisted emulsification is based on cavitational effects. When ultrasonic energy is applied to a mixture of two immiscible liquids the bubbles collapse near the surface of the phase boundary layers of two liquids, and produces extremely efficient homogenization due to a cavitational mixing (Mason et al., 1996). Ultrasonic emulsification is used for the commercial production of a wide variety of products in the cosmetics, pharmaceuticals, textile, and chemical industries mainly because of the relatively low energy input in the production of very fine and highly stable emulsions (Canselier et al., 2002; Freitas et al., 2006). The food processing industry has shown great interest in ultrasound-assisted emulsification, and the technology has been incorporated in the

processing of various products including mayonnaise, tomato ketchup and fruit juice (Patist and Bates, 2008; Wu et al., 2000).

2.5.3 Defoaming

Foam is a dispersion of a gas in a liquid where the distance between individual bubbles are very small. Foaming occurs usually when liquids with foaming tendency are aerated or gases are released under conditions of a sudden pressure relief in a chemical reactor. Foaming is usually considered as an undesirable phenomenon in many food industries due to its adverse effect on product quality and/or yield. Therefore great effort has been made either to prevent foam formation or to control once the foam has been formed. Several thermal, chemical and mechanical defoaming methods have been developed. Mechanical method is the most widely used method in defoaming process, where bubbles collapse is produced by any mechanical shock (rapid pressure change, shear force, compressive force and centrifugal force). The use of high-power ultrasound can be considered within the mechanical method of defoaming (Gallego, 1998). Even though substantial work has been done in the past on ultrasonic defoaming based on aerodynamic acoustic sources such as Hartmann whistle and the rotator siren but apparently only the use of stepped-plate transducer for air-borne ultrasound has been applied for the large scale industrial application (Gallego et al., 1978; Rodriguez et al., 1985). The air-borne power ultrasound is powerful and compact piezoelectric equipment with no moving parts, no air flow and readily sterilizable whereas, Hartmann whistle type defoamer causes the problem of noise, requires high air generation capacity and energy consuming. The high-power

ultrasonic technology is commercially applied in some food processes, such as application of ultrasound in defoaming of carbonated beverages and fermentation systems (Morey et al., 1999) and to control the excess foam produced on high-speed canning lines and in the dissipation of foams in reactors (Gallego, 1998). Even though various attempts have been made at explaining the underlying mechanism by which foam is destroyed under ultrasonic radiation, the exact mechanism of ultrasound defoaming is still unclear. However, ultrasound based defoaming is assumed to be due to combination of following factors: high acoustic pressures, radiation pressure, resonance of the foam bubbles and acoustic streaming (Gallego, 1998). This area still needs to be explored.

2.5.4 Food cutting

The application of high-power ultrasound in food cutting processes is another example of diverse applications of high-power ultrasonics in food processing. The foods such as cheese, candy bars, bakery and confectionary products and convenience foods are mainly targeted for ultrasonic cutting (Schneider et al., 2002). In commercial scale foods are usually cooked or baked in large blocks or in bulks, thus they need to be reduced in size to portions suitable for handling or eating. Several knives and machines have been developed to achieve easy and effective cutting. However, different factors affect the conventional cutting process such as knife shape, sharpness, cleanliness, knife motion and speed throughout the product and applied force (Rawson, 1998). Incorrectly set cutting assemblies results in miscutting and a damaged product, which, adds a significant cost to the production and/or restricts the range of the product. Thus, incorporating the ultrasound-supported cutting assemblies into an

automated food manufacturing line or as a free standing machine can improve cutting In ultrasonic cutting, specially designed knife blades or probes (horns) excited by ultrasound are used. The efficiency of ultrasonic cutting depends upon the factors such as ultrasonic amplitude, ultrasonic frequency, blade geometry and the direction of vibration relative to the cutting direction (Rawson, 1998). The ultrasonic cutting effect is due to occurrence of mechanical and thermal effect in the separation zone at the cutting edge and/or flanks of the blade (Schneider et al., 2002; Zahn et al., 2006). However, physical, chemical and structural properties of the food determine whether the reduction in the cutting force is due to the effect of vibrating edge or due to the reduced friction (Zahn et al., 2006). Many difficult-to-cut foods such as hot bread, sticky confectionary can be cut successfully with ultrasonic cutting; however, heat dissipated during ultrasonication can cause the structural or chemical modifications of liquid-rich food ultimately affecting the food quality (Schneider, et al., 2002).

2.5.5 Crystallization

High-power ultrasound has been widely used in the crystallization process because it plays an effective role in the initiation of nuclei, in the crystal growth rate by disrupting the nuclei present in the medium and in the formation of small and even-sized crystals (Li and Sun, 2002; Luque De Castro and Capote, 2007). Since size of the material/particle is the major quality parameter in crystallization of food items or additives, these processes need to be well-controlled. Thus, high-power ultrasound can benefit food freezing processes and lead to products of better quality by accelerating the freezing process of fresh food products

mainly by heat and mass transfer (Li and Sun, 2002). Li and Sun (2002) applied high-power ultrasound intermittently during immersion freezing of potato slices, where temperatures were reduced from 0 to -7 °C. Their results indicated that high-power ultrasound can lead to an increase in freezing rate. This is attributed to the cavitation phenomena of ultrasonication, which results in the occurrence of microstreaming and enhances the heat and mass transfer accompanying the freezing process. Similarly ultrasound has been greatly used to clarify wine, which reduces the precipitation time of potassium bi-tartrate from 4-10 days to 1.5 to 2 h (Mason et al., 1996).

2.5.6 Modification of functional properties of proteins

The properties of proteins that determine their uses in foods are called functional properties. According to Kinsella (1976), “functional properties denote those physical and chemical properties that influence the behavior of proteins in food systems during processing, storage, cooking and consumption”. Furthermore, Damodaran (1997) defined functionality as “the physico-chemical properties that influence functional behavior of proteins in food include their size, shape, amino acid composition and sequence, net charge, charge distribution, hydrophobicity, hydrophilicity, structures (secondary, tertiary and quaternary), molecular flexibility/rigidity in response to external environment (pH, temperature, salt concentration) or interaction with other food constituents”. Solubility, emulsification capacity, foaming capacity, rheological properties are some of the important functional properties of proteins. The functional properties of proteins are affected by several factors. These factors can be categorized into three groups, intrinsic, environmental and

processing. Some of the processing factors influencing the functional properties most are heating, drying, pH, ionic strength, presence of reducing agents, storage conditions and physical, enzymatic or chemical modifications (Kinsella, 1979). Most of the native proteins do not possess the functional properties as desired by the food industry, which explains the need for novel food processing technologies to modify the protein functional properties. Among the alternative technologies that have been developed, ultrasound technology is the one that is the most used and is applied for a wide range of commercial applications (Leadley and Williams, 2006; Patterson et al., 2006). In a recent study, Krešić et al. (2008) investigated the effect of high-power ultrasound on rheological and thermophysical properties of whey protein concentrate (WPC) and whey protein isolates (WPI). Both the solubility and apparent viscosity of WPC and WPI were improved significantly after sonication of 10% protein dispersion for 15 min. This increase in protein solubility was attributed to the changes in the structure and conformation of protein caused by the ultrasound treatment. Ultrasonication of protein unfolds the protein structure, which exposes their hydrophilic sites to water increasing the binding of water molecules (Morel et al., 2000). This additional water binding increases the viscosity of sonicated proteins. However, ultrasound can also decrease the protein viscosity. Indeed if the treatment provides enough energy, the treatment can reduce the protein molecular mass, which decreases viscosity (Seshadri et al., 2006). Jambrak et al. (2008) determined the effect of low intensity (500 kHz) and high intensity (20 kHz probe and 40 kHz bath) ultrasound on solubility and foaming properties of whey protein suspension. Ultrasound significantly increased the solubility and foaming capacities and foaming stabilities of whey protein isolate treated at 20 kHz, while

treatment at 40 kHz had less effect on functionality, and treatment at 500 kHz had no impact on foaming ability but improved the protein solubility. These increases in solubility of whey protein isolates are conferred to the changes in the three-dimensional structure of globular protein, which resulted in increased numbers of charged groups. These conditions cause electrostatic forces to be higher, so more water interacts with the protein molecule, the polarity increases, and hence solubility increases. The improvement in foaming capacities and foaming stability of whey protein was due to the homogenization effect of ultrasound as generated by the cavitation effect of ultrasound. The homogenization effect of ultrasound usually disperses the protein and fat particles more evenly, thus improving the foaming properties such as foaming capacity and foaming stability. The use of high-power ultrasound in food processing can lead to the several advantages like increase in protein solubility, foaming capacity, viscosity. However, disadvantages may arise if ultrasound is applied without testing optimal power for treatment time, which may lead to destructive effects of ultrasound, such as protein denaturation.

2.6 Industrial equipment

Ultrasound is emerging as an innovative tool in the field of food science and technology. Though the potential of power ultrasound for industrial use was recognized long ago, industries seemed to be somewhat reluctant to adopt this technology until recently. One major factor that limited the industrial use of ultrasound was the lack of commercial ultrasonic instruments designed specifically to automatically optimize the effects of ultrasonication in food processing. However, recent advancements in ultrasonic technology

have resulted in more industrially robust food processing equipment (Patist and Bates, 2008). The manufacturers of high-power ultrasound equipment have been focusing on designing equipment by including specific operational features. Firstly, incorporation of automated frequency scanning enables maximum power delivery during fluctuation processing conditions. Romdhane and Gourdan (2002) demonstrated the benefit of an automated frequency system as compared to fixed frequency, where the former system achieved a 32% increase in pyrethrine extraction and 30% increase in power delivery to the product. Secondly, the focus has been on the construction of large flow-continuous treatment chambers (flow-cells) to reduce the cost per volume of material to be treated. Currently, 16 kW is the highest power available in a single ultrasound flow cell, with flow capacity ranging from 5 to 500 L/min. Multiple cell provide the flexibility to operate at larger flow rates by combination in series or in parallel mode (Patist and Bates, 2008). Mixed frequency reactors are reported to be more effective with respect to process efficiency and energy distribution (Delgadino et al., 2002; Feng et al., 2002; Moholkar et al., 2000). Additionally, construction of radial, donut and hybrid sonotrodes are desirable as they provide a greater range in the application of design and product opportunities.

2.7 Conclusion

Recent research has clearly demonstrated the usefulness of high-power ultrasound in the food industry, as it creates novel methodologies which are often complementary to the existing traditional techniques. Ultrasound cannot only improve the quality of processed food, but also provides a ground for developing new products with unique functionality.

However, the effect of ultrasonication depends on many physical parameters including the duration, intensity or frequency of the treatment. Hence more fundamental research is needed to establish their relationship to the ultrasonic efficiency. Moreover, commercial viability of high-power ultrasound depends on the availability of appropriate instruments that are easy to operate, and cost-effective. Thus the future development of this technology is directly dependent on the progress in designing and developing large-scale ultrasound equipment.

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CHAPTER 3: ENHANCING PROTEIN AND SUGAR RELEASE FROM DEFATTED SOY FLAKES USING ULTRASOUND TECHNOLOGY

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3.1 Abstract

This research focused on the use of high-power ultrasound prior to soy protein extraction to simultaneously enhance protein and sugar release in soy extract. Defatted soy flakes dispersed in water were sonicated for 15, 30, 60 and 120 s using a bench-scale ultrasound unit. The ultrasonic amplitude was varied from 0, 21, 42, 63 and 84 $\mu m_{pp\ (peak\text{-}to\text{-}peak)}$. The respective power densities were 0.30, 0.87, 1.53 and 2.56 W/ml. Scanning electron micrographs of sonicated samples showed the structural disruption of soy flakes. The flakes particle size was reduced nearly 10-fold following ultrasonic treatment at high amplitude settings. Treatment at high amplitude for 120 s gave the highest increase in total sugar release and protein yield, which was 50% and 46%, respectively, when compared to non-sonicated samples (control). Ultrasonic pretreatment was also carried out with and without cooling for

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temperature moderation. The heat generated during sonication had no significant effect on protein and sugar release from defatted soy flakes. Integration of ultrasound in soy processing plants can significantly improve soy protein yield and reduce the overall production costs.

Keywords: Defatted soy flakes; protein yield; sugar release; ultrasonic pretreatment; particle size distribution

3.2 Introduction

Soybean *(Glycine max)* is a protein-rich leguminous oilseed and soy proteins are usually extracted from defatted soy flakes, also known as residual soy meal, a by-product of soybean oil solvent extraction (Grieshop et al., 2003; Lusas and Riaz, 1995). Hexane extraction is performed on soy flakes to obtain maximum oil extraction yield due to cell disruption promoted during flaking. Most of the cells in the residual soy meal are therefore disrupted. However, this flaking is not sufficient to promote maximum protein extraction yield during the conventional alkaline aqueous extraction, as approximately half of the available protein from the soy flakes remains in the insoluble fraction (Jung et al., 2006). Because water is used to recover the proteins, soluble sugars are simultaneously extracted, some of which associated with flatulence problems, which constrains the use of soybean and soy protein ingredients in food applications. Flatulence results from the presence of significant amounts of α-linked oligosaccharides, mainly raffinose and stachyose (De Lumen, 1992). Several studies have examined the extraction methods and removal of these unwanted

oligosaccharides from the soy protein (Fischer et al., 2001; Ouhida et al., 2002). During extraction, many parameters including the nature of the solvent, the temperature, the pH, the agitation speed and extraction time can be optimized for optimum protein recovery (Kasai and Ikehara, 2005; Mason et al., 1996). Location of the protein inside the soybean structure was found to add to the difficulty of extracting proteins (Kasai et al., 2005). Different extraction processes such as microwave heating (Choi et al., 2006), enzymatic modifications (Jung et al., 2006) and chemical modifications were investigated to improve protein extractability from soy substrates. In general, high-power ultrasound has the potential to improve the extraction of intracellular compounds from plant material. Ultrasound pretreatment produces localized cavitation, which facilitates disintegration of particles (Khanal et al., 2007). Ultrasound technology has been applied widely in various biological and chemical processes. For example, ultrasound has been used to enhance oil extraction from soybeans (Li et al., 2004), active xylan and heteroxylan extraction from corn cobs and corn hulls (Ebringerova et al., 1998), and glucose release from corn slurry from dry-grind ethanol plants (Khanal et al., 2007). To date, the potential of high-power ultrasound to enhance protein and sugar release from defatted soy flakes has not been clearly established. Because the release of protein is governed by disintegration of lamellar structures binding the protein molecules, it is possible to hypothesize that the use of high-power ultrasound improves protein and sugar release.

The key objectives of this paper are to report on a laboratory-scale study investigating

(i) the effect of different sonication conditions (e.g. power input and sonication time) on protein and sugar release from defatted soy flakes with and without temperature moderation, and

(ii) the optimization of sonication conditions for maximum recovery of protein.

3.3 Materials and methods

3.3.1 Soybean samples

Hexane-defatted soy flakes (50 kg) were obtained from the Center for Crops Utilization Research (CCUR), Iowa State University (Ames, IA, USA). The soy flakes were stored in air-tight plastic bags at 4°C until use. The moisture content of soy flakes was 5.2% on a wet basis.

3.3.2 Ultrasonic equipment

The ultrasonic system was a Branson 2000 Series bench-scale ultrasonic unit (Branson Ultrasonics Corporation, Danbury, CT, USA), with a maximum power output of 2.2 kW. It was operated at a frequency of 20 kHz. Other components included the booster (gain 1:1.5) and the catenoidal titanium horn (gain 1:2.8), with a flat 13 mm diameter face.

3.3.3 Sonication of soy flakes

Defatted soy flakes (100 g) were dispersed in 500 ml tap water in a customized a 1.2-L stainless steel sonication chamber with a cooling water jacket. The samples were treated in

batch-mode at four different amplitude levels, 21, 42, 63 and 84 $\mu m_{pp\ (peak\text{-}to\text{-}peak)}$, designated as very low, low, medium, and high amplitude, respectively. The power levels were changed by varying the amplitude at the horn tip. For each amplitude, the samples were sonicated for 15, 30, 60 and 120 s with and without temperature moderation. Temperature was moderated by circulating cold water (4°C) through a cooling jacket and by pulsing after 30 s of sonication. Temperature moderation by pulsing was carried out only for 60 and 120 s treatment at each amplitude, where sonication was on for 30 s and off for 30 s. A schematic of sonication set-up is shown in Figure 1. After sonication, the slurry was extracted as described below.

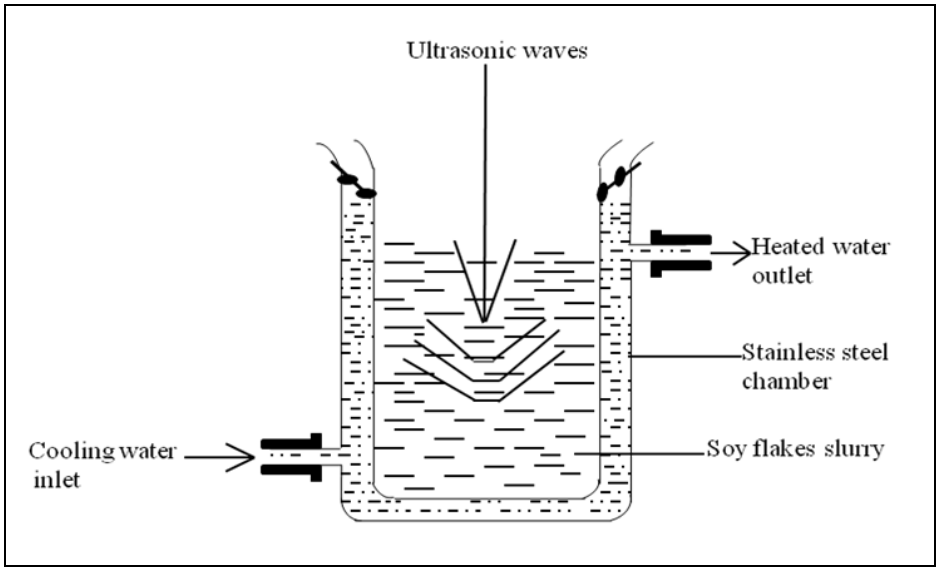


Figure 1: Experimental set-up for temperature moderated sonication system

3.3.4 Extraction procedure

The details of sonication and soy protein isolate (SPI) preparation are summarized in Figure 2. Sonicated soy flakes slurry samples were dispersed in additional 500 ml of warm

water (~65°C) in a beaker to maintain a flakes-to-water ratio of 1:10 (w/w basis). Controls were similarly prepared from unsonicated (i.e., 0 s sonication) soy flakes. The initial pH of approximately 6.2 was raised to 8.5 by adding 2N NaOH. The slurry was placed in a 60^{o}C water bath and stirred for 30 min and the pH was maintained at 8.5. The samples were then centrifuged at 10, 000 g for 20 min at 20°C. The supernatant was collected for protein and sugar determination. All experiments were performed in triplicate.

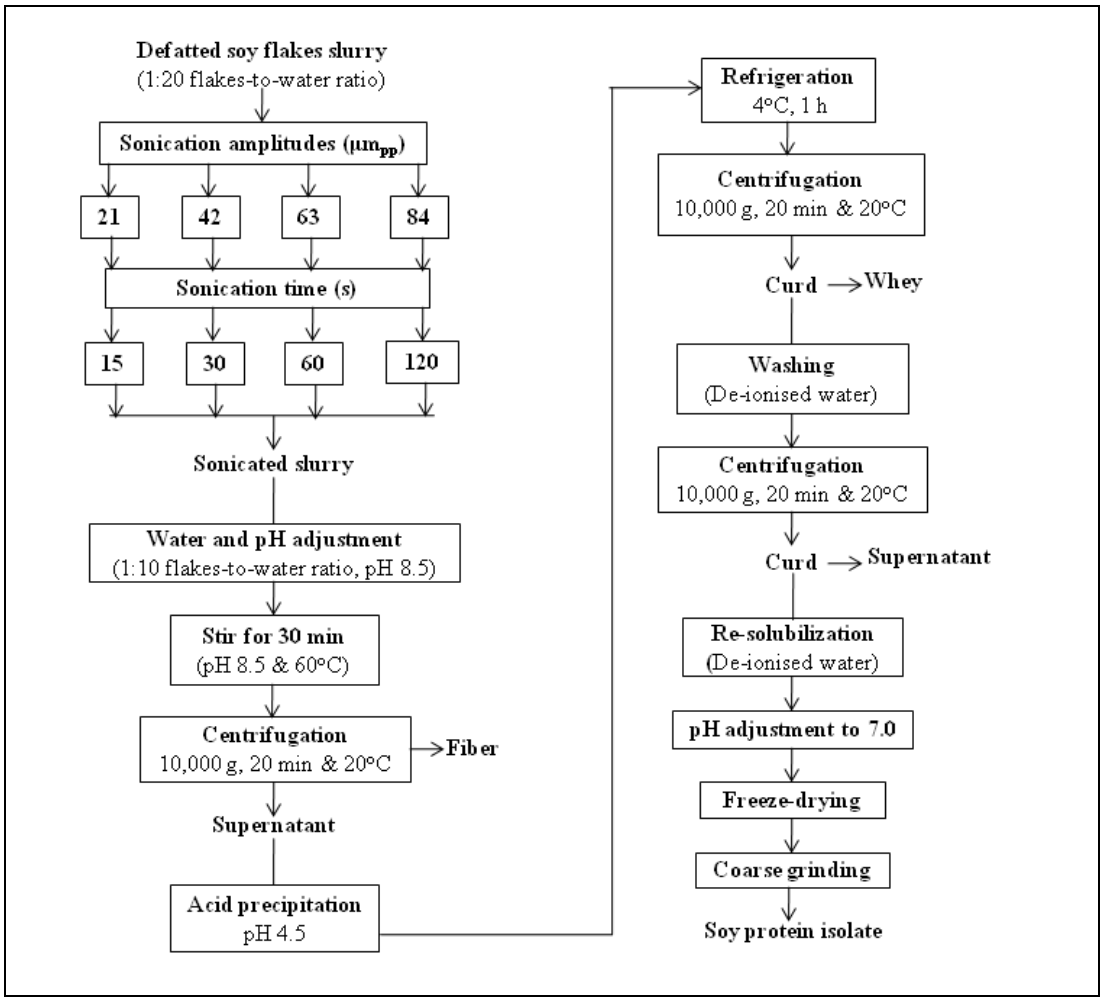


Figure 2: Condition applied to the defatted soy flakes to obtain supernatant and summary of the steps to obtain soy protein isolate

3.3.5 Protein determination

Protein content in the supernatant and soy flakes was determined by a combustion-type Rapid Nitrogen Analyzer Rapids N III (Elementar Americas, Inc., Mt. Laurel, NJ, USA) according to the Dumas method (AOAC, 1995). Liquid samples were collected in tin capsules and sealed. Aspartic acid (A9, 310-0; Sigma-Aldrich, St Louis, MO, USA) was used as the nitrogen reference calibration. Oxygen dosing for optimal combustion was selected based on sample type. Dosing for blanks was 50 ml O_2/min, whereas dosing for samples was 150 ml O_2/min. After analysis of 15 samples, an aspartic acid run-in was analyzed to verify satisfactory system performance. Protein content was calculated from the nitrogen content of the material using a nitrogen conversion factor of 6.25. The yield of protein was calculated from the measured protein content in the recovered supernatant relative to the total protein content of starting flakes. The protein yield in the sample was calculated as:

$$\text{Protein yield (\%)} = \left\lfloor \frac{\text{weight of protein in supernatant (g)}}{\text{weight of protein in defatted flakes (g)}} \right\rfloor \text{x } 100 \qquad \text{[Eq. 1]}$$

3.3.6 Total sugar determination

Total sugar in the aqueous extract was determined by a phenol sulfuric acid assay (Crawford and Pometto, 1988). Appropriately diluted supernatant samples (1.0 ml) were directly transferred into a test tube (1.5 x 15 cm). Phenol reagent, 5% (v/v) (1.0 ml) was added followed by 5 ml of concentrated sulfuric acid using a fast-flow pipette. The solution was mixed immediately by shaking and allowed to cool down to room temperature. After 30 min, the absorbance at 490 nm was determined using a spectrophotometer

(ThermoSpectrogenic Genesys 2 model W1APP11, Rochester, NY, USA). Total sugar concentrations were calculated using a D-glucose standard curve. The total sugar release was calculated as:

$$\text{Total sugar release} = \left[\frac{\text{weight of total sugar in supernatant (g)}}{\text{100 g of defatted soy flakes}} \right] \qquad \text{[Eq. 2]}$$

All samples were analyzed in triplicate.

3.3.7 Particle size distribution

Slurry obtained after sonication was passed through a mesh of 1,000 µm pore size. The filtered samples were then analyzed for particle size distribution using a particle size analyzer (Mastersizer 2000 S, Malvern Inc., Worcestershire, United Kingdom). The particle size distributions were calculated using a refractive index ratio of 1.47. The sonicated slurry was diluted with water to obtain an obscuration of about 12-14%. All analyses were performed in triplicate.

3.3.8 Scanning electron microscopy

The slurry recovered after sonication was fixed with 3% glutaraldehyde (w/v) and 2% paraformaldehyde (w/v) in 0.1 M cacodylate buffer (pH 7.2) for 48 h at 4°C. Samples were rinsed 3 times in this buffer and post-fixed in 1% osmium tetroxide for 1 h, followed by two 5-min washes in buffer. The samples were then dehydrated in a graded ethanol series up to 100% ultra-pure ethanol followed by substitution into hexamethyldisilazane and allowed to air dry. When dried, the samples were placed on carbon adhesive coated aluminum stubs,

sputter coated (Denton Desk II sputter coater, Denton Vacuum, LLC, Moorestown, NJ, USA) with palladium/gold alloy (60/40), and imaged using a JEOL 5800LV SEM (Japan Electron Optics Laboratory, Peabody, MA, USA) at 10 kV with a SIS ADDA II for digital image capture (Soft Imaging Systems Inc., Lakewood, CO, USA).

3.3.9 Ultrasound energy dose calculation

Ultrasound energy dose is the amount of energy supplied per unit volume of defatted soy flakes slurry and is expressed as Ws/ml or kWs/ml (J/ml or kJ/ml). Ultrasonic dose is mainly used to express the energy input for the disintegration of sample on a volume basis assuming the solid content of the sample remains fairly constant. The ultrasonic dose was calculated as,

$$P_{avg} = \left[\frac{P_{initial} + P_{final}}{2} \right] - P_{air} \qquad \text{[Eq. 3]}$$

$$Q_{avg} = \frac{P_{avg}}{V} \qquad \text{[Eq. 4]}$$

$$E\text{-}_{density} = Q_{avg}. \, t \qquad \text{[Eq. 5]}$$

Where P is the electrical power (W) as displayed by the power system, Q is average power density (W/ml), V is the volume of sample (ml), t is the sonication time (s), E-*density* is the energy density (J/ml). $P_{initial}$ and P_{final} were the initial and final powers, respectively, indicated by the power supply system. P_{air} is the power required to run the system in an unloaded condition.

3.3.10 Statistical analysis

The data were analyzed by using one-way Analysis of Variance (ANOVA) and means for each pair were compared at 5% significance level by using Student's t-test, in the JMP system (Version 6.0.3, SAS Institute INC., Cary, NC, USA).

3.4 Results and discussion

3.4.1 Scanning electron microscopic (SEM) examination

SEM examination of sonicated soy flakes slurry sample was carried out for three different amplitude levels; low (42 μm_{pp}), medium (63 μm_{pp}) and high (84 μm_{pp}). At each amplitude level, samples were sonicated for 15, 60 and 120 s. The SEM images of defatted slurry samples at low, medium and high amplitudes are shown in Figures 3, 4 and 5 along with non-sonicated control samples, respectively. SEM for the control group showed intact cells and presence of intracellular material (Figures 3a, 4a and 5a). Several micro-fractures appeared in the soy flakes following ultrasound pretreatment. The severity of disintegration improved progressively with increase in amplitude and sonication time. At high amplitude (84 μm_{pp}), there was near complete rupture of defatted soy flakes cell with large numbers of fragmented cell matter. The SEM study showed particulate surface material with a sponge-like texture rather than prominent protein bodies.

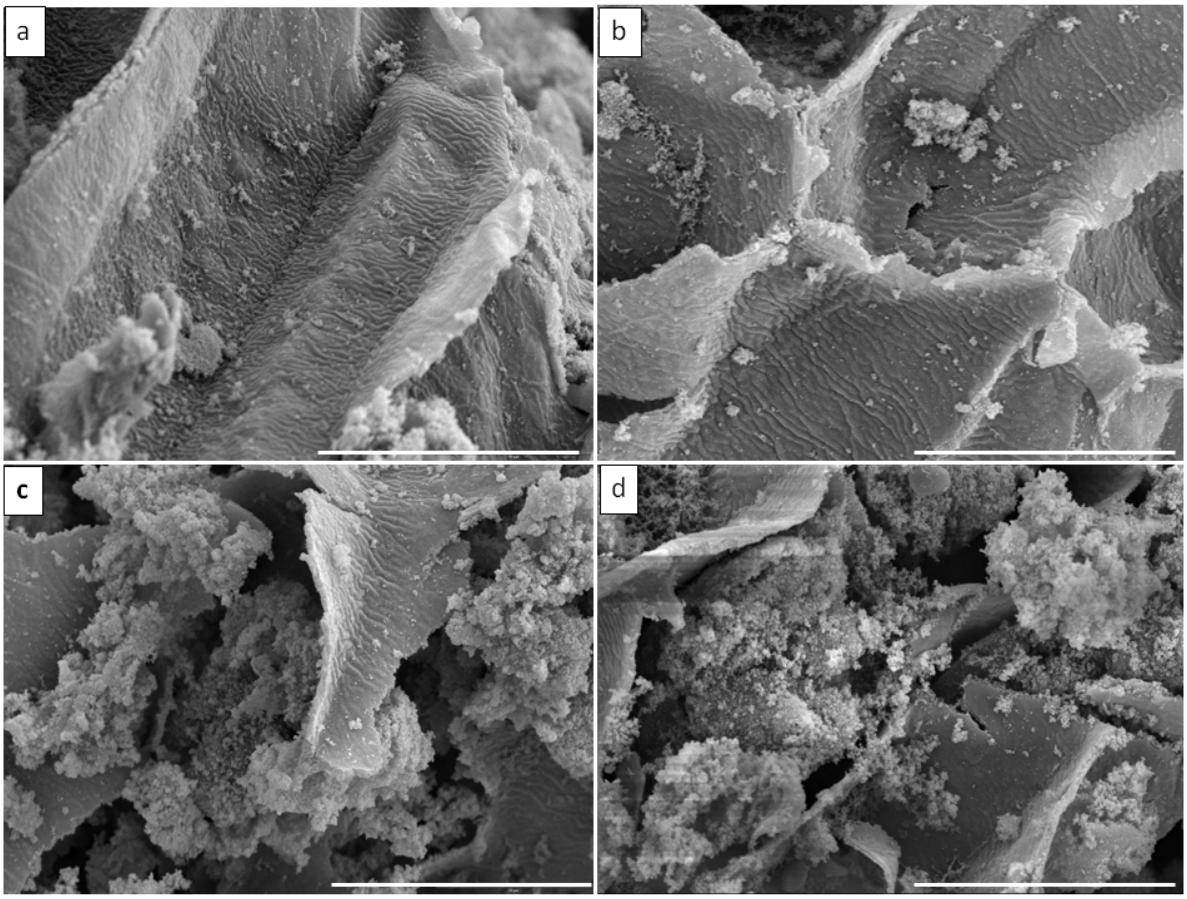


Figure 3: Scanning electron microcopy images of defatted soy flakes at low amplitude (42 μm_{pp}): (a) control (0 s); (b) 15 s; (c) 60 s; (d) 120 s
(bars on diagrams represent 10 µm)

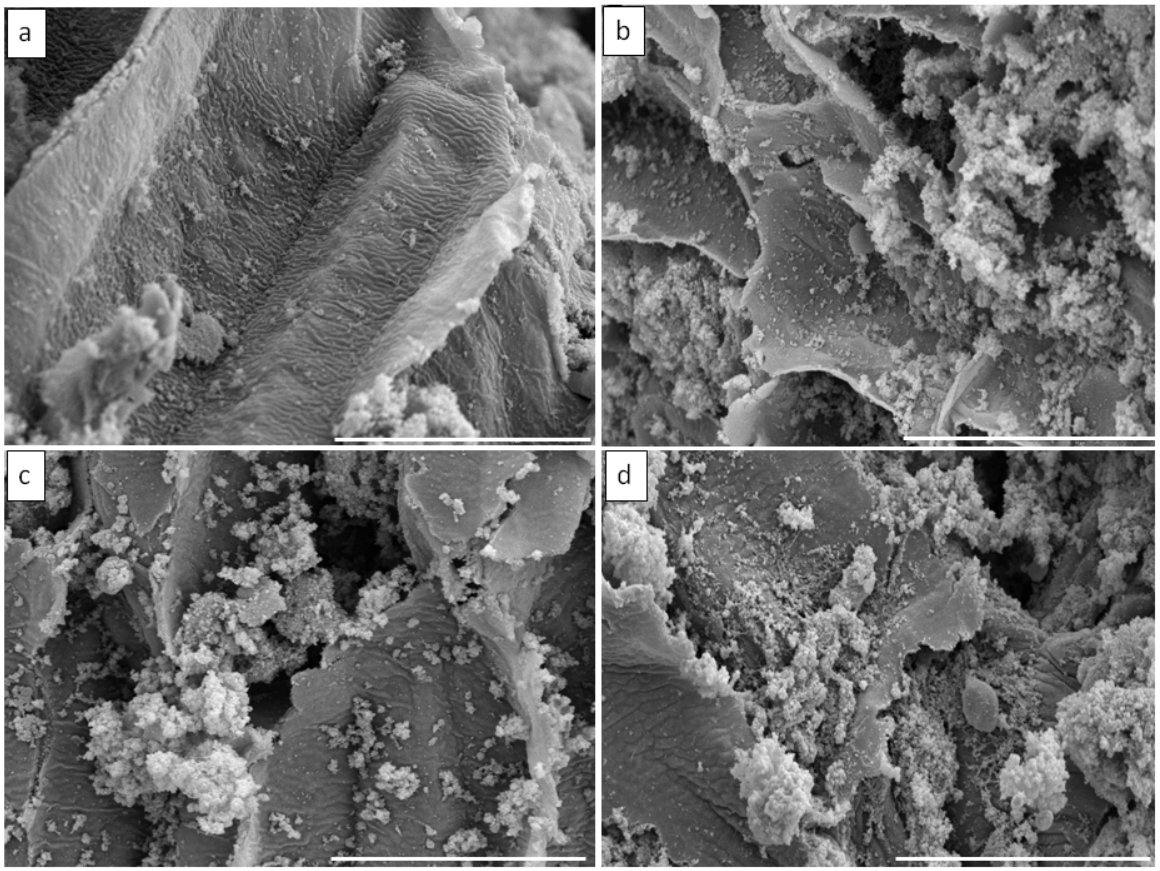


Figure 4: Scanning electron microcopy images of defatted soy flakes at medium amplitude (63 μm_{pp}): (a) control (0 s); (b) 15 s; (c) 60 s; (d) 120 s
(bars on diagrams represent 10 µm)

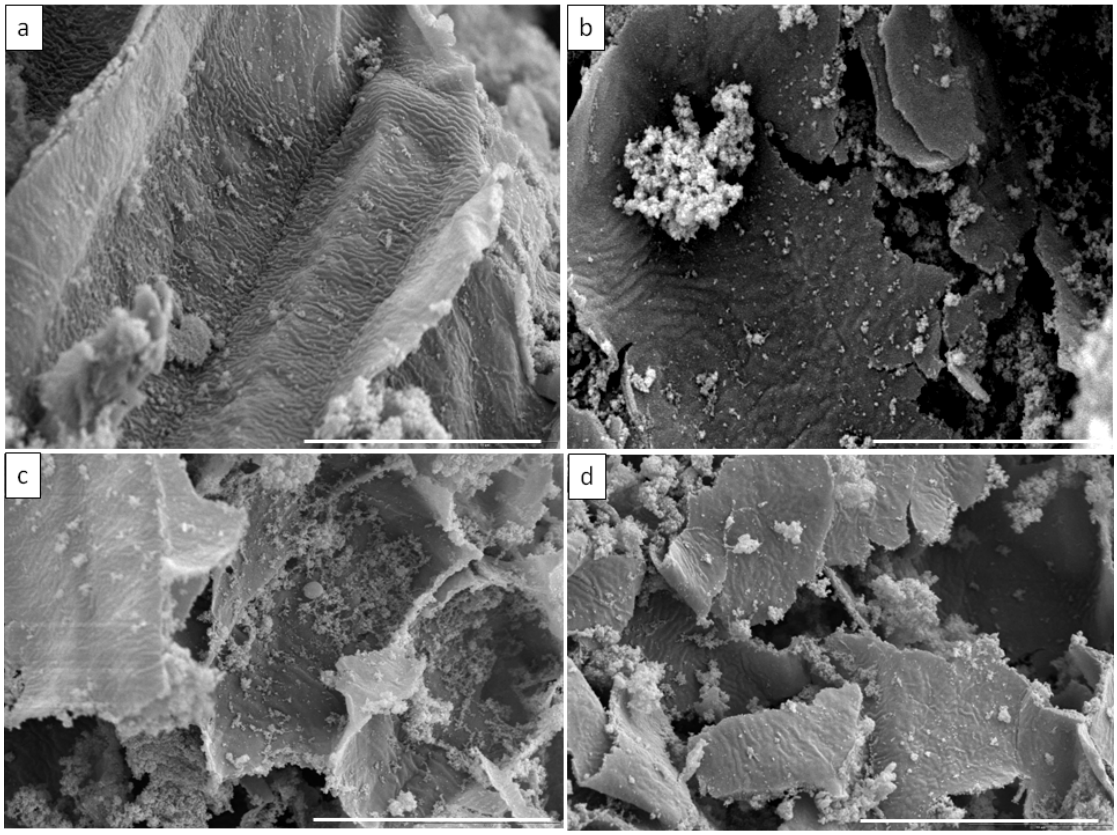


Figure 5: Scanning electron microcopy images of defatted soy flakes at high amplitude (84 µm): (a) control (0 s); (b) 15 s; (c) 60 s; (d) 120 s
(bars on diagrams represent 10 µm**)**

3.4.2 Particle size distribution

For particle size distribution, the soy flakes samples were subjected to low- (42 μm_{pp}), medium- (63 μm_{pp}), and high- (84 μm_{pp}) amplitude sonication. For all amplitudes, the samples were sonicated for 15, 60, and 120 s without temperature moderation. The peak of particle size distribution curve shifted from 1,000 µm to approximately 90 µm when soy flakes were sonicated at high amplitude (84 μm_{pp}) during a longer sonication period of 2 min (Figure 6c). Particle size reduction was directly related to sonication amplitude and time. For example, at low amplitude level and a longer sonication period of 2 min (Figure 6a), the peak at 1,000 µm decreased whereas the 10 µm peak increased. The soybean protein body diameter was reported to range from 8-10 µm but could range from 2 to 20 µm (Snyder and Kwon, 1987). Thus the particle size of 10 to 20 µm resulting from ultrasound treatment could be protein bodies.

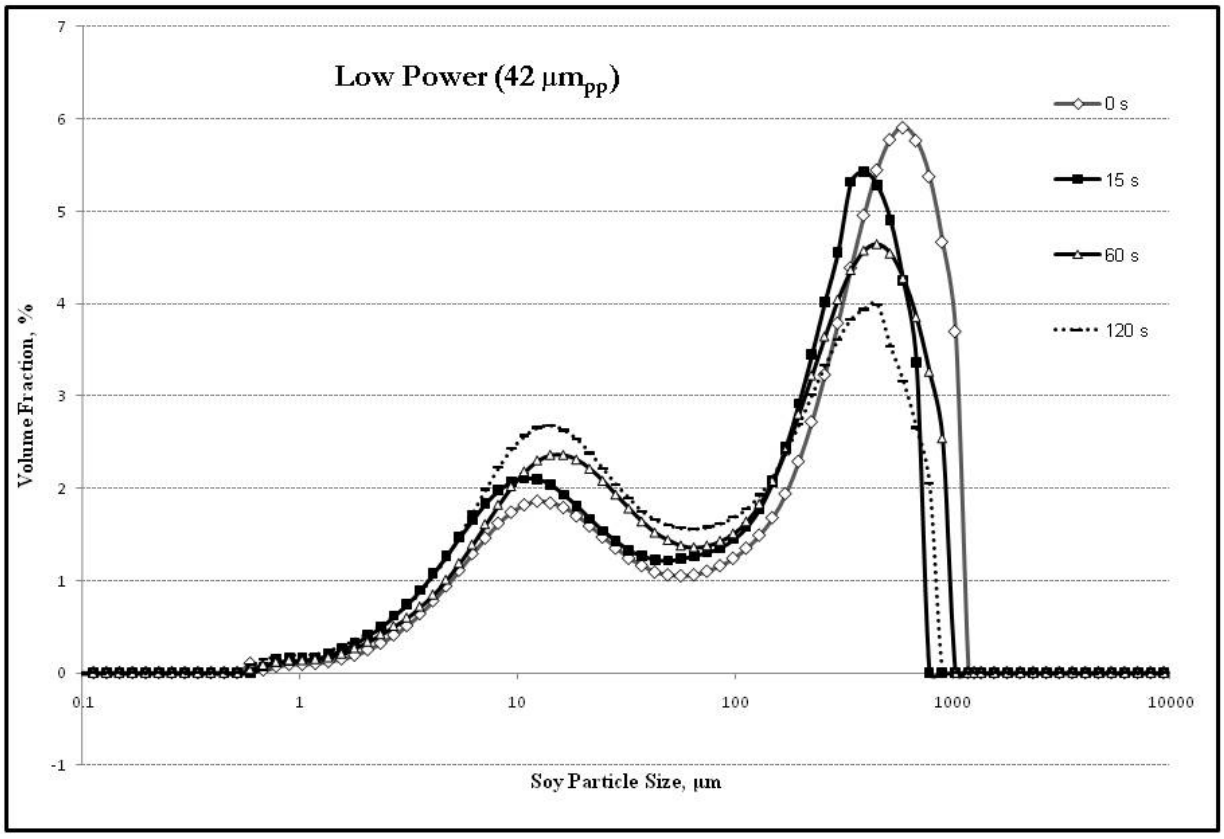


Figure 6a: Particle size distribution of soy flakes slurry treated at low amplitude (42 μm$_{pp}$)

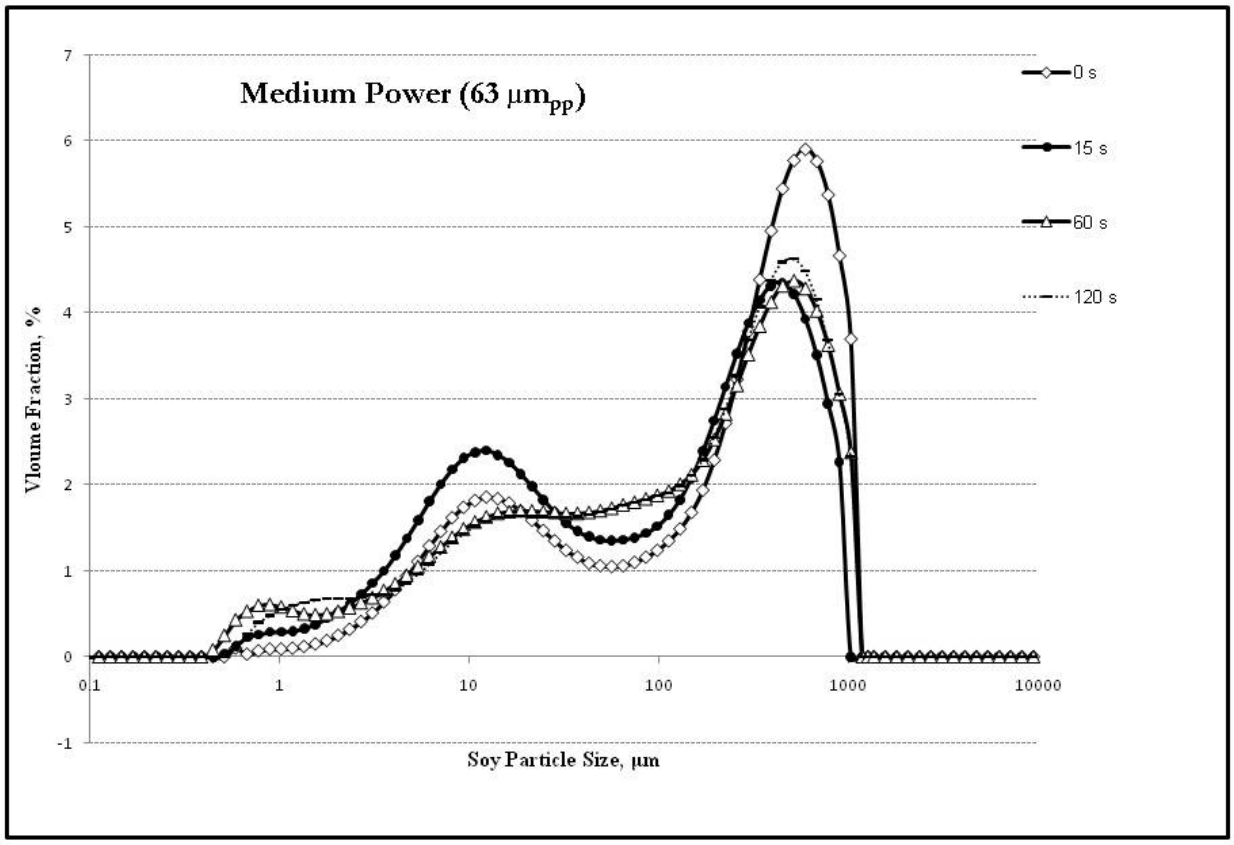


Figure 6b: Particle size distribution of soy flakes slurry treated at medium amplitude (63μm$_{pp}$)

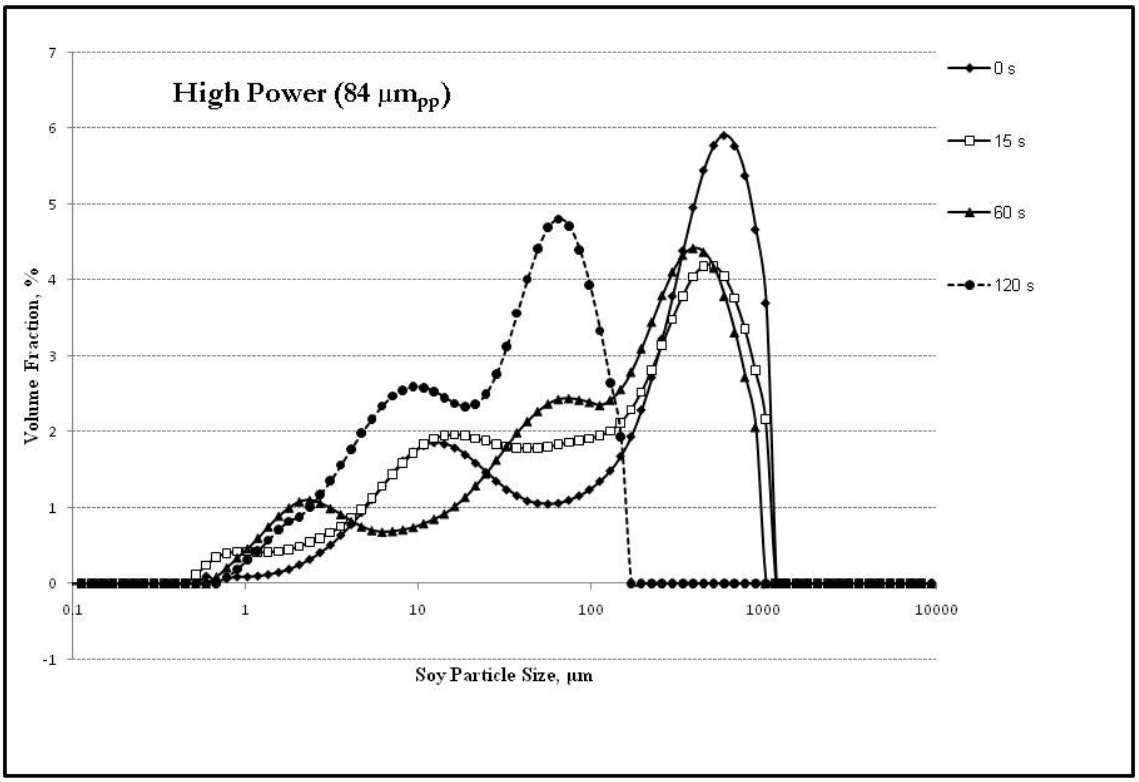
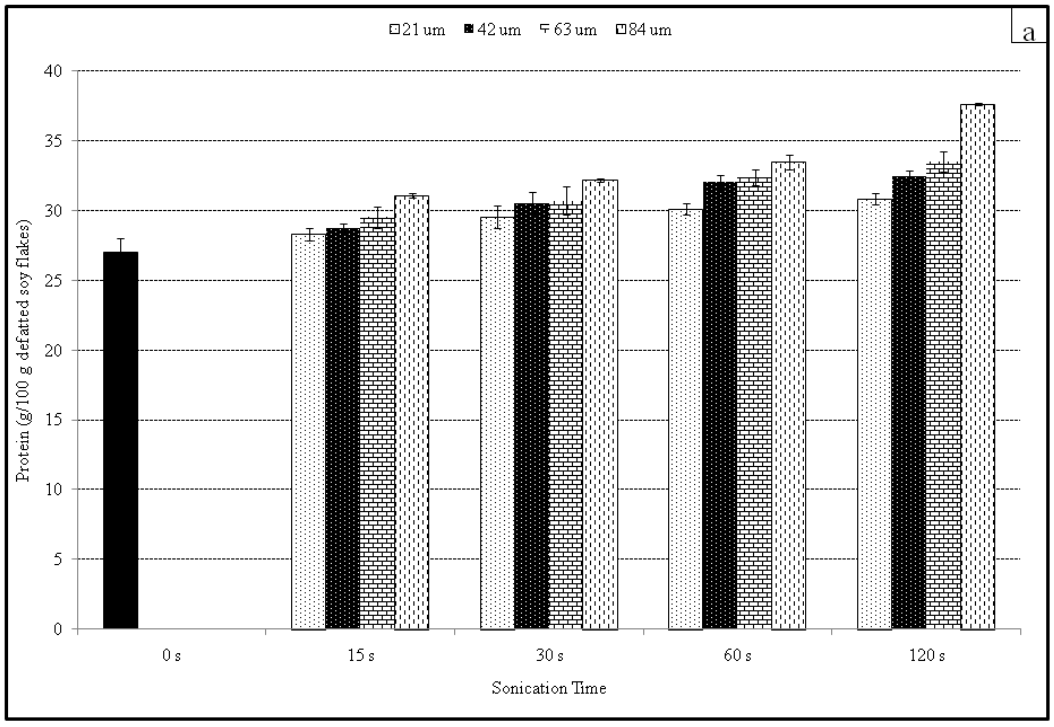


Figure 6c: Particle size distribution of soy flakes slurry treated at high amplitude (84μm$_{pp}$)

3.4.3 Effect of ultrasound on protein release

The effect of ultrasonic pretreatment, with and without temperature moderation, on protein release is shown in Figure 7a and Figure 7b, respectively. Protein released is generally proportional to sonication time both with and without temperature moderation. After 120 s of sonication at amplitude 84 μm$_{pp}$, the protein release increased with respect to the control by 40% and 46% with and without temperature moderation, respectively. There was a small decrease in protein release in samples obtained with temperature moderation in

comparison with samples obtained without temperature moderation. Although there was an increase in protein release with increasing amplitude from (21 μm_{pp}), low (42 μm_{pp}), medium (63 μm_{pp}) to high amplitude (84 μm_{pp}) inputs, the trend of protein release was not linear. Protein release improved by 13, 23, 27 and 46%, respectively, with respect to control for 2 min sonication time at four different amplitude levels, i.e, very low (21 μm_{pp}), low (42 μm_{pp}), medium (63 μm_{pp}) and high (84 μm_{pp}) respectively. The treatment at amplitudes of 21 μm_{pp}, 42 μm_{pp} and 63 μm_{pp} gave similar protein releases. By contrast, the protein contents at 84 μm_{pp} and 2 min sonication were statistically different (p<0.05) in both temperature moderated and non-moderated systems. In addition to the structural damage caused by the treatment, the reduction in size of the flakes, as seen in Figure 6c, may have contributed to the increased protein extraction yield at high amplitude and 2 min of sonication. This finding was in close agreement with that of Russin et al. (2007).



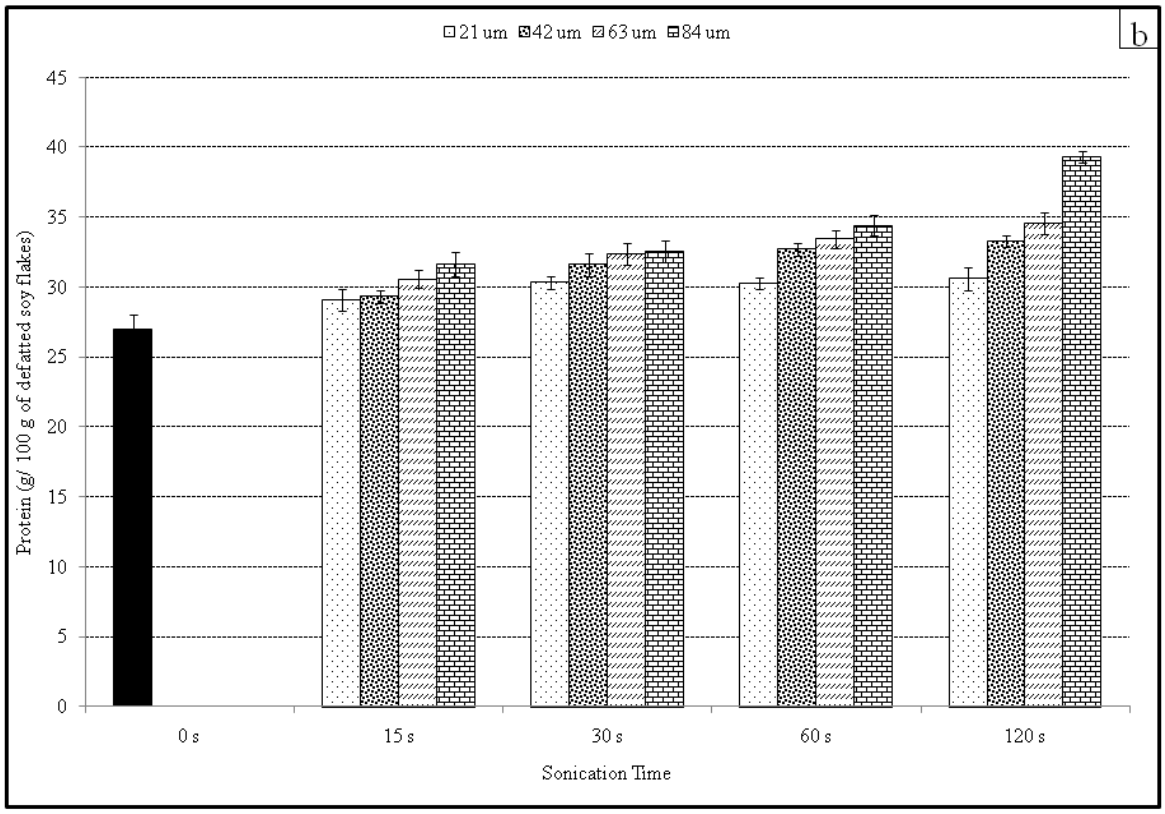


Figure 7: Protein yield at various sonication conditions: (a) with temperature moderation system; (b) without temperature moderation system (n=3)

3.4.4 Effect of ultrasound on total sugar release

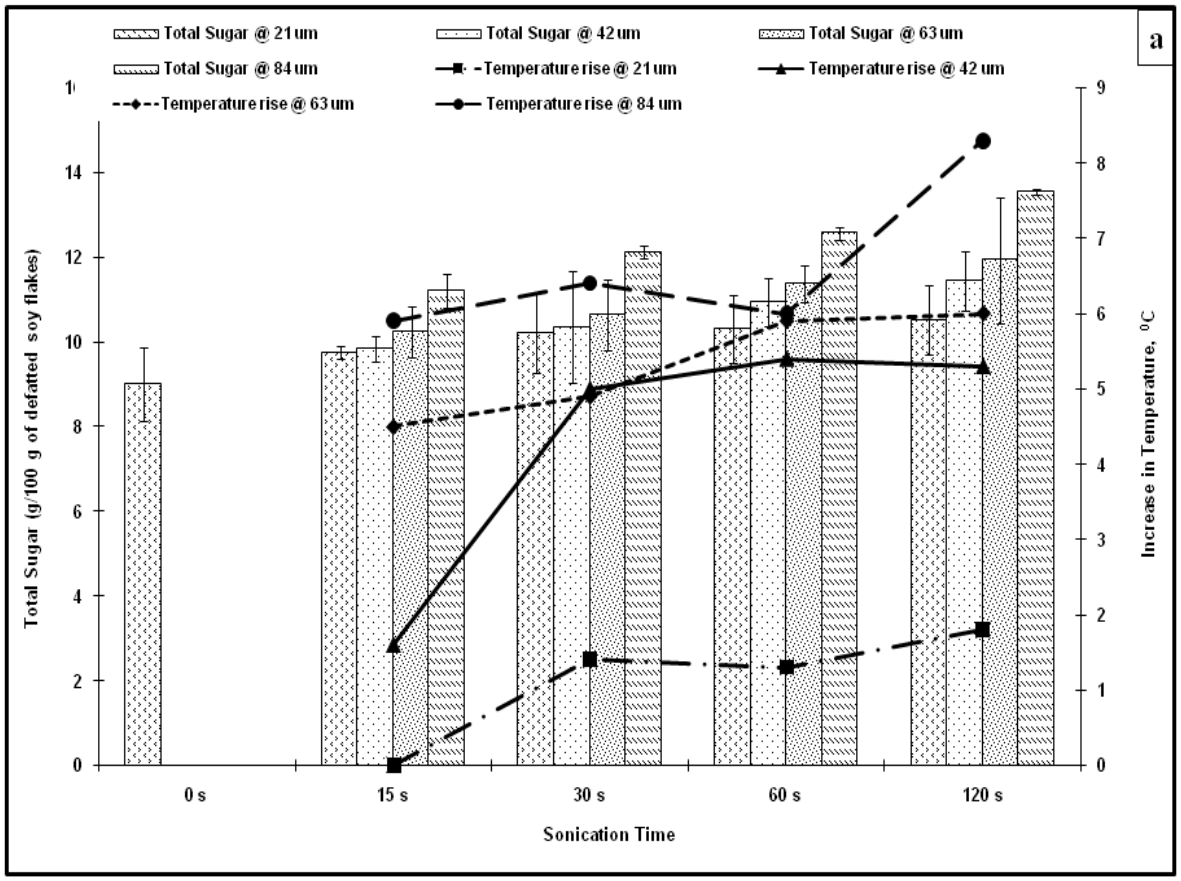
Total sugar release for control and sonicated samples at different sonication amplitudes and times, with and without temperature moderation, is shown in Figures 8a and 8b, respectively. Total sugar release was generally proportional to sonication time. However, a significant difference (p<0.05) in total sugar release was obtained only between 15 and 120 s of sonication time. The sugar release values at four different amplitude levels increased significantly (p<0.05), with increased amplitude settings. The highest sugar release of approximately 13 ±0.3 g/100 g of defatted soy flakes was obtained after 120 s of sonication at high amplitude, i.e., 84 μm_{pp}. The highest sonication amplitude setting increased the total

sugar release by 45 and 50%, respectively, in temperature moderated and non-moderated systems, respectively. These findings have economical implications, as the sugar-rich soy whey generated during soy protein isolate production could be used as a fermentation media for the production of high-value products such as nisin, enzymes, lactic acids, and probiotics. It could also be expected that the insoluble residue would contain less flatulence-causing sugars, as more sugar is extracted with water. The sugar profile needs to be determined to verify this hypothesis. The decrease in carbohydrate content could enhance the value of the insoluble fraction as a food ingredient.

Soy protein extraction yield is affected by the extraction temperature (Rickert et al., 2004). We therefore determined the temperature history during treatment. Figure 8a and 8b show the increase in temperature (°C) of the defatted soy flakes slurry during sonication with and without a temperature moderation system. The temperature history showed that the maximum increase in temperature was 38°C when temperature was not moderated and the increase was only ~ 9°C when temperature was moderated. This temperature fluctuation during sonication, however, did not significantly affect the total protein and total sugar release. Thus, the increase in protein and sugar release was not due to the thermal effect of sonication but was due to the cellular rupture and reduction in particle size. Extensive cellular disruption was evident from SEM examination and lends support to our hypothesis of cell disruption and resulting release of cell constituents into the aqueous phase.

Ultrasound technology provides a unique opportunity to improve the extraction of protein and sugar from defatted soy flakes. It is more likely that the cavitation effect was the major governing factor in improving the protein and sugar release following sonication. The

shear forces generated due to cavitation, as well a shock waves, physically disrupt the plant cell walls thereby facilitating the release of extractable compounds (Vinatoru, 2001).



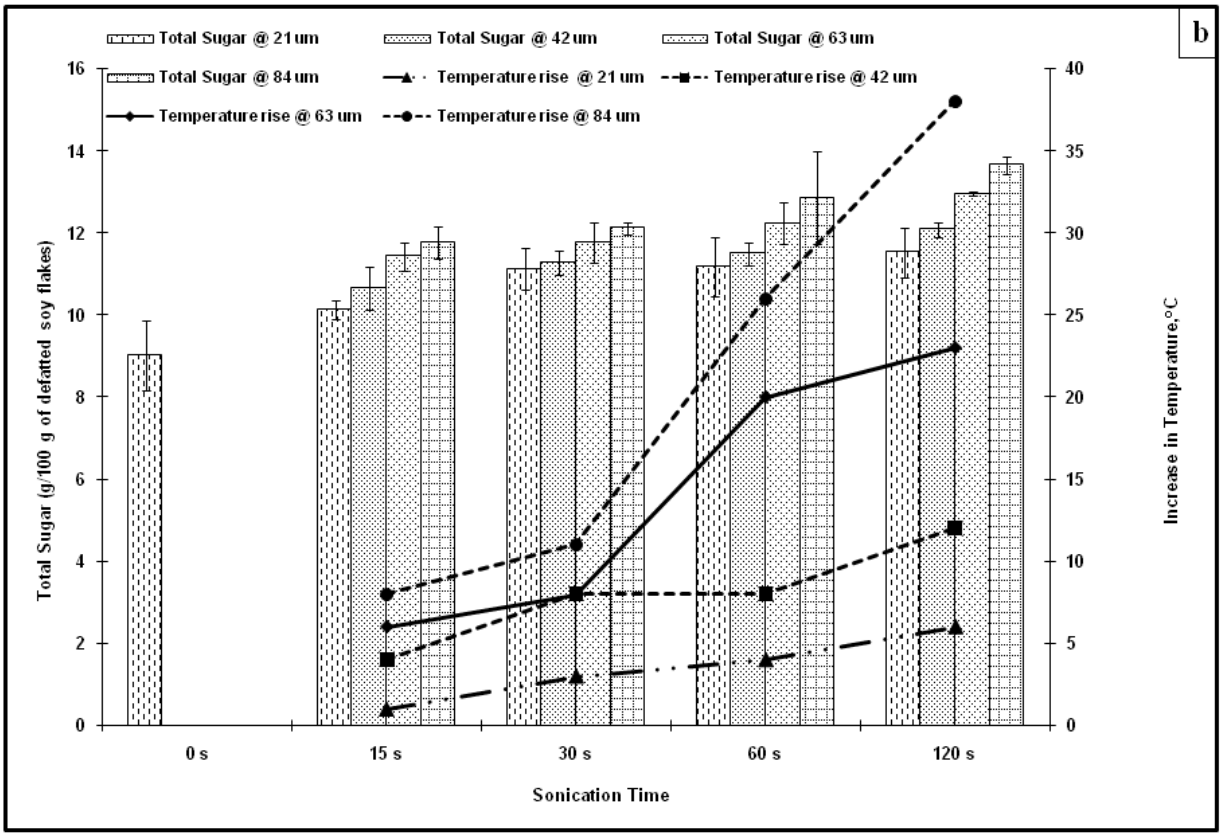


Figure 8: Total sugar yield at various sonication conditions: (a) with temperature moderation system; (b) without temperature moderation system (n=3)

3.4.5 Ultrasonic energy requirement

Ultrasonic disintegration is affected by several factors; however, energy is one of the key determinants in selecting the ultrasound system for commercial application. Thus, it is essential to correlate the protein and sugar release with ultrasonic energy input for scale-up of the process to achieve the maximum recovery with minimum energy input. We estimated the ultrasonic density (W/ml) and ultrasonic energy (J/ml) at four different power levels (amplitude settings) and 2 min sonication time using Eqs. 3 to 5. The ultrasonic densities,

energy inputs and amounts of protein and sugar released at different power levels and 2 min sonication time are summarized in Table 1.

Table 1: Ultrasonic energy inputs at different sonication conditions and total sugar and protein release at respective energy inputs (n=3)

Pretreatment	Dissipated power (Watt)	Ultrasonic density (W/ml)	Energy dose (J/ml)	Protein yield (%)	Total sugar (g/100 g of defatted soy flakes)
No Sonication	0	-	-	53.50±0.31	9±0.86
	Very low: 154	0.30	36.00	61.5±0.56	11.51±0.81
	Low: 438	0.87	104.40	66 ± 0.38	12.07±0.69
	Medium: 765	1.53	183.60	69±0.71	12.95±1.48
	High: 1280	2.56	307.20	78±0.40	13.57±0.07

As evident from Eq. 5, ultrasonic energy is a function of sonication power, time and volume. Other variables such as substrate properties and pressure also effect energy requirements, but it was assumed these factors remained constant. It was observed that protein and sugar release was loosely proportional to dissipated energy; however, the relationship was not directly proportional. The results showed a protein yield of 61% and 78% and sugar release of 11.5 and 13.5 g per 100 g of defatted soy flakes at an energy input of 36 J/ml and 307 J/ml respectively. Thus, relative to energy efficiency, low power (amplitude setting) setting is more energy efficient compared to a high power setting (amplitude setting), in spite of higher protein and sugar releases. In order to fully realize the economic benefits of this technology, a detailed economic analysis should be made based on protein and sugar release obtained from a continuous-flow system. As sonication exposure is directly related to the flow rate (L/h), design and incorporation of large continuous-flow

chambers may reduce the cost per volume of the material to be treated. Studies are currently underway to examine the efficacy of ultrasound system in a continuous-flow mode.

3.5 Conclusion

The ultrasonic pretreatment of defatted soy flakes resulted in a nearly 10-fold reduction in particle size. SEM images showed the disruption of the cell wall and release of intracellular materials. Improvement in protein and sugar release in water extracts was proportional to the sonication time and amplitude. The total sugar release was improved by as much as 50% and total protein by 46% with respect to controls. Heat generated during sonication did not affect the protein and sugar release, indicating the cell rupture caused by the ultrasound to be the main mechanism for better separation and product yield. Results obtained in this study will have significant implications in terms of quality of human food and animal feed. Ultrasonication has the potential to improve protein and sugar extraction compared to conventional extraction systems, and could significantly reduce the cost of extraction in traditional soy processing plants.

3.6 Acknowledgements

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and the Bessey Microscopy Facility for scanning electron micrographs. The defatted soy flakes sample was provided by Cargill through CCUR.

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CHAPTER 4: FUNCTIONAL PROPERTIES OF SOY PROTEIN ISOLATE PRODUCED FROM ULTRASONICATED DEFATTED SOY FLAKES

A paper published in *Journal of American Oil Chemists' Society*

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4.1 Abstract

This study aimed to determine the effect of pretreating defatted soy flakes with ultrasound on soy protein isolate (SPI) yield and functionality. Defatted soy flakes dispersed into water (16% w/w) were sonicated for 30, 60 and 120 s at ultrasonic amplitudes of 21 and 84 μm_{pp} (peak to peak amplitude in μm), representing low and high power, respectively. The power densities were 0.30 and 2.56 W/mL, respectively. The SPI yield increased by 13 and 34%, after sonication for 120 s at low and high power, respectively. The sonication of defatted soy flakes for 120 s at the higher power level improved the SPI solubility by 34% at pH 7.0, while decreasing emulsification and foaming capacities by 12% and 26%, respectively when compared to the control SPI. Rheological behavior of the SPI was also

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modified with significant loss in consistency coefficient due to sonication. Some of these results could be explained by the loss of the protein native state with increased sonication time and power.

Keywords: Defatted soy flakes; Ultrasound; Functional properties; Soy protein isolate

4.2 Introduction

Soy protein isolate (SPI) is a commercial soy protein product having at least 90% protein [dry basis (db)] [1]. SPI holds a unique place in the human diet, not only because of its use as a low-cost substitute for animal food proteins, but also because of the health benefits associated with soy protein consumption, including blood cholesterol reduction and cardiovascular disease prevention [2]. SPI can also be used in non-food applications including the production of biodegradable plastics and paper coatings and sizing [3]. The versatile uses of SPI can be attributed to the wide range of functional properties that SPI can confer to a food product [4, 5]. These properties are affected by the intrinsic, extrinsic and process parameters [6], and could be modified by using alternative processing techniques [5]. Extensive research has been done on enzymatic [7, 8, 9], mechanical [5] and thermal [1, 10] modifications of soy proteins to improve their functional properties.

The first step in SPI production is an alkaline aqueous extraction of the defatted soy flakes. This extraction step is far from being efficient as about half of the available protein remains in the insoluble fraction [9]. Microwave heating [11], enzymatic [9] and chemical modifications were applied to the defatted soy flakes, improving the protein extraction yield

by up to 50% during the aqueous alkali extraction. High-power ultrasound has recently been reported as a powerful method to increase extraction of intracellular compounds from plant materials, including oil from soy flakes [12], water-soluble polysaccharides from roots of valerian (*Valeriana officinalis L*.) [13] and saponin from ginseng [14]. While high-power ultrasound seems to be a promising technology in improving the extraction efficiency of plant material, there is little information as to whether ultrasound-assisted extraction alters the physicochemical and biological properties of plant extract, especially proteins. During ultrasonication, mechanical effect occurs; therefore protein might undergo structural changes [15], which could result in changes in SPI functional properties.

The objectives of this study were firstly, to determine the effects of sonication power and time applied on the SPI yield. Secondly, the functional properties and some structural properties of the SPI obtained from sonicated defatted soy flakes were determined.

4.3 Experimental procedures

4.3.1 Defatted soy flakes

Hexane-defatted soy flakes were obtained from the Center for Crops Utilization Research (CCUR), Iowa State University (Ames, IA, USA). The soy flakes were stored in air-tight plastic bags at 4 °C until use. The moisture content of the soy flakes was 5.2%.

4.3.2 Ultrasound treatment

Defatted soy flakes (100 g) were dispersed into 500 mL tap water in a customized 1.2-L stainless steel sonication chamber. The samples were treated in batch-mode using a Branson 2000 Series bench-scale ultrasonic unit (Branson Ultrasonics Corporation, Danbury, CT, USA), with a maximum power output of 2.2 kW. A standard 20-kHz half wavelength titanium horn with a gain of 1:2.8 and a booster with a gain of 1:1.5 were used. Samples were sonicated at two different amplitude (power) levels, 21 and 84 μm_{pp} (peak to peak amplitude in µm), and designated as low and high power level, respectively. The power levels were changed by varying the amplitude at the horn tip through pulse-width modulation voltage regulation to the converter. For each power input (amplitude), the samples were sonicated for 30, 60 and 120 s without temperature control. The temperature of the slurry was 25, 26 and 28 °C, after sonication at a low power level for 30, 60 and 120 s, respectively. At high power level, the temperature of slurry was 31, 48 and 65 °C after sonication for 30, 60 and 120 s, respectively.

4.3.3 Extraction and SPI procedure

After sonication, 500 mL of water at 65 °C was added to the flakes slurry to obtain a flakes-to-water ratio of 1:10 (w/w). Controls were similarly prepared from unsonicated soy flakes (Fig. 1). The initial pH of approximately 6.2 was raised to 8.5 by adding 2N NaOH. The slurry was placed into a 60 °C water bath and stirred for 30 min while maintaining a constant pH. The sample was then centrifuged at 10,000 x g for 20 min at 20 °C. The supernatant was acidified to pH 4.5 with 2N HCl and stored at 4 °C for 1 h, before

centrifuging at 10,000 x g for 20 min at 20 °C. The supernatant was discarded while the curd (precipitate) was collected. The precipitate was washed two times with distilled water and centrifuged again at the same condition. The washed precipitate was dispersed into distilled water and neutralized to pH 7.0 with 2N NaOH. The neutralized curd was freeze-dried, powdered, sealed in plastic bag and stored at 4 °C until use. These procedures were performed in triplicate.

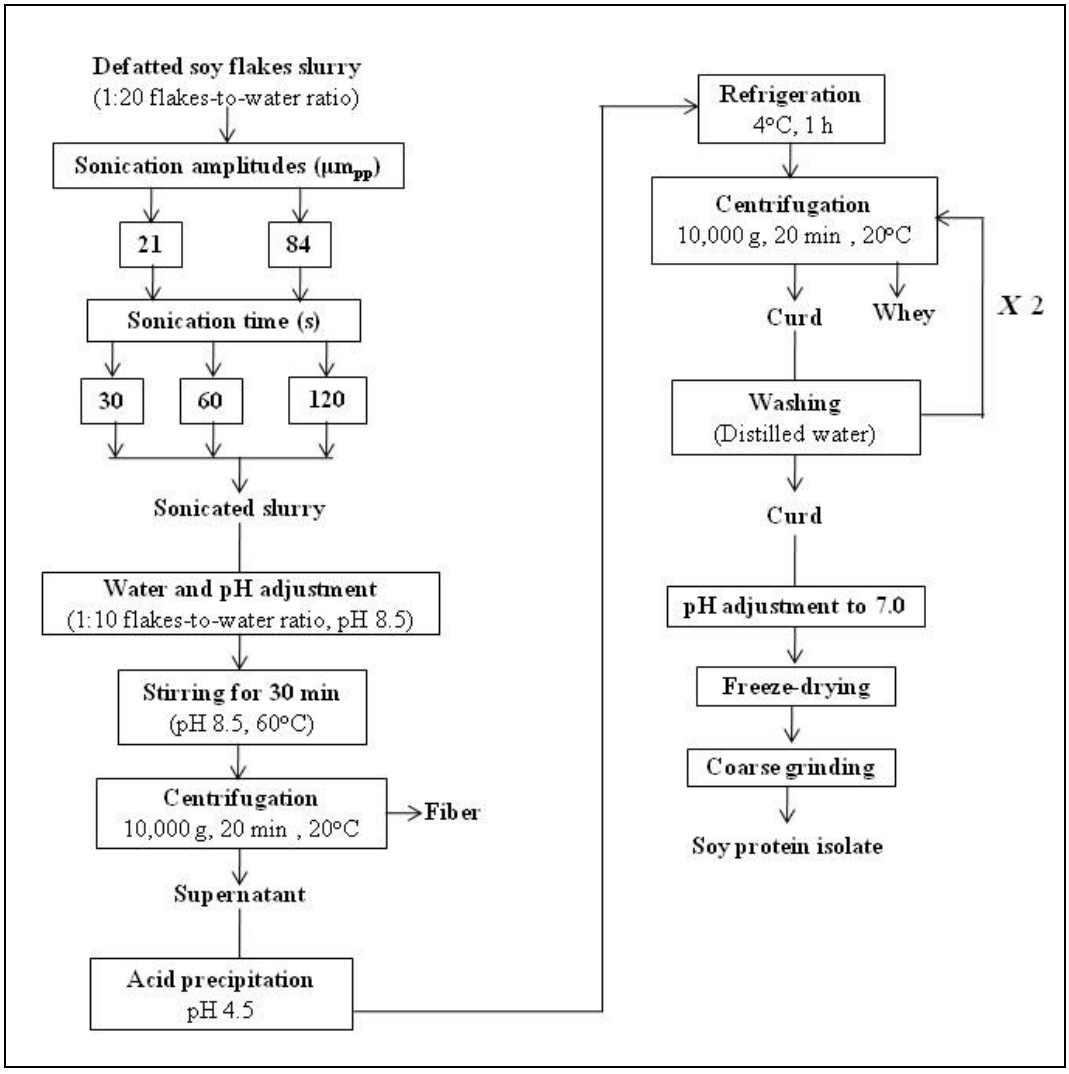

Figure 1: Flow chart of sonication condition and soy protein isolates production

4.3.4 Protein and moisture determination

Protein contents of the SPI and soy flakes were determined with a Nitrogen Analyzer (Rapids N III, Elementar Americas, Inc., Mt. Laurel, NJ, USA) as per the Dumas method [16] with aspartic acid (A9, 310-0; Sigma-Aldrich, St. Louis, MO, USA) as the nitrogen reference calibration. Crude protein content was calculated from the nitrogen content of the material using a nitrogen conversion factor of 6.25.

The protein yield was calculated as:

$$\text{Protein yield}\,(\%) = \left[\frac{\text{weight of protein in isolates}\,(\text{g})}{\text{weight of protein in defatted flakes}\,(\text{g})}\right] \text{x}\,100$$

For moisture determination, approximately 1.0 g of sample was heated in a vacuum-oven at 110 °C for 3 h and the moisture content then determined gravimetrically [17].

4.3.5 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed on isolated soy proteins obtained from sonicated and non-sonicated defatted soy flakes using SDS-Tris-glycine buffer system with Bio-Rad Ready Gels (Mini Protean II Gel, Bio-Rad Inc. CA). Protein samples were diluted to a concentration of 1 mg/mL in a solution containing 125 mM THAM, 5.0 M urea, 2% β-mercaptoethanol, 0.20 % SDS, 20% glycerol, 0.01% bromophenol blue and pH 6.8. Ten µg of a M.W. marker (Sigma M4038) and 5 µg of protein sample were loaded into a lane. Gel electrophoresis was carried out at a constant 200 V. The gels were stained according to the method of Neuhoff et al. [18].

4.3.6 Thermal behavior

Differential scanning calorimetry (DSC) measurement of the isolated protein was performed with a DSC 7 PerkinElmer Thermal Analyzer (PerkinElmer, Inc., Shelton, CT, USA). Twenty-five µl of 10% (w/w, db) protein dispersion prepared in 0.01 M phosphate buffer (pH 7.0) was hermetically sealed in an aluminum pan and an empty sealed pan was used as reference. The sample was heated from 10 to 120 °C at a rate of 10 °C /min. Peak denaturation temperature (T_d in °C) and thermal denaturation enthalpies (ΔH in Joules per gram of protein) were calculated from the endothermic curves using Pyris software (version 7.0, PerkinElmer, Inc., Shelton, CT, USA). T_d is the maximum temperature of the peak in the curve and ΔH is the area under the endothermic curve. All samples were analyzed in triplicate.

4.3.7 Water solubility profile

An aliquot of 0.50 g of freeze-dried protein was dispersed into distilled water at a 1% concentration. The pH of the protein dispersion was adjusted to 3, 4, 5, 7 and 9 with either 2N HCl or 2N NaOH. The protein dispersion was stirred for 1 h and within this hour the pH was adjusted, if necessary, after 15 and 30 min of stirring. Twenty-five mL of the dispersion was loaded into 50 mL centrifuge tubes and centrifuged at 10,000 x *g* for 10 min at 20 °C. Supernatant was measured for protein content using the Biuret method with Bovine Serum Albumin (BSA) as standard. Solubility was calculated as:

$$\text{Protein Solubility (\%)} = \left[\frac{\text{Protein in supernatant (g)}}{\text{Protein in starting material (g)}} \right] \text{x } 100$$

All samples were analyzed in triplicate.

4.3.8 Emulsification capacity (EC)

Twenty-five mL of a 2% (w/w) sample dispersion adjusted to pH 7.0 with 2N HCl or 2N NaOH was transferred to a 400-mL plastic beaker. Canola oil dyed with approximately 4 µg/mL Sudan Red 7B (Sigma, St. Louis, MO, USA) was continuously blended into the dispersion at a 36 mL/min flow rate by using a Bamix hand mixer (ESGE AG Model 120, Mettlen, Switzerland) at low setting until phase inversion was reached. Phase inversion was identified by the abrupt decrease in homogeneity and loss of viscosity. The weight of oil needed to reach the phase inversion was determined and EC (g oil/g sample) was calculated as the weight of oil used to cause inversion multiplied by 2. All samples were analyzed in triplicate.

4.3.9 Foaming capacity (FC)

Dispersion of 0.5 % (w/w, db) of soy protein at pH 7.0 was prepared. An 80 mL aliquot was loaded into a custom-designed glass column with a coarse fritted glass disk at the bottom and N_2 gas was purged through the sample at a 100 mL/min flow rate. The time for the foam to reach a 300-mL volume, the volume of the liquid incorporated into the foam, and the time for one half of the liquid incorporated into the foam to drain back were measured. The following parameters were calculated:

$$\text{Foaming capacity} = \left[\frac{V_f}{f_r \times t_f} \right]$$

$$\text{Foaming stability} = \left[\frac{1}{(V_{max} \text{ x } t_{1/2})} \right]$$

$$\text{Foaming rate} = \left[\frac{V_{max}}{t_f} \right]$$

Where, V_f is a fixed volume of 300 mL, f_r is the flow rate of the gas and t_f is time to reach V_f, V_{max} is volume of liquid incorporated into foam and $t_{1/2}$ is the time to drain one half of the liquid incorporated into the foam. All the samples were analyzed in triplicate.

4.3.10 Rheological properties

A 10 % protein dispersion of SPI was prepared in distilled water at pH 7.0. The sample was analyzed with a RS-150 Rheo Stress Rheometer (Haake, Germany) equipped with a cone-plate sensor of 60 mm diameter and 2° angle. The shear was applied at rate of 10-500 s^{-1} at room temperature. The power law model, $\sigma = k(\dot{\gamma})^n$ was used to model the experiment flow curves, where, σ was shear stress (Pa), k was the consistency coefficient (mPa.s^n), $\dot{\gamma}$ was shear rate (s^{-1}) and n was the flow behavior index. All samples were measured in triplicate.

4.3.11 Statistical analysis

Three independent batches of SPIs were prepared from defatted soy flakes for each sonication condition (power level and sonication time) and used for experiments. Data were analyzed by using the General Linear Model (GLM) in SAS system (version 9.1, SAS

Institute, Inc., USA) to compare means and calculate Least Significant Difference (LSD) at $p<0.05$.

4.4 Results and discussion

4.4.1 Protein extraction yield and protein content of SPI

The protein yield significantly increased with increase in sonication time and power level as compared to the control (Table 1).

Table 1: Protein yield and protein content of soy protein isolates as a function of sonication conditions

Sample	Sonication time (s)	Protein yield (%, db)	Protein content (%, db)
Control	0	54.24 ± 0.40^{a}	89 ± 0.80^{a}
Low power level	30	57.27 ± 1.23^{b}	$90\pm1.52^{a,b}$
	60	58.23 ± 0.80^{b}	94 ± 0.30^{c}
	120	61.28 ± 1.05^{c}	91 ± 1.48^{b}
High power level	30	62.39 ± 0.98^{c}	91 ± 0.82^{b}
	60	65.89 ± 1.53^{d}	86 ± 0.41^{d}
	120	72.91 ± 0.65^{e}	85 ± 1.30^{d}
LSD		1.42	1.45

LSD denotes the least significant difference. Means within each column followed by different superscript are significantly different at $p<0.05$ (n=3). db stands for dry basis.

Protein extraction yield increased by 13% and 34% when defatted soy flakes were treated for 120 s at low and high sonication power level, respectively. This increase could be attributed to cavitation induced by the ultrasound treatment, promoting a turbulent flow and enhancing mass transfer of the cell content [19]. The physical damage caused by the

hydrodynamic shear force produced by the cavitational effect of the ultrasound might also have contributed to the protein extractability increase [20]. The best condition for protein extractability, i.e., high power for 120 s was, however, not favorable to a high protein content of the recovered isolated proteins. Indeed this SPI had a significantly lower protein content than the one obtained for SPI from untreated flakes, 85% vs. 89%. While the appellation SPI could only be used if protein content is higher than 90%, db [21], this term was used for isolated proteins from high power sonication applied for 60 and 120 s, to simplify the description of the isolated protein fractions. The protein content of SPI being affected by the amount of associated and conjugated non-protein constituents precipitating as impurities with the protein [22], the lower protein content of SPI at high power and longer sonication time could be explained by an increase in extracted non-protein constituents. The ultrasound-treated proteins solubility might also have increased at pH 4.5, i.e., pH applied for SPI production. A non-linear increase in protein extractability was observed with sonication power and sonication time. For instance, at 25% power output, i.e., low power level, as sonication time increased from 60 to 120 s, the mean protein extraction yield increased by 3% (from 58 to 61%) instead of 7% at 100% power output, i.e., high power level (from 66 to 73%). The nonlinearity of the effects of sonication power and sonication time on wheat flour protein extractability was previously reported by Singh et al. [23].

4.4.2 SDS-PAGE

SDS-PAGE profile of isolated soy proteins obtained with sonication and without sonication of defatted soy flakes are shown in Fig. 2. The profile of the SPI recovered from

treated defatted soy flakes was similar to the one of the control, revealing that sonication did not modify the peptide profile, regardless of the sonication conditions.

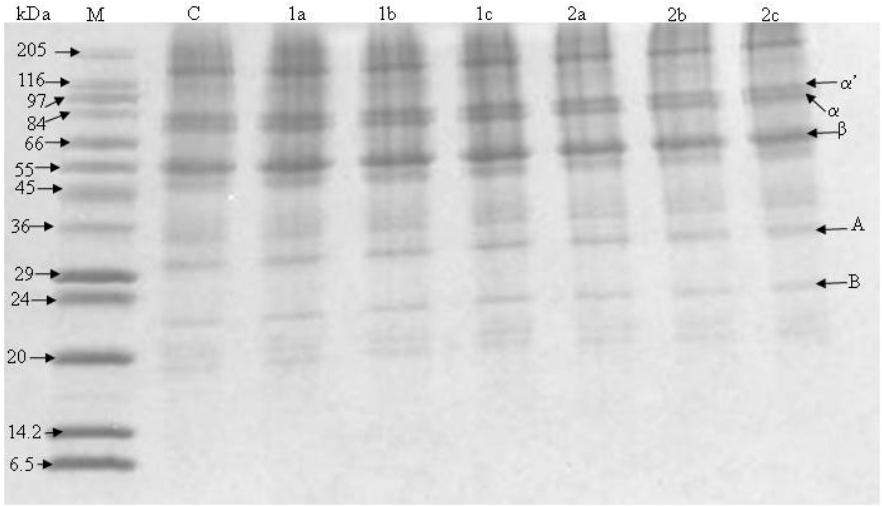

Figure 2: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profiles of soy protein isolates. M: molecular weight marker; C: control; 1a, 1b and 1c: SPI sonicated at low power level for 30, 60 and 120 s respectively; 2a, 2b and 2c: SPI sonicated at high power level for 30, 60 and 120 s, respectively; α',α and β: subunits of β-conglycinin; A: acidic subunit of glycinin; B: basic subunit of glycinin

4.4.3 Thermal properties of SPI

The effect of sonication conditions (power and time) on thermal properties of SPI is shown in Table 2. The control SPI exhibited two thermal transitions at approximately 77 °C and 92 °C corresponding to the denaturation peak temperature (T_d) of β-conglycinin (7S) and glycinin (11S), respectively [24-25]. The enthalpy (ΔH) value of β-conglycinin and glycinin for control SPIs were consistent with those of Deak and Johnson [4]. After 60 s at low power level, T_d of β-conglycinin and glycinin decreased significantly, while increased after 120 s at high power level when compared to the control. At low power level, the ΔH of β-conglycinin

and glycinin remained unchanged compared to the control regardless of the sonication time, except for 60 s sonication, where ΔH of β-conglycinin increased significantly. Alternatively, at a high power level, ΔH of β-conglycinin at 120 s sonication periods remained unchanged as compared to the control, but ΔH of both proteins decreased significantly for all treatment times as compared to the control.

Table 2: Thermal properties of soy protein isolates as a function of sonication conditions

Sample	Sonication time (s)	Peak temperature, T_d [°C]		Enthalpy, ΔH [J/g]	
		β-conglycinin	Glycinin	β-conglycinin	Glycinin
Control	0	76.66±0.43[a]	92.32±0.60[a]	0.98±0.06[a,d]	5.19±0.30[a]
Low power level	30	75.60±0.52[b]	92.72±1.00[a]	0.80±0.26[a,d]	4.95±0.78[a]
	60	73.16±0.47[c]	90.08±0.83[b]	1.37±0.30[b]	4.73±0.48[a,b]
	120	77.15±0.90[a,d]	91.80±0.50[a]	0.81±0.12[a,d]	5.22±0.12[a]
High power level	30	77.33±0.40[d]	92.91±1.24[a]	0.39±0.14[c]	4.21±0.61[b]
	60	76.81±0.71[a,d]	93.03±0.49[a]	0.71±0.15[d]	4.37±0.51[b]
	120	78.18±0.31[d]	95.11±0.34[c]	0.83±0.24[a,d]	1.73±0.23[c]
LSD		0.472	0.740	0.196	0.578

LSD denotes the least significant difference. Means within each column followed by different superscript are significantly different at $p<0.05$ (n=3)

The significant reduction in ΔH (from 5.19 to 1.78 J/g) and increase in T_d (from 92.32 to 95.11 °C) of glycinin from SPI obtained with 120 s sonication at high power level (Table 2) suggested that although temperature of SPI during high power and 120 s was much lower (~65 °C) than T_d of glycinin (~92 °C), its protein conformation was altered to a certain extent and hydrophobic regions from the interior could be partially exposed. As a result, partially dissociated glycinin components would refold to form more stable aggregates resulting in

higher T_d [25]. Our findings differed from the results reported by Deak and Johnson [4] reporting important reduction in enthalpies of β-conglycinin (2.07 to 0.52 J/g) and slight reduction in enthalpies of glycinin (6.51 to 6.21 J/g), when the temperature was increased from 25 °C to 60 °C during aqueous extraction of SPI. These discrepancies in the results were probably caused by the cavitational effect of the ultrasound, a phenomenon of formation and collapse of minute gas bubbles. The energy is transferred into the liquid as gas bubbles collapse during the low-pressure part of the sonic pressure wave and results in producing heat, generating chemical changes, electrical charges and providing mechanical agitation. The amount of mechanical energy transferred into the liquid system largely depends on the experimental conditions and usually may give unpredictable results. Thus, these findings indicated that sonication of defatted soy flakes did not completely denature the proteins recovered in the SPI; however, denaturation occurred to some extent with an increase in sonication power and sonication time.

4.4.4 Protein solubility

All samples exhibited the typical U-shaped solubility profile characteristic of soy proteins, with the lowest solubility at the isoelectric pH (4-5) (Fig. 3). Regardless of sonication conditions, increased solubility of the treated SPI was observed at pH 3.0, 7.0 and 9.0, while for other pH values and treatment conditions, no such increment pattern was observed. Among the tested samples, the SPI obtained from sonication of defatted soy flakes for 120 s at a high power level had the highest solubility (~95%) at pH 9.0. This increase in solubility could not be attributed to the changes in the peptide profiles as none of the

treatment altered the SDS-PAGE profile of the samples (Fig. 2). Thus, part of the increased solubilities for the samples treated under the more drastic conditions can be related to the denatured state of the proteins (Table 2), which might have contributed to changes in the number of hydrophobic residues, charge, electrostatic repulsion and ionic hydration, which are parameters affecting protein solubility [26].

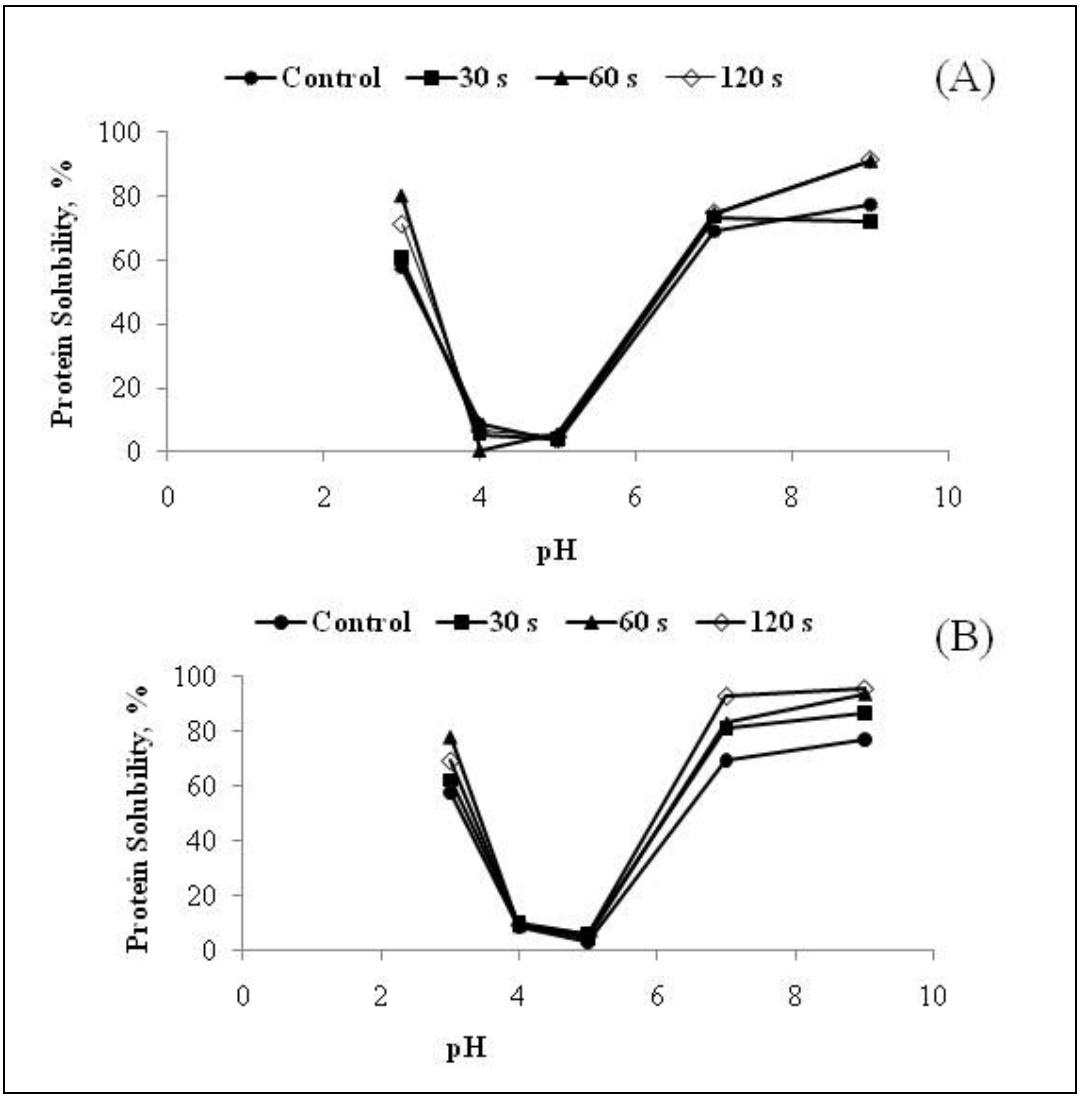

Figure 3: Protein solubility of soy protein isolates in water as a function of pH at different sonication conditions: (A) low power level (21 µm); (B) high power level (84 µm)

In principle, it is usually understood that denatured protein has a lower solubility as compared to native protein; however, Zheng et al. [27] also reported the higher solubility of fully denatured protein as compared to partly denatured and native spray-dried proteins, suggesting that protein solubility is influenced by several factors not only by the degree of exposed hydrophobic groups.

4.4.5 Emulsification capacity and foaming properties

The average emulsification capacity (EC) obtained for the control SPI was 445 g oil/ g protein at pH 7.0. Under some conditions (low power for 60 and 120 s and high power for 120 s), the EC of the recovered SPIs was significantly decreased (Fig. 4), while for other conditions, the SPIs retained their EC. No clear correlation could be made with DSC results (Table 2) or protein solubility at pH 7.0 (Fig. 3), which increased regardless of the treatment.

Foaming capacity (FC) of the control SPI was 0.98 ± 0.08 at pH 7.0. The FC and foaming rate (FR) of SPI were decreased after ultrasound treatment of flakes at low and high power levels (Table 3). However, no change in foaming stability (FS) of SPI was observed after sonication treatment to defatted soy flakes. Although increase in protein solubility would increase the EC and FC [28], sonication might have altered the conformation of protein in a way that prevents the ability of protein to unfold at interface resulting in poor surface activity.

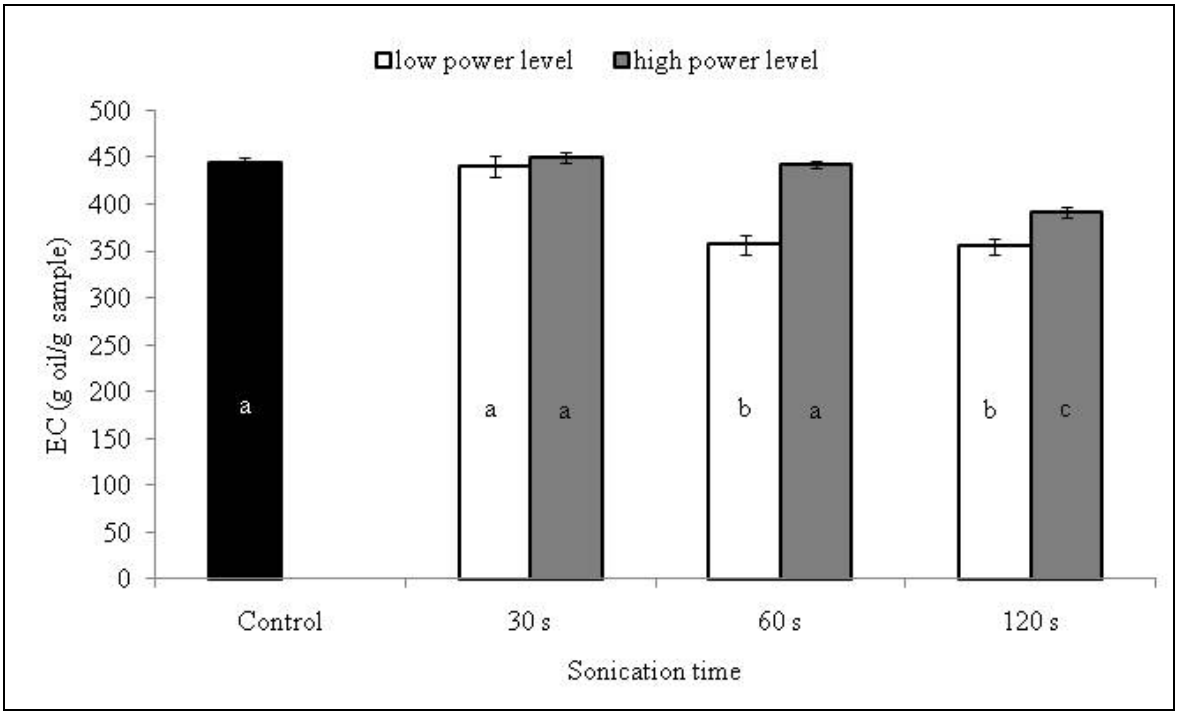

Figure 4: Emulsification capacity of soy protein isolates under various sonication conditions

Emulsification capacities bearing the same letters are not significantly different at $p<0.05$ (n=3)

Table 3: Foaming properties of soy protein isolates under various sonication conditions

Sample	Sonication time	FC (*)	FR (mL/min)	FS (1/mL.min)
Control	0	0.98 ± 0.06^{a}	8.30 ± 0.51^{a}	0.0121
Low power level	30	0.83 ± 0.01^{b}	7.00 ± 0.18^{b}	0.0106
	60	0.81 ± 0.05^{b}	6.82 ± 0.26^{b}	0.0117
	120	0.76 ± 0.05^{c}	6.92 ± 0.63^{b}	0.0103
High power level	30	0.71 ± 0.05^{d}	6.11 ± 0.71^{c}	0.0100
	60	0.84 ± 0.01^{b}	6.66 ± 0.41^{b}	0.0119
	120	$0.72\pm0.02^{c,d}$	6.19 ± 0.49^{c}	0.0101
LSD		0.05	0.56	Not different

LSD denotes the least significant difference. Means within each column followed by different superscripts are significantly different at $p<0.05$ (n=3). FC, FR and FS represents foaming capacity, foaming rate and foaming stability of SPI.

(*) Represents mL of foam formed by 1 mL of a 0.5% SPI dispersion.

4.4.6 Rheological properties

The Power Law parameters (consistency coefficient k, and flow behavior index, n) were determined from flow behavior of SPI prepared from non-sonicated and sonicated defatted soy flakes. All SPI dispersion exhibited a shear-thinning non-Newtonian flow behavior ($n<1$) up to 500 s^{-1}. Ultrasound treatment of the soy flakes significantly decreased the SPI consistency and increased their flow behavior except for the sample treated for 30 s at low power level. Some of these changes could be due to the effect of the treatment on the protein native state [4]. The changes in apparent viscosity of the SPIs at 500 s^{-1} shear rate are reported in Table 4.

Table 4: Rheological properties of soy protein isolates for different sonication conditions

Sample	Sonication time (s)	Flow consistency index (k, $mPa.s^n$)	Flow behavior index (n)	Apparent viscosity (Pa.s) At 500 s^{-1}
Control	0	0.232 ± 0.020^{a}	0.626 ± 0.008^{a}	0.03 ± 0.001^{a}
Low power level	30	0.199 ± 0.020^{b}	0.641 ± 0.010^{a}	0.05 ± 0.001^{b}
	60	0.188 ± 0.004^{b}	0.663 ± 0.010^{b}	0.02 ± 0.001^{c}
	120	$0.175\pm0.005^{b,c}$	0.789 ± 0.002^{c}	0.05 ± 0.001^{b}
High power level	30	$0.170\pm0.001^{b,d}$	0.814 ± 0.010^{d}	0.02 ± 0.001^{c}
	60	0.148 ± 0.006^{c}	0.721 ± 0.010^{e}	0.03 ± 0.002^{a}
	120	$0.157\pm0.02^{c,d}$	0.665 ± 0.020^{b}	0.02 ± 0.001^{c}
LSD		0.02	0.01	0.001

LSD denotes the least significant difference. Means within each column followed by different superscript are significantly different at $p<0.05$ (n=3).

Some samples (60 s at low power and 30 s and 120 s at high power level) had a lower apparent viscosity as compared to the control, which might be due to the increased

protein solubility (Fig. 3) following sonication. Compared to the control, the apparent viscosity of SPI remained unchanged after sonication for 60 s at a high power level but the viscosity increased after 30 s and 120 s sonication at low power level. Under these last conditions, proteins probably underwent some structural changes such as increased hydration properties [28] that impacted the viscosity behavior without modifying DSC values and peptide profile, as reported in the preceding sections.

4.5 Conclusion

The results of this study showed that high-power ultrasound is an efficient tool to improve the recovery of soy protein isolate from defatted soy flakes while only slightly modifying some functional properties including solubility and emulsification and foaming capacities. These changes were made without peptide profile changes and with some alterations in the native state of glycinin and β-conglycinin as illustrated by the DSC results, which is a unique advantage of this technology compared to protease treatment of soy flakes, which increases protein extraction yield but also dramatically affect functionality of the recovered proteins.

4.6 Acknowledgements

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CHAPTER 5: PROCESS ECONOMICS AND SIGNIFICANCE

5.1 Introduction

Soybeans are a major US crop, grown primarily for edible vegetable oil, protein and livestock feed. Solvent extraction is used to recover the oil from seed and the proteins are usually recovered from the resulting defatted soybean flakes (Grieshop et al., 2003; Lusas and Riaz, 1995). There are four major categories of protein commercially available: flour, concentrates, isolates and textured soy protein, each varying in fat, protein, and carbohydrate content and in functionality. Soy protein isolate (SPI) is the most refined forms of soy protein containing at least 90% protein on dry basis (Wang and Johnson, 2001) and can be used in food and non-food application (Deak and Johnson, 2007).

An alkaline aqueous extraction of defatted soy flakes is the first step in SPI production. This extraction step alone is not efficient in extracting all available protein as about half of the available protein remains in the insoluble fraction (Jung et al., 2006). In the past several pretreatment methods such as microwave heating (Choi et al., 2006), enzymatic (Babiker, 2000) and chemical modifications were applied to the defatted soy flakes improving the protein extraction yield by up to 50% during aqueous alkali extraction method. The study by Karki et al. (2009) evaluated the use of high-power ultrasound prior to protein extraction in soy processing and improved the protein yield approximately by 46% with respect to control. While results were promising, the uncertainty about economic feasibility of high-power ultrasound has been the principal factor limiting its widespread application in

food processing. Thus, this section presents an economic analysis based on protein release obtained from sonication of defatted soy flakes.

5.2 Economic analysis

An analysis was conducted to evaluate the economic feasibility of high-power ultrasound as pretreatment technique during soy protein isolate production from defatted soy flakes. In this analysis, continuous flow ultrasonics using donut horn was assumed because of the extensive use of this system in various large scale applications. The economic analysis was based on the biobased economic analysis of Brown (2003). Since this study focused on the pretreatment of defatted soy flakes prior to protein extraction during aqueous extraction processing, the principle components of the cost-benefit analysis were the value of additional soy protein isolate vs. the cost of ultrasonication. Thus capital cost amortization and operational costs were developed for ultrasonication method.

Table 1 summarizes the assumptions of the economic analysis. The soy processing plants were set to operate 24 h/day with 35 days of predetermined shutdown period for maintenance annually. Thus a basis of 7,920 h per year operating time was used in this analysis. The soy processing plants were designed to process 3 million lbs (1,500 tons) of defatted soy flakes annually. Material module factor (MMF) is defined as “the ratio of cost of labor to install a particular piece of equipment to the combined cost of the installed equipment “, and labor module factor (LMF) is “the ratio of the cost of labor to install a particular piece of equipment to the combined cost of installed equipment and materials used to perform the installation “(Brown, 2003).

Table 1: Assumptions of economic analysis in 2009 dollars

Parameters	Ultrasonics
Annual processing of defatted soy flakes (lbs/yr)	3,00,0000
Annual operating hours	7,920
Material module factor (MMF)	0.27
Labor module factor (LMF)	0.27
Electricity cost (cents/kW.h)	7.12
Number of ultrasonics unit to be installed	43
Ultrasonics power required (W/unit)	3,000
Maintenance and repair (% of capital cost)	10
Ultrasonics cost ($/unit)	10,000
Interest rate (%)	15

Brown (2003) has defined a MMF and LMF for common industrial equipments, however, high-power ultrasonics is not included in the list, and thus an MMF and LMF of 0.27 was assumed. While computing operating cost, labor cost was not considered in the computation as it requires only portion of operator's work coverage. While electricity cost were used to account for the utility cost of ultrasonics. A maximum flow rate in a full-scale plant was assumed to be 300 gal/min and numbers of ultrasonic donut horn calculated were 43. The industrial electric utility costs were obtained from the US energy information administration (EIA, 2009) and ultrasonic unit cost was determined. The percentage scale from capital cost was used for maintenance and repair assumptions (Brown, 2003). Ten percent of capital cost was set for repair and maintenance cost of ultrasonics as it is known that ultrasonic horn will wear when continuously operated.

Summary of economic analysis of ultrasonic pretreatment during aqueous extraction processing of soybean is shown in Table 2. The total capital cost was categorized into direct cost and indirect cost. "Direct cost include the purchase price of the equipment, cost of

Table 2: Economic analysis of high-power ultrasound as pretreatment

Fixed Capital Cost		Ultrasonics (2009) $
Direct cost		
Equipment, C_p		4,300,000
Materials for installation, C_m	MMF x C_p	116,100
Direct installation labor, C_l	LMF x (C_p + C_m)	147,447
Total direct cost, C_d	$C_p + C_m + C_l$	**693,547**
Indirect cost		
Freight, insurance, taxes, C_{fit}	0.08 x C_p	34,400
Construction overhead, C_o	0.70 x C_l	1,032,129
Engineering expenses, C_e	0.15 x (C_p + C_m)	81,915
Total indirect cost, C_{id}	$C_{fit} + C_o + C_e$	**1,148,444**
Fixed capital cost, C_f	$C_{id} + C_d$	1,841,991
Annual capital charges	$C_{capital}$	806749
Operating cost		
Utilities		193,550
Maintenance and repair		184,199
Total annual operating cost	$C_{operating}$	**377,749**
Annual pretreatment cost	$C_{capital} + C_{operating}$	1,184,499
Product cost (cents/lbs) due to pretreatment	Annual production cost/annual production	39.00

materials required for installation and salary for installation labor" (Brown, 2003), while indirect costs included "freight, insurance and taxes; construction overhead and engineering expenses' (Brown 2003). According to Brown (2003), 8% of the total equipment cost (C_p) can be used to estimate freight, insurance and taxes in US. However, construction overhead (C_o) can be as high as 70% of the labor cost (C_l), which included fringe benefits on labor, construction machinery cost and site cleanup cost. The salaries and benefits for design engineers, office supply expenses and associated overhead were included in the engineering

cost, which was assumed to be 15% of combine equipment (C_p) and installation material (C_m) cost (Brown, 2003).

The sum of annual capital charge ($C_{capital}$) and annual operating cost ($C_{operating}$) was the total pretreatment cost. The annual capital charge ($C_{capital}$) is the cost a company has to pay the bank if a fixed capital cost was secured through a loan with an annual interest rate of 15% over a payment period of 3 years. The annual pretreatment cost was then divided by 3 million lbs to determine the pretreatment cost per lbs of protein produced. Since the information regarding continuous flow system on soy protein extraction was lacking, we analyzed by assuming that continuous flow system will require lesser energy as compared to the batch process to extract the same amount of protein. The estimated weight of protein produced due to ultrasonics was 3, 00,000 lbs per year. Annual revenue (A_r) from additional SPI resulted due to ultrasonication of defatted soy flakes was calculated from market price of SPI and the protein yield. The selling price of $5.50 per pounds of SPI was chosen after surveying the current prices of similar product being marketed in June 2009.

The capital cost for ultrasonication was estimated to be $1,841,991 as shown in table 2. The profitability of ultrasonic pretreatment was further evaluated by calculating net present value (NPV) and cost benefit ratio (BCR) method. NPV is the total present value of a time series of cash flow while BCR method measures the total expected benefit against total expected cost (Munasinghe and Cleveland, 2007). Assuming the SPI price of $5.50 per lbs, the annual rate of return for the soy processing plant using ultrasonics would be 39%. In addition to that the net present value of ultrasonics is $819,826 with discount rate of 10% for a period of 3 years. Cost benefit ratio of 1.4 using ultrasonics indicate that investment in

ultrasonics is cost effective. This study confirmed that defatted soy flakes can be profitably processed with ultrasonic pretreatment to recover high-value protein.

5.3 Conclusion

The economic feasibility of a hypothetical soy processing plant, which was sized to process 3 million lbs of defatted soy flakes annually, was evaluated. The use of a continuous flow system using donut horn was assumed as the treatment point of analysis. It was found that installation of ultrasonics in soy processing plant results in economically attractive (39%) annual rate of return. However, more detailed analysis based on pilot scale study should be made to fully appreciate the economic benefits of using high-power ultrasound technology in soy processing.

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CHAPTER 6: GENERAL CONCLUSIONS AND RECOMMENDATIONS

The effect of ultrasonication was evaluated for its ability to enhance the protein and total sugar yield, soy protein isolate yield and functionality of the protein isolate obtained during bench-scale aqueous extraction of defatted soybean flakes pretreated at various high-power ultrasound intensity and time. This work also aimed to determine if temperature raise during treatment significantly impacted the extractability of protein and sugar from defatted soybean flakes.

Our results clearly showed that sonication power had a significant effect on protein and sugar release. Indeed, the total protein release increased by 13, 23, 27 and 46%, respectively, with respect to control, after 2 minutes at very low (21 μm_{pp}), low (42 μm_{pp}), medium (63 μm_{pp}) and high (84 μm_{pp}) power level, respectively. Similarly, total sugar release improved by 50% after 2 minutes of sonication at high power level when compared to control. The comparison of the total protein and total sugar release obtained with and without temperature control systems during sonication showed that the temperature fluctuation during sonication had no impact on the extractability of these components. Therefore, it was established that the increase in protein and sugar release was not due to the thermal effect of sonication. We provided evidence that improved protein and sugar release was primarily due to the cellular rupture of defatted soybean flakes and reduction in particle size induced by the cavitation phenomenon occurring during treatment. Extensive cellular disruption of defatted soybean flakes was observed by SEM of the sonicated samples and nearly 10-fold reductions in the particle size of defatted soybean flakes were observed. These findings might have

important economical implications. It could indeed be assumed that the insoluble fraction recovered after aqueous extraction of sonicated defatted soybean flakes would contain less flatulence-causing sugars, as more sugar was released in the water extract. Sugar profile of insoluble fractions, however, needs to be determined to verify this hypothesis. Soybeans are rich in α-linked oligosaccharides mainly raffinose and stachyose, thus, α-1,6-galactosidase enzyme is required to breakdown these sugar molecule. Since humans and other mono-gastric animals lack this enzyme in their intestinal mucosa, these sugars are metabolized in the lower intestine by intestinal micro flora containing enzymes, which leads to gas production. Therefore excessive accumulation of intestinal gas has been a significant limiting factor to utilizing soybeans and soy protein ingredients in food and feed. Thus decrease in carbohydrate content could enhance the value of the insoluble fraction as food ingredients.

Soy protein isolate yield increased by 34% when defatted soybean flakes were treated for 120 seconds at high sonication power. This increase was attributed to cavitation induced by the ultrasound treatment, which promotes a turbulent flow and higher penetration of the solvent into the cellular material and thus, enhances mass transfer of the cell content to and from the interfaces. However the best conditions for protein extractability, i.e., high power for 120 seconds, were not favorable to obtain a soy protein isolate with high protein content. In these conditions the protein content of soy protein isolate as compared to that of isolated protein from untreated flakes were 85 vs. 89%. This could be attributed to an increase in extracted non-protein constituents that were precipitated as impurities with protein. With the use of other techniques such as membrane filtration, this problem might not occur and therefore, the higher protein extractability will lead to higher soy protein isolate recovery.

A non-linear increase in protein extractability was observed with sonication power and sonication time. For example, at low power level, as sonication time increased from 60 to 120 seconds, the mean protein extraction yield increased by 3% (from 58 to 61%) instead of 7% at high power level (from 66 to 73%). This could be due to the ultrasonic parameters other than sonication amplitude and duration such as vapor pressure and temperature, which affect the overall process output. Thus it is essential to optimize the sonication parameters to maximize extractability with a minimum amount of energy.

Since energy is the key parameter in selecting the ultrasound technology for commercial application we estimated the ultrasonic energy input as function of sonication power, time and volume. The results showed that an energy input of 36 J/mL of slurry was required to obtain a protein yield of 61 and sugar release of 11.5 per 100 g of defatted soy flakes. Increasing the protein yield and sugar release to 78% and 13.5 g per 100 g of defatted soy flakes increased the energy input to 307 J/mL of slurry. Therefore from an energy input point of view, the low power level is more energy efficient than the high power level, in spite of higher protein and sugar releases. However in order to fully appreciate the economic benefits of this technology, detailed economic analysis should be made based on protein and sugar release obtained from a commercial-size continuous-flow system.

The study on the functional properties of soy protein isolates resulting from ultrasonication of defatted soy flakes provided interesting results regarding the impact of sonication time and power level. Some differences in thermal behavior were observed between the soy protein isolates produced by sonication and the control. A significant reduction in enthalpy (from 5.19 to 1.78 J/g) and increase in peak temperature (from 92.32 to

95.11°C) of glycinin, one of the major soy proteins in soy protein isolates, was obtained with 120 seconds of sonication at high power level suggesting that the protein conformation was altered to a certain extent. These changes were attributed to the cavitational effect of ultrasound, a phenomenon of formation and collapse of minute gas bubbles. However, no change in the peptide profile was revealed from sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Thus the results of this study showed that high-power ultrasound is an efficient tool to improve the extractability of protein and sugar as well as the recovery of soy protein isolate from defatted soybean flakes, while only slightly modifying some functional properties including solubility and emulsification and foaming capacities. These changes were made without peptide profile changes and with minor alterations in the native state of glycinin and β-conglycinin, which is a unique advantage of this technology compared to protease treatment of soybean flakes, which increases protein extraction yield but can also dramatically affect functionality of the recovered proteins.

6.1 Future studies

Several conclusions were drawn from this research on high-power ultrasound effect on protein and total sugar yield, soy protein isolate yield and functionality of the protein isolate obtained during bench-scale aqueous extraction of defatted soybean flakes, yet more understanding of mechanisms occurring during ultrasound treatment is needed to optimize the better use of this technology for soy protein production and utilization. The use of high-power ultrasound prior to protein extraction from defatted soybean flakes has been discussed

at a bench-scale (500 mL working volume). Thus one area of research would be to apply high-power ultrasound in a continuous mode in a large scale to see the feasibility of this technology in commercial scale, and also to optimize the ultrasonic parameters such as flow rate, sonication time and power. As explained above, it is expected that the fiber fraction will contain less amount of indigestible oligosaccharides than those of regular soybean fiber, making their usage more attractive for animal feed. At the same time, sugar-rich soy whey generated during soy protein isolate production could be used as a fermentation media for the production of high-value product such as nisin, enzymes, and lactic acids. The extraction of important components of soybeans, such as isoflavones, saponins and phytic acids are greatly affected by the processing parameters, thus, it would be interesting to determine the effect of high-power ultrasound on their extractability. From the standpoint of utilization of soy proteins, heat treatment of flakes is required to remove undesirable factors such as lipoxygenase and trypsin inhibitors. However, such heat treatment of flakes results in loss of protein solubility due to heat denaturation. It could be interesting to determine whether ultrasonication helps in removal of these unwanted compounds, without affecting the protein native state. The heat inactivation of lipoxygenase activity during soybean processing usually helps in removing the beany flavor of soy protein isolate so it would be a good idea to evaluate sensory properties of soy protein isolates prepared from ultrasonicated flakes.

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